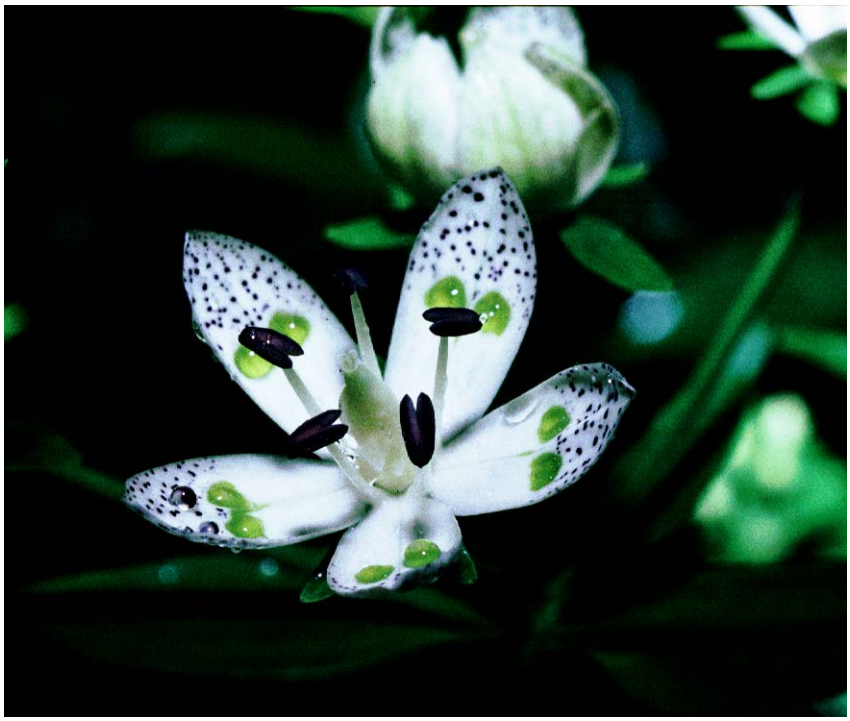
Molecular Phylogenetic, Karyological and Palynological Studies in Subtribe Swertiinae (Gentianaceae)



par Philippe Chassot sous la direction du Prof. Philippe Küpfer

Thèse présentée à la faculté des sciences de l'Université de Neuchâtel pour l'obtention du grade de docteur ès sciences

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Molecular phylogenetic, karyological and palynological studies in subtribe Swertiinae (Gentianaceae)

M. Philippe CHASSOT

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel, sur le rapport des membres du jury

Mme L. Struwe (Rutgers University, New Jersey USA),
MM. P. Küpfer (directeur de thèse), M. Aragno,
Y.-M. Yuan et F. Bretagnolle (Dijon F)

autorise l'impression de la présente thèse.

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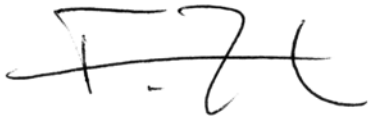

François Zwahlen

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SUMMARY

In its most recent circumscription, subtribe Swertiinae (Gentianaceae–Gentianeae) is a polymorphic assemblage of 14 genera (15 including the controversial genus *Lomatogoniopsis*) representing over 500 species distributed almost world-wide and mostly in montane, alpine or arctic habitats. The centres of generic diversity are in Asia and North America, but species diversity is concentrated in the mountains of Eurasia and the southern hemisphere (*Gentianella*, *Halenia*), with secondary diversification centres in western North America (*Frasera*) and the mountains of tropical Africa (*Swertia*). Seven genera are monotypic or count less than 5 species (*Bartonia*, *Jaeschkea*, *Latouchea*, *Megacodon*, *Obolaria*, *Pterygocalyx*, *Veratrilla*), 4 have not more than 25 species (*Comastoma*, *Frasera*, *Gentianopsis*, *Lomatogonium*), *Halenia* is of moderate size (ca 40-80 spp.), while *Swertia* (ca 140 spp.) and *Gentianella* (ca 250 spp.) are by far the most speciose. Whereas the circumscription of tribe Gentianeae has remained rather stable over the time (only *Bartonia* and *Obolaria* were controversial), the delimitation of the Swertiinae from the Gentianinae is more recent, and the systematic position of some taxa (*Bartonia*, *Latouchea*, *Megacodon*, *Obolaria*) relies much on molecular phylogenetic results. Within the Swertiinae, flower morphological homoplasy and varying emphases of perceived similarities and differences have led to considerable transfer, lumping and splitting. Many genera are now widely accepted, that were segregated from *Gentianella* (*Comastoma*, *Gentianopsis*, *Megacodon*, *Pterygocalyx*), and for which reliable delimiting characters have been convincingly brought out. For the genus *Swertia*, however, limits with accepted segregates (*Halenia*, *Lomatogonium*, *Veratrilla*) are less convincing, as they are often based on very few and sometimes overlapping characters. In their current circumscription, *Gentianella* and *Swertia* are still largely polymorphic. Phylogenetic issues in *Gentianella* have been addressed elsewhere (Hagen & Kadereit, 2001), and it is focused here on the affinities among species of *Swertia* and with other taxa of the Swertiinae. This study was thus undertaken in the aim of addressing 1) phylogenetic relationships in subtribe Swertiinae using non-coding nuclear ribosomal and chloroplast DNA sequences, 2) chromosomal evolution in the subtribe in regard of molecular results, 3) pollen morphological relevance to infer phylogenetic affinities, and 4) generic limits and the relevance of flower morphological characters commonly used in the taxonomy of the Swertiinae, in particular in the broad *Frasera-Lomatogonium-Swertia-Veratrilla* complex.

Previous phylogenetic hypotheses among taxa of the Swertiinae based on phytochemical, karyological, morphological, anatomical and molecular data are often contradictory, mainly due the small number of species studied and restrained amount of data available. Molecular phylogenetic relationships among 128 species of 15 genera of subtribe Swertiinae (Gentianaceae-Gentianeae) were

thus studied using chloroplast *trn*L intron, *trn*L-F and *trn*S-*ycf*9 intergenic spacers, and nuclear ITS sequences, as well as published *mat*K sequences for a reduced taxon sample. Phylogenies obtained by unweighted parsimony were similar to those inferred with the Bayesian method. Incongruent relationships inferred from nuclear and chloroplast data were explored in some detail, but found to be inconclusive. The phylogeny of the Swertiinae based on the analyses of combined sequence data consisted of a basal grade comprising *Bartonia*, *Frasera*, *Gentianella* p. p. (1 lineage), *Gentianopsis*, *Halenia*, *Latouchea*, *Megacodon*, *Obolaria*, *Veratrilla* and *Swertia* p. p. (8 lineages), and of a more derived highly supported clade (clade A) including *Comastoma*, *Gentianella* p. p. (4 lineages), *Jaeschkea*, *Lomatogoniopsis*, *Lomatogonium* and *Swertia* p. p. (6 lineages). Polyphyly was confirmed for *Gentianella*, *Jaeschkea*, *Lomatogonium* and *Swertia*. The North American *Obolaria* and Chinese *Latouchea* were disjunct sister taxa basal to all other Swertiinae. Other clades corresponding to genera and lineages of polyphyletic genera were individually well supported, but deeper level relationships among them were often not resolved confidently. Species of *Swertia* belonged to 14 different lineages, that were scattered across the phylogeny and either distantly or closely interrelate, or related to other genera. From a biogeographical point of view, the molecular phylogeny suggests that migrations between Asia and North America happened several times in different taxa, while an altitudinal vicariance was observed in 2 lineages of *Swertia*. A penalised likelihood estimation of divergence times led to the hypothesis that critical geological and climatic events at ca 8 mya (rising of the Himalaya and onset of the Asian monsoon) may have played a major role in the history of the Swertiinae, and that the last common ancestor of the Swertiinae could have lived in the early Neogene. In addition, migrations of *Swertia* from the Sino-Himalayan region to South India, Indonesia and Melanesia probably occurred only recently, while migrations to Africa took place twice and at different dates.

To compare these results with karyological data, we counted the chromosome numbers for 60 species and reviewed the data available from literature for 171 species. 25 species were examined for the first time, mainly in the genera *Swertia* and *Lomatogonium*. Eight numbers were new for a genus, i. e. the tetraploid level (2n = 52) for *Frasera*, 2n = 10, 12, 16 and 38 for *Gentianella*, 2n = 24 for *Pterygocalyx*, and 2n = 12 and 42 for *Swertia*. Polyploidy was found to affect mainly genera that have diversified in North America, such as *Bartonia* (4x), *Frasera* (2x, 4x, 6x), and *Obolaria* (4x), or in South America, such as *Gentianella* (2x, 4x, 6x). Polyploidy is absent from Africa except for 1 species of *Swertia* in Madagascar, and rare in Asia. Primary basic chromosome numbers in the Swertiinae range from x = 5 to x = 15, the most frequent being x = 8, 9, 10 and 13, while 3 species only have the secondary basic numbers x = 15, 19 and 21. The variability in basic chromosome numbers is concentrated in *Comastoma*, *Lomatogonium*, *Jaeschkea*, binectariate species of *Gentianella*, and

Swertia, a genus which shows a level of variation nearly equal to that of the entire subtribe. The basic numbers x = 13 or x = 14 could be ancestral in the subtribe, and lower basic numbers are likely to be derived from x = 13. Species sharing the basic numbers x = 13 or x = 14 were found to be restricted to the basal lineages in the molecular phylogeny of the subtribe, while lower basic chromosome numbers characterise species occupying a more derive position. Frequent dysploidy in the latter derived lineages often results in overlapping chromosome numbers in distantly related taxa, whereas occasional severe descending dysploidy may bring about rather different chromosome numbers in closely related species. Of the various lineages identified in molecular phylogenetic studies, none confines to a unique and distinctive chromosome number. However, the relatively clear-cut cleavage between basal taxa of *Swertia* with x = 13 or 14, and those with a lower basic chromosome number is consistent with molecular phylogenetic results.

The significance of exine pattern variation in subtribe Swertiinae was re-evaluated in comparison with molecular phylogenetic results. Pollen grains of 65 representative species of all but 2 (*Megacodon* and *Veratrilla*) genera of the Swertiinae, with a particular focus on the genus *Swertia*, were examined by scanning electron microscopy as a complement of Nilsson's (1967b) survey of tribe Gentianeae using light microscopy. The main exine patterns found in the Swertiinae are striate, striato-reticulate, rugulate, reticulate, punctate, perforate, verrucate, spinose, shortly ridged or scabrate to smooth. Many distantly related genera such as *Frasera*, *Halenia*, *Gentianella*, and lineages of *Swertia* share a common striato-reticulate to reticulate exine pattern, whereas some closely related taxa such as *Comastoma* and *Lomatogonium* p. p. or *Latouchea* and *Obolaria* have very different pollen types. In general, relationships between genera and lineages of a genus revealed by molecular analyses were not well supported by pollen morphology, except for *Pterygocalyx* with some species of *Gentianopsis*. However, in combination with other morphological and karyological characters, pollen morphology was particularly useful in characterising several lineages of the polyphyletic genus *Swertia*.

Of the 14 lineages of *Swertia* identified by molecular data, 13 are well supported by morphological, karyological and palynological data, but, for most of them, do not correspond to existing infrageneric taxa, or only partly. In one clade for which no corroboration from other data was found, 3 species of *Swertia* were related to species of *Gentianella* and *Lomatogonium*. All but a few species of *Swertia* not sampled here could be unequivocally related to one of the lineages defined for the genus, for which a simple key was provided. The taxonomic relevance of *Swertia* in its current circumscription is questioned, and the opportunity to define smaller genera is envisaged. The generic status of *Lomatogoniopsis* and *Pterygocalyx* is briefly touched upon, and some recent generic segregates of Asian binectariate species of *Gentianella* are discussed in a phylogenetic perspective.

A new species of *Swertia* from Nepal (*S. barunensis* P. Chassot) is described and illustrated.

RÉSUMÉ

Dans sa circonscription la plus récente, la sous-tribu des Swertiinae (Gentianaceae–Gentianeae) est un assemblage polymorphe de 14 genres (15 en considérant le genre controversé *Lomatogoniopsis*), représentant plus de 500 espèces distribuées presque dans le monde entier, et essentiellement dans des habitats montagnards, alpins ou arctiques. Au niveau générique, les centres de diversité sont en Asie et en Amérique du Nord, alors que la diversité spécifique est maximale dans les montagnes d'Eurasie et de l'hémisphère sud (*Gentianella*, *Halenia*), avec des centres de diversification secondaires dans l'ouest de l'Amérique du Nord (*Frasera*) et dans les montagnes d'Afrique tropicale (*Swertia*). Sept genres sont monotypiques ou comptent moins de 5 espèces (*Bartonia*, *Jaeschkea*, *Latouchea*, *Megacodon*, *Obolaria*, *Pterygocalyx*, *Veratrilla*), 4 ont moins de 25 espèces (*Comastoma*, *Frasera*, *Gentianopsis*, *Lomatogonium*), *Halenia* est de taille modérée (ca 40-80 spp.), tandis que *Swertia* (ca 140 spp.) et *Gentianella* (ca 250 spp.) sont de loin les plus riches en espèces. Bien que la circonscription de la tribu des Gentianeae soit restée plutôt stable au cours du temps (l'inclusion de *Bartonia* et *Obolaria* seulement fut controversée), la délimitation entre la sous-tribu des Swertiinae et celle des Gentianinae est plus récente, et la position systématique de quelques genres (*Bartonia*, *Latouchea*, *Megacodon*, *Obolaria*) est basée presque uniquement sur des résultats phylogénétiques moléculaires. Au sein des Swertiinae, une forte homoplasie dans la morphologie florale, ainsi qu'une appréciation variable de la signification des ressemblances ou des différences observées ont conduit à de nombreux transfères, scissions ou regroupements entre taxa. Actuellement, de nombreux genres qui furent ségrégés de *Gentianella* (*Comastoma*, *Gentianopsis*, *Megacodon*, *Pterygocalyx*) sont communément acceptés, puisque des caractères fiables les délimitant ont pu être mis en évidence. Pour le genre *Swertia* par contre, les limites avec les genres proches reconnus (*Halenia*, *Lomatogonium*, *Veratrilla*) sont moins convaincantes, étant souvent basées sur des caractères peu nombreux et qui se chevauchent entre genres. Dans leur circonscription actuelle, *Gentianella* et *Swertia* sont encore des genres largement polymorphes. La problématique phylogénétique dans le genre *Gentianella* a été traitée ailleurs (Hagen & Kadereit, 2001), et nous nous concentrons ici sur les affinités des espèces de *Swertia* entre elles et avec celles des autres taxa des Swertiinae. Cette étude a donc été entreprise dans le but d'explorer 1) les relations phylogénétiques dans la sous-tribu des Swertiinae au moyen de séquences non-codantes d'ADN nucléaire ribosomique et chloroplastique, 2) l'évolution caryologique dans la sous-tribu en regard des résultats moléculaires, 3) l'utilité taxonomique et phylogénétiques de la morphologie du pollen, et 4) les limites génériques et la pertinence des critères de morphologie florale communément utilisés dans la taxonomie des Swertiinae, en particulier dans le complexe *Frasera-Lomatogonium-Swertia-Veratrilla*.

Résumé

Parmi les taxa des Swertiinae, les hypothèses phylogénétiques précédentes basées sur des données phytochimiques, caryologiques, morphologiques, anatomiques et moléculaires sont souvent contradictoires, principalement dû au nombre restreint d'espèces étudiées et au manque de données. Les relation phylogénétiques moléculaires entre 128 espèces de 15 genres des Swertiinae sont donc étudiées au moyen de séquences non-codantes chloroplastiques (intron *trn*L, espaceurs *trn*L-F et *trn*S-*ycf*9) et nucléaires ribosomiques (ITS), et de séquences *mat*K publiées par des tiers pour un nombre réduit d'espèces. Les phylogénies déduites de l'analyse par parcimonie non-pondérée des données sont semblables à celles déduites par l'analyse Bayésienne. Les différences entre les affinités déduites des séquences nucléaires et celles déduites des séquences chloroplastiques sont explorées en détail mais ne semblent pas être d'importance significative. La phylogénie des Swertiinae basée sur l'analyse des séquences combinées consiste en un grade basal comprenant *Bartonia*, *Frasera*, *Gentianella* p. p. (1 lignée), *Gentianopsis*, *Halenia*, *Latouchea*, *Megacodon*, *Obolaria*, *Veratrilla* et *Swertia* p. p. (8 lignées), et en clade dérivé hautement supporté (clade A) comprenant *Comastoma*, *Gentianella* p. p. (4 lignées), *Jaeschkea*, *Lomatogoniopsis*, *Lomatogonium* et *Swertia* p. p. (6 lignées). La polyphylie de *Gentianella*, *Jaeschkea*, *Lomatogonium* et *Swertia* est confirmée. *Obolaria* d'Amérique du Nord et *Latouchea* de Chine sont des taxa sœurs disjoints et basaux par rapport au reste des Swertiinae. Les autres clades, correspondant à des genres ou à des lignées de genres polyphylétiques, sont individuellement bien supportés, mais les relations entre eux aux niveaux plus profonds de la phylogénie ne sont pas résolues avec assurance. Les espèces du genre *Swertia* appartiennent à 14 lignées différentes, lesquelles sont présentes à tous les niveaux de la phylogénie, et ont des affinités proches ou distantes entre elles ou avec d'autres genres des Swertiinae. D'un point de vue biogéographique, les phylogénies moléculaires suggèrent que des migrations entre l'Asie et l'Amérique du Nord ont eu lieu à plusieurs reprises dans différents taxa. Une vicariance altitudinale est observée dans 2 lignées de *Swertia*. L'estimation par vraisemblance pénalisée des dates de divergence mène à l'hypothèse que des événement géologiques et climatiques majeurs (surrection de l'Himalaya et renforcement de la mousson Asiatique) survenus il y a ca 8 ma pourraient avoir eu une incidence importante sur l'histoire évolutive des Swertiinae. Le dernier ancêtre commun des Swertiinae pourrait avoir existé au début du Néogène. En outre, la migration d'espèces de *Swertia* depuis la région Sino-Himalayenne vers le sud de l'Inde, l'Indonésie et la Mélanésie ne semble s'être produite qu'au Pléistocène, alors que des migrations vers l'Afrique ont eu lieu 2 fois et à des dates différentes.

Afin de comparer ces résultats avec des données caryologiques, le nombre chromosomique de 60 espèces est établi, et les données disponibles dans la littérature sont compilées pour 171 espèces. 25 espèces sont examinées pour la première fois, surtout dans les genres *Swertia* et *Lomatogonium*. Huit nombres chromosomiques sont nouveaux pour un genre, i. e. la tétraploïdie (2n = 52) pour *Frasera*,

2n = 10, 12, 16 et 38 pour *Gentianella*, 2n = 24 pour *Pterygocalyx*, et 2n = 12 et 42 pour *Swertia*. La polyploïdie affecte principalement des genres qui se sont diversifiés en Amérique du Nord tels que *Bartonia* (4x), *Frasera* (2x, 4x, 6x) et *Obolaria* (4x), ou en Amérique du Sud avec *Gentianella* (2x, 4x, 6x). Elle est absente en Afrique, sauf chez 1 espèce de *Swertia* de Madagascar, et rare en Asie. Les nombres chromosomiques de base primaires documentés pour les Swertiinae s'échelonnent de x = 5 à x = 14, les plus fréquents étant x = 8, 9, 10 et 13, tandis que 3 espèces seulement ont des nombres de base secondaires x = 15, 19 et 21. La variabilité des nombres chromosomiques et forte chez *Comastoma*, *Lomatogonium*, *Jaeschkea*, les espèces binectariées de *Gentianella*, et *Swertia*. Ce dernier genre montre un niveau de variation presque égal à celui de l'entier de la sous-tribu. Les nombres de base x = 13 ou x = 14 sont confinés aux lignées basales dans la phylogénie moléculaire et pourraient être ancestraux chez les Swertiinae, tandis que les nombres de bases plus bas et vraisemblablement dérivés de x = 13 caractérisent les espèces occupant une position plus dérivée. En outre, la dysploïdie est fréquente chez ces dernières, et se traduit souvent par l'occurrence de nombres chromosomique identiques mais non homologues dans des lignées distantes, ou par des nombres différents chez des espèces apparentées de près. Aucune des différentes lignées identifiées par les analyses phylogénétiques moléculaires n'est caractérisée par un nombre chromosomique qui lui soit propre. Cependant, la distinction nette entre les espèces de *Swertia* avec x = 13 et 14, et celles dont le nombre de base est plus bas est en accord avec leur position basale et dérivée respectivement dans les phylogénies moléculaires.

L'importance de la variation de la structure de l'exine du pollen est réévalué en comparaison avec les résultats moléculaires. Le pollen de 65 espèces représentant tous les genres des Swertiinae sauf 2 (*Megacodon* et *Veratrilla*) et en particulier le genre *Swertia* est examiné par microscopie électronique à balayage en complément aux travaux de Nilsson (1967b) réalisés par microscopie photonique. Les types d'exine principaux trouvés chez les Swertiinae sont strié, strié-réticulé, rugulé, réticulé, ponctué, perforé, verruqueux, épineux, brièvement crêté, ou scabre à lisse. De nombreux genres distants tels que *Frasera*, *Halenia*, *Gentianella* et plusieurs lignées de *Swertia* partagent un type d'exine strié-réticulé commun, tandis que certains taxa proches comme *Comastoma* et *Lomatogonium* p. p., ou *Latouchea* et *Obolaria* on des types de pollen très différents. En général, les affinités entre genres ou lignées d'un genre révélées par les analyses moléculaires ne sont pas corroborées par la morphologie pollinique, sauf pour *Pterygocalyx* avec certaines espèces de *Gentianopsis*. Cependant, en combinaison avec d'autres données morphologiques et caryologiques, la morphologie pollinique est d'importance dans la caractérisation de plusieurs lignées du genre polyphylétique *Swertia*.

Résumé

Des 14 lignées de *Swertia* identifiées par les données moléculaires, 13 sont bien corroborées par les données morphologiques, caryologiques et palynologiques. Mais, pour la plupart, ces lignées ne correspondent à aucun taxon infra-générique, ou seulement partiellement. Dans un clade seulement, pour lequel aucune corroboration par d'autres types de données n'a pu être mise en évidence, 3 espèces de *Swertia* sont apparentées à des espèces de *Gentianella* et *Lomatogonium*. Toutes sauf quelques espèces de *Swertia* non échantillonnées dans cette étude peuvent être attribuées sans équivoque à une des lignées définies pour ce genre, pour lesquelles une clé simple est proposée. La pertinence taxonomique du genre *Swertia* dans sa circonscription actuelle est mise en doute, et l'opportunité d'une scission en plusieurs genres plus homogènes est envisagée. Le statut de genre de *Lomatogoniopsis* et *Pterygocalyx* est brièvement débattu, et quelques genres récemment séparées de *Gentianella* pour accommoder certaines espèces binectariées asiatiques sont discutés dans une perspective phylogénétique.

Une nouvelle espèce de *Swertia* du Népal (*S. barunensis* P. Chassot) est décrite et illustrée.

INTRODUCTION

Gentianaceae – Brief Overview

The Gentianaceae are a cosmopolitan family of over 1600 species and 87 commonly accepted genera, absent only from Antarctica. Its description is attributed to Jussieu (1789: 141), but the name *Gentiana* is accredited to Tournefort (1700) who used a Dioscoridean name commemorating the Illyrian king Gentius (180-167 BC) (Gunther, 1934). The boundaries of Gentianaceae have remained quite stable over the year, although with the addition of numerous newly discovered genera, our present day understanding of this family has considerably evolved since Jussieu's (1789) concept of a dicotyledonous, sympetalous and hypogynous natural order «Gentianeae».

In *Genera et Species Gentianearum*, Grisebach (1839) offered the first complete account of all known genera and species of the family, soon followed by his treatment of Gentianaceae in De Candolle's *Prodromus* (Grisebach 1845), which still is the only complete species-level review of the family. Since then, other treatments of the Gentianaceae have been proposed (e. g. Bentham, 1876; Huxley, 1888), but it is Gilg's (1895) epoch-making classification of the family based essentially on palynological characters that has been referred to for slightly over a century.

More recently, Gentianaceae were not spared by the ever increasing recourse to genomic characters using molecular phylogenetic techniques. These methods allowed to elaborate new hypotheses about morphological and anatomical character evolution, and to address issues about classification in a more reliable phylogenetic context. Much progress has been achieved in terms of delimiting monophyletic lineages both in the Gentianales and in the Gentianaceae (summarised in Struwe & Albert, 2002). The position of the few taxa for which their position in the Gentianaceae was controversial was clarified, new boundaries were thus set for this family, and a new tribal and sub-tribal classification was worked out that integrated a considerable amount of molecular and also morphological results (Struwe et al., 2002) (Fig.

1). Notably, Saccifoliaceae were included in Gentianaceae, (tribe Saccifolieae), as well as the Potalieae often considered as part of the Loganiaceae since Bentham (1857). Menyanthaceae, treated as a separate family by Dumortier (1829) but often retained in the Gentianaceae (e. g. Grisebach, 1845; Bentham, 1876; Gilg 1895) were excluded, as well as *Mitreola* L. and *Spigelia* L.

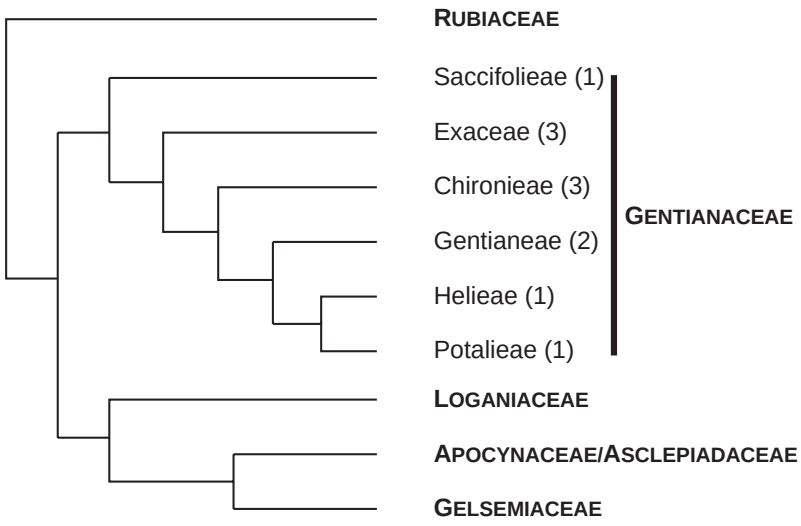

Fig. 1 Position of the Gentianaceae in the Gentianales and relationships among the 6 tribes of Gentianaceae. Number of subtribes are in parenthesis. (summarised from Backlund *et al.*, 2000 and Struwe *et al.*, 2002.)

Yet, in spite of the indubitable monophyly of the Gentianaceae in their current definition (Struwe *et al.*, 1994, 1998, 2002), there is no particularly striking synapomorphic diagnostic feature confined to the entire family. Morphological diversity is surprisingly high, such that the Gentianaceae may be viewed as a complex assemblage of taxa that are, however, for most of them rather well defined as not fitting in other families of Gentianales.

Gentianeae – Tip of the Iceberg ?

Tribe Gentianeae is probably the most known representative of Gentianaceae, as it comprises ca 939-968 species (out of over 1600 for the entire family), that have plentifully colonised temperate, alpine or arctic habitats of the globe, and thus contributed much to confer on the family its «markedly temperate» label (e. g. Good, 1947). Yet, ca 80% of these plants belong to 4 genera only: *Gentiana* L. (400 spp), *Gentianella* Moench (250), *Swertia* L. (135) and *Halenia* Borkh. (80) (counts from Struwe & Albert, 2002). In addition, with only 17 genera (18 with the erection of *Gentiana* sect. *Stenogyne* Franchet to genus level as *Metagentiana* T.-N. Ho & S.-W Liu), tribe Gentianeae only accounts for ca 20 % of the generic diversity in the Gentianaceae. Paradoxically, and notwithstanding the fact that numbers may greatly depend on the different taxonomists concept of genus and species, generic diversity and endemicity in the Gentianaceae are concentrated in tropical regions, in particular in the Neotropics (Favarger, 1985; Struwe & Albert, 2002). Also from a

Table 1. Circumscription of the Gentianeae (sensu Struwe *et al.*, 2002) according to Grisebach (1845), Gilg (1895) and Struwe *et al.* (2002). Taxa on a same background colour encompass more or less equivalent groups of species.

Grisebach (1845)	Gilg (1895)	Struwe *et al.* (2002)
Tribe Gentianeae	**Tribe Gentianeae**	**Tribe Gentianeae**
Subtribe Swertieae	**Subtribe Gentianinae**	**Subtribe Gentianinae**
Genus *Crawfurdia*	Genus *Crawfurdia*	Genus *Crawfurdia*
	Subgenus *Pterygocalyx*	
	Subgenus *Dipterospermum*	
Genus *Tripterospermum*	Subgenus *Tripterospermum*	Genus *Tripterospermum*
Genus *Gentiana*	Genus *Gentiana*	Genus *Gentiana*
	Subgenus *Eugentiana*	
		Subtribe Swertiinae
Genus *Eudoxia*	Subgenus *Gentianella*	Genus *Gentianella*
		Genus *Comastoma*
		Genus *Gentianopsis*
		Genus *Pterygocalyx*
		Genus *Megacodon*
	Genus *Jaeschkea*	Genus *Jaeschkea*
Genus *Pleurogyne*	Genus *Pleurogyne*	Genus *Lomatogonium*
		Genus *Veratrilla*
Genus *Swertia*	Genus *Swertia*	Genus *Swertia*
Genus *Anagallidium*		
Genus *Ophelia*		
Genus *Stellera*		
Genus *Frasera*		Genus *Frasera*
Genus *Halenia*	Genus *Halenia*	Genus *Halenia*
Genus *Exadenus*		
Genus *Centaurella*	in Subtribe Erythraeinae	Genus *Bartonia*
in Orobanchaceae	in Subtribe Erythraeinae	Genus *Obolaria*
undescribed	undescribed	Genus *Latouchea*
in Subtribe Chloreae	Genus *Ixanthus*	in Subtribe Chironiinae

phylogenetic point of view, the Gentianeae occupy a derived position in the family, sister to tribes Helieae and Potalieae. In sum thus, and in spite of its impressive number of species, tribe Gentianeae only encompasses a minor portion of the generic diversity encountered throughout the Gentianaceae. However, as more studies are realised on temperate Gentianaceae (e. g. Chironiinae [Mansion, 2001], Gentianinae [Yuan, 1996], Swertiinae [present study]), evidence accumulates suggesting that generic limits in these taxa are broad compared to tropical Gentianaceae, thus inducing an important bias in the comparison of diversity.

Gentianeae – Where is the Problem ?

However specious this tribe may be, its circumscription has remained remarkably stable over the years. New genera were described while others were abandoned, but inter-familial (!) / tribal / subtribal transfers involved only *Bartonia* Willd., *Ixanthus* Griseb. and *Obolaria* L.

(Table 1). A first outline of Gentianeae was proposed by Don (1837-1838), who included all genera of the modern Gentianeae in his subtribe Gentianeae-verae, but also several taxa now clearly excluded from this tribe (e. g. *Blackstonia* Huds., *Canscora* Lam., *Tachia* Aubl., etc.). With the description of tribe Swertieae from which Don's (1837-1838) alien elements were excluded, Grisebach's (1839) concept of this group already considerably resembled our present day one. In De Candolle, Grisebach (1845) retained the same generic composition but reduced Swertieae to a subtribe. Bentham's (1876) and Gilg's (1895) circumscription of the group brought little change. *Bartonia* and *Obolaria* that had been included in Bentham's (1876) Swertieae were removed by Gilg (1895), who additionally transferred *Ixanthus* to his subtribe Gentianinae. Recent molecular studies confirmed the affinities of *Bartonia* and *Obolaria* with genera of the Swertiinae (Struwe *et al.*, 1998, 2002; Hagen & Kadereit, 2002; I; IV) as suggested by Bentham (1876), whereas the affinities of *Ixanthus* with *Blackstonia* of the Chironiinae were established by Thiv *et al.* (1999). The monotypic genus *Latouchea* was described (Franchet, 1899: 212) after the completion of Gilg's (1895) classification. The plant was ascribed to the Gentianinae (Smith in Nilsson, 1967b; Ho & Liu, 1990), a position that was also recently verified by molecular data (I; IV). Thus, with exception of the cases detailed above, tribe Gentianeae does not appear to be particularly problematic, at least as regards its delimitation at tribal rank.

The subdivision of Gentianeae into two subtribes, Gentianinae and Swertiinae, mainly results from the separation of *Gentianella*, with, among other characters, nectariferous glands situated at the base of the corolla, from *Gentiana*, with glands at the base of the ovary. *Gentianella* was at first considered as a subgenus of *Gentiana* (Kusnezow, 1894, 1895), but the emergence of the concept of a *Gentiana*-lineage, as opposed to a *Gentianella*-lineage (see Gillett, 1957; Toyokuni, 1965b), laid the foundations for the two subtribes. The *Gentiana*-lineage included *Crawfurdia* Wall., *Gentiana* and *Tripterospermum* Blume, characterised by nectaries on the ovary, corollas with plicae (elongated folds between the corolla lobes), and an intracalycine membrane (membrane extending between the bases of the calyx lobes), the latter absent in some species of *Gentiana* and inconspicuous or absent in *Tripterospermum*. This lineage now forms subtribe Gentianinae. Its monophyly, generic composition and subdivisions have been established convincingly both by morphological (Smith, 1965) and molecular (Yuan & Küpfer, 1995, 1997; Yuan *et al.*, 1996) studies. The *Gentianella*-lineage was defined as to include those genera with epipetalous nectaries, a corolla without plicae and lacking an intracalycine membrane. Taxa such as (at the taxonomic rank accepted in Struwe *et al.* [2002]) *Comastoma* Toyok., *Gentianella*, *Frasera* Walter, *Halenia*, *Jaeschkea* Kurz,

Lomatogonium A. Braun, *Swertia* and *Veratrilla* Baill. were easily ascribed to this lineage, as they shared all of the above characters. The discontinuous intracalycine membrane of *Gentianopsis* Ma was not considered as homologous of that found in the *Gentiana*-lineage and this taxon was also related to the *Gentianella* lineage, as it has all of its other distinctive traits (Gillett, 1957). *Pterygocalyx* Maxim. was separated from *Gentiana* by Marquand (1931) and considered as closely related to *Gentianopsis* (Smith in Nilsson, 1967b; Toyokuni, 1965b) although the epipetalous nature of its nectaries was assessed only recently (Chen & Ho, 1998). Yet a number of genera have ambiguous or altogether unique morphologies so that their affinity with the *Gentianella*-lineage was only established convincingly by DNA sequence data (Yuan & Küpfer, 1995; Hagen & Kadereit, 2002; I; IV). These taxa lack plicae and an intracalycine membrane, but *Latouchea, Megacodon* (Hemsl.) Harry Sm. and *Obolaria* have nectaries on the ovary as in the *Gentiana*-lineage, whereas nectaries have never been convincingly reported for *Bartonia* (Lindsey, 1940). In sum thus, and in spite of the fact that no unique synapomorphic trait unambiguously characterises this group of taxa now recognised as subtribe Swertiinae, the phylogenetic coherence of this lineage as a monophyletic clade sister to the Gentianinae is well supported (Yuan & Küpfer, 1995; Hagen & Kadereit, 2002; Struwe *et al*., 2002; I; IV).

While phylogenetic relationships, generic circumscription and subdivisions within Gentianinae have been extensively studied and almost completely solved (see Yuan, 1996; Struwe *et al*., 2002 and references therein), similar problems have been much less successfully addressed in the Swertiinae, principally because the subtribe was never treated in its entirety but rather genus by genus, and also because most studies were based on a restricted number of species precluding generalised conclusions (e. g. Löve, 1953; Hedberg, 1957; Rao & Nagaraj, 1982; Ho & Liu, 1985; Omer *et al.*, 1988; Omer & Qaiser, 1991; Liu & Ho, 1992; Yuan, 1993; Xue et al., 1999, 2000, 2001; Liu *et al.*, 2001; Liu & Ho, 2002). Prior to initiating this study, phylogenetic affinities between taxa of the Swertiinae had been proposed based on karyological (e. g. Löve, 1953; Toyokuni, 1965b; Löve & Löve, 1976; Pringle, 1990), phytochemical (e. g. Favarger, 1985 and references therein), and morphological (e. g. Lindsey, 1940; Devi, 1962, 1967; Meszaros, 1996) data but were either based on relatively scanty data and/or included too few taxa (Fig. 2). An extensive palynological survey of Gentianeae was carried out (Nilsson, 1964, 1967ab; Nilsson & Skvarla, 1969; Jonsson, 1973), but the interpretation of pollen morphological similarities admittedly (Nilsson, 1967b) suffered from the poor understanding of phylogenetic affinities in subtribe Swertiinae, and in particular in the genus *Swertia*.

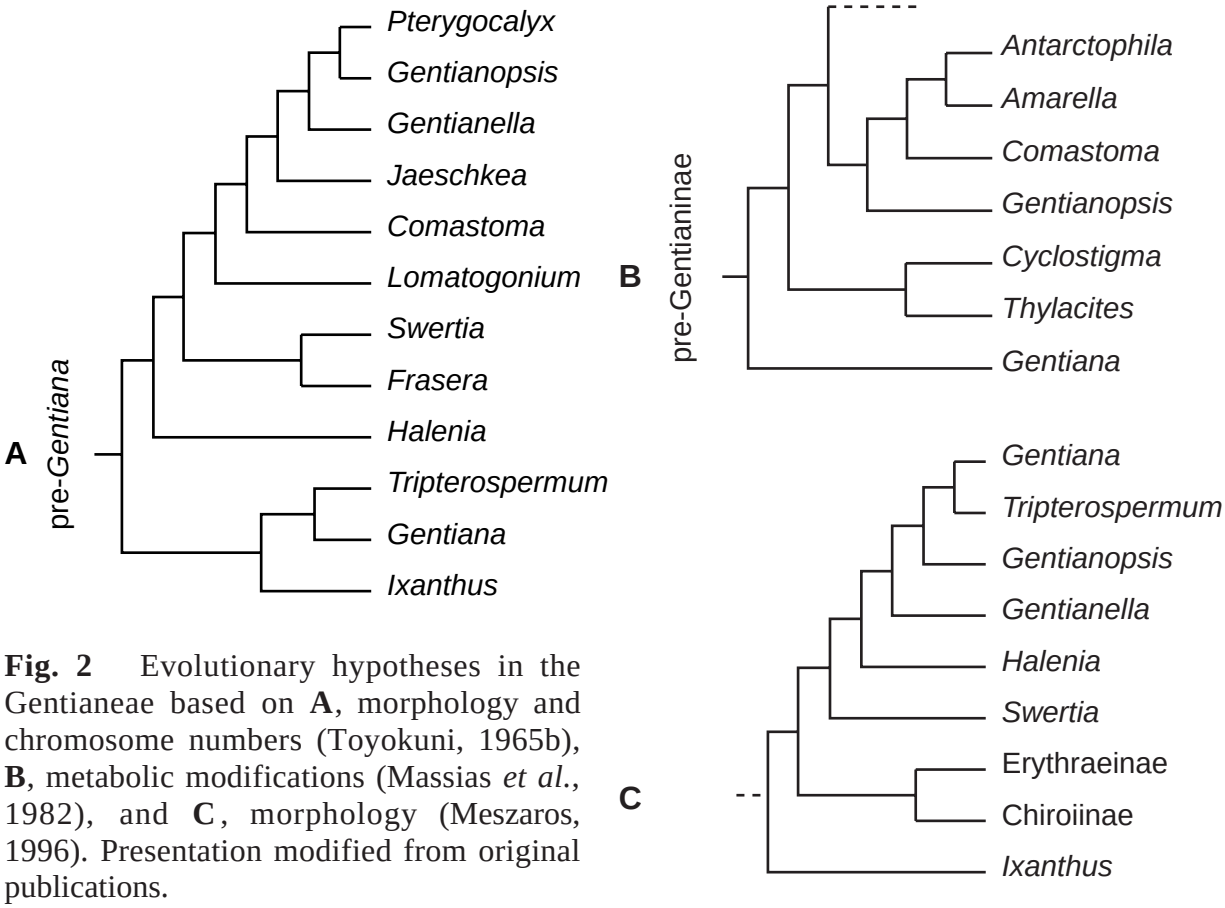

Fig. 2 Evolutionary hypotheses in the Gentianeae based on **A**, morphology and chromosome numbers (Toyokuni, 1965b), **B**, metabolic modifications (Massias *et al.*, 1982), and **C**, morphology (Meszaros, 1996). Presentation modified from original publications.

In addition to the general lack of knowledge about generic affinities in the Swertiinae, several genera per se are poorly defined, their identity often relying on very few diagnostic characters (e. g. *Frasera*, *Jaeschkea*, *Lomatogonium*, *Veratrilla*, see below). As appears from the comparison of generic composition in different classifications through time (Table 1), taxonomic instability is most important in *Gentianella* s. l. and *Swertia* s. l. Whereas the circumscription of *Gentianella* was narrowed by the segregation of *Comastoma* (Toyokuni, 1961) and *Gentianopsis* (Ma, 1951) (but still leaving a *Gentianella* containing ca 250 species), that of *Swertia* was greatly broadened as all but one (*Veratrilla*) of its early segregates were merged with it, resulting in a polymorphic genus of ca 140 species.

Genera of the Swertiinae

The following synopsis of the genera of Swertiinae is based on the classification of Struwe *et al.* (2002). Genera are presented in an order not reflecting phylogenetic affinities but their taxonomic history, for which a brief review is provided. Although some genera are well defined, this outline is intended to explain how weakly others are characterised, while still others may be viewed as grab-bags, the circumscription of which reflects more over 200 years of ambiguous taxonomic history than convincing evidence. A short morphological description is provided, and detailed accounts on karyology and palynology may be found in the other

chapters (II and III respectively). The first three genera, *Obolaria*, *Latouchea* and *Bartonia*, have always been regarded as well defined and clear-cut genera. The next 6 genera, *Jaeschkea*, *Gentianella*, *Megacodon*, *Pterygocalyx*, *Gentianopsis* and *Comastoma* have a taxonomic history intermingled with that of *Gentiana* (mainly due to the corolla lobes fused into a conspicuous tube in most species) and for this reason are considered here together. The remaining 5 genera, *Swertia*, *Frasera*, *Halenia*, *Lomatogonium* and *Veratrilla* all have a taxonomic history closely associated to *Swertia*, and may be viewed as a broadly defined «Swertioid» complex.

***Obolaria* L.** *Obolaria virginica* (Fig. 3), the only species of this genus, is an uncommon perennial and mycotrophic herb with morphological characteristics unique in the Swertiinae. Its corolla is tetramerous, with epipetalous stamens inserted in the sinuses of the lobes. Holm (1897) reports fimbriate scales on the lower part of the portion of the filaments adnate to the corolla tube (Fig. 4 A) and considered them as «nectaries» homologous of those of *Swertia*, but Lindsey (1940) situated nectariferous tissue at the base of the ovary. The plant has often been considered as lacking a calyx (e. g. Knoblauch, 1894; Baillon, 1889) but Holm (1897) brings convincing evidence that the two foliaceous elements subtending the corolla (Fig. 4 B) are sepals, although Lindsey (1940) found no vestige of vascular bundles to the two presumably aborted lobes. The aestivation is imbricate, a rare case in the Swertiinae otherwise known

Fig. 3 *Obolaria virginica* (Photography David Smith, www.delawarewildflowers.org).

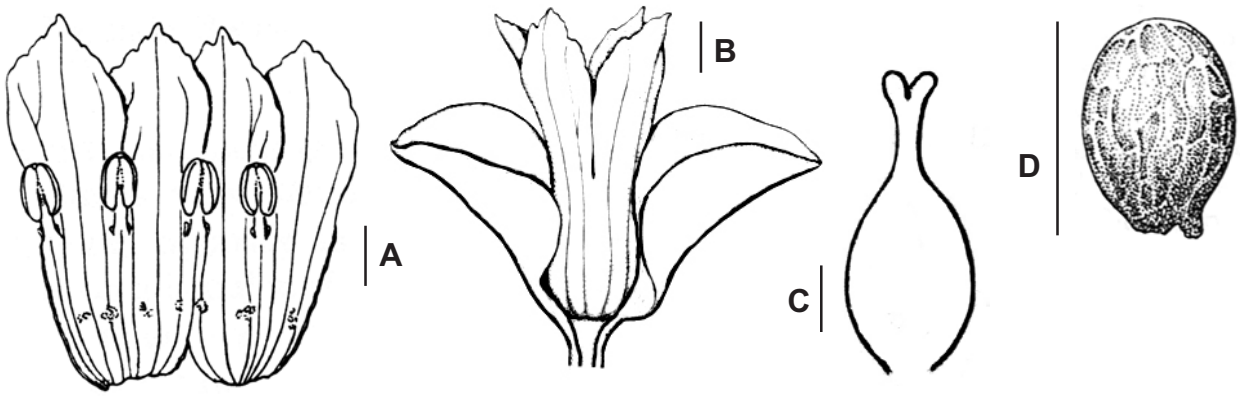

Fig. 4 *Obolaria virginica*. A: corolla, B: particular calyx with only 2 foliaceous sepals, C: ovary, D: seed. Scale bar 2 mm (A, B, C), 0.2 mm (D). Redrawn from Wood & Weaver (1982).

only from *Bartonia* and *Jaeschkea*. Seeds are ca 1600 per capsule, minute and delicately reticulate.

This genus was described by Linnaeus (1753, 2: 632), who thought it to be related to Orobanchaceae. This erroneous association was perpetuated by later botanists until Kuntze (1891) recognised its position in the Gentianaceae (as *Schultzia* Rafin.). Gillett (1959) confirmed the morphological differences between *O. virginica* L. and Orobanchaceae. *Obolaria* was related to the Swertiinae by Bentham (1876), and confirmed in this subtribe by molecular data (Struwe *et al.*, 1998; Hagen & Kadereit, 2002; I; IV). It grows in eastern North America, and is the closest relative of the Chinese monotypic genus *Latouchea* according to molecular data (I; IV), although Hagen & Kadereit argued for a close relationship with *Bartonia* based on the shared imbricate corolla aestivation and mycotrophic habit.

***Latouchea* Franch.** *Latouchea fokienensis* (Fig. 5) is an endemic perennial plant of south-eastern and south-western China. Its rosette leaves are large and dark green to reddish, reflecting its undergrowth habitat. The cauline leaves are much smaller and fewer, and axilate clusters of 1-3 tetramerous flowers. The corolla segments are fused into a relatively long tube, to which the filaments are adnate until shortly under the sinuses of the corolla lobes (Fig. 6 A). The calyx is divided to the base, and the sepals are asymmetrical, the two outer lobes being distinctly longer and somewhat decurrent on the pedicel (Fig. 6 B). Corolla aestivation is convolute. The ovary is attenuated into a long and curved stylar beak, and borne on a very short gynophore.

Fig. 5 *Latouchea fokienensis* (Photography Y.-M. Yuan).

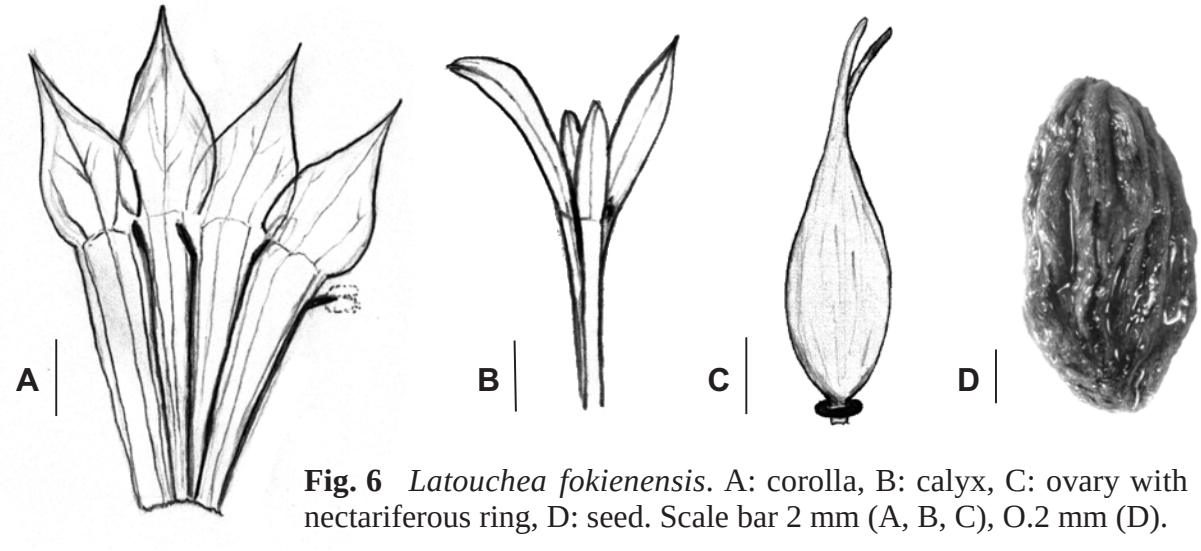

Fig. 6 *Latouchea fokienensis*. A: corolla, B: calyx, C: ovary with nectariferous ring, D: seed. Scale bar 2 mm (A, B, C), O.2 mm (D).

Nectaries form a conspicuous ring on the gynophore (Fig. 6 C). The seeds are longitudinally corrugate (Fig. 6 D).

The genus was described by Franchet (1899: 212). It was ascribed to the Gentianeae by Smith (in Nilsson, 1967b) and Ho & Liu (1990) on morphological grounds. Molecular results (I; IV) confirmed this position and related *Latouchea* to the Swertiinae as sister taxon to *Obolaria* in the basalmost lineage of the subtribe.

***Bartonia* Mühl. ex Willd.** Plants of *Bartonia* are restricted to eastern North America. They are of very slender habit, wiry stem and minute, subular, opposite or alternate leaves (Fig. 7). Holm (1906) considered the species of *Bartonia* investigated by him to be mycotrophic based on the coralloid roots. The flowers are tetramerous and solitary or arranged in terminal or axillary dichasia. The calyx is lobed nearly to the base and the sepals are unequal, the two inner lobes overlapped by the two outer ones. The corolla is campanulate, the lobes are fused in the lower third and of imbricate aestivation. The stamens are epipetalous and the filaments are adnate to the corolla tube up to the sinus of the lobes. No nectariferous tissue has been reported in *Bartonia* (see Holm, 1906; Lindsey, 1940; Gillett, 1959), but in very few flowers of *B. virginica*, a thickening of the basal part of the corolla segments could be observed, although a potentially nectariferous function of these anomalies could not be assessed (Fig. 8 A). The ovary is sessile and of variable shape, but consistently lacks a style, the stigmatic surface being broadly decurrent along the apical part of the carpelar suture (Fig. 8 B). The seeds are very numerous, minute and smooth to minutely reticulate.

Fig. 7 *Bartonia virginica* (Photography USDA-NRCS [2003]).

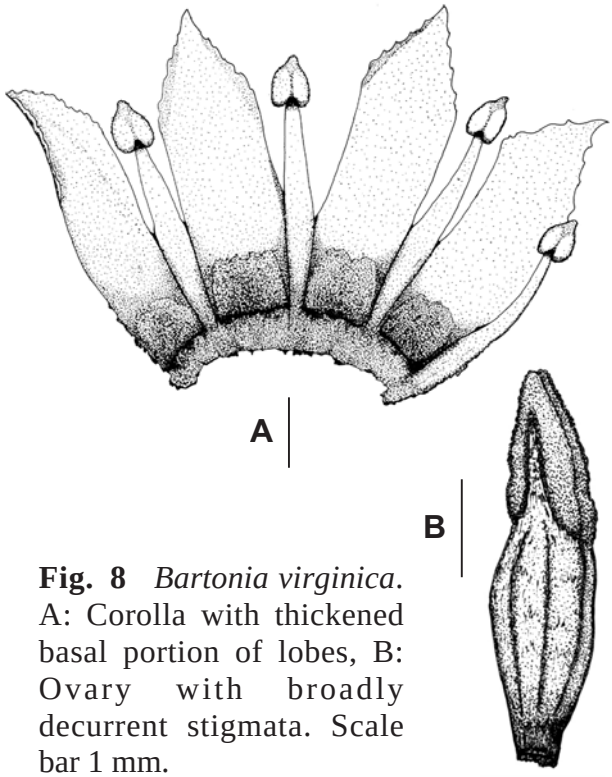

Fig. 8 *Bartonia virginica*. A: Corolla with thickened basal portion of lobes, B: Ovary with broadly decurrent stigmata. Scale bar 1 mm.

Bartonia is a *nomem conservandum* described by Willdenow (1801: 444) who

credited Muhlenberg with the discovery of the genus. The taxonomic history of its 3 or 4 species (depending on the views) consists of a series of confusions clarified in Fernald & Weatherby (1932) and Gillett (1959), but its homogeneity as a generic unit is evident. *Bartonia* was included by Grisebach (1839, 1845) (as *Centaurella*) in the Swertieae near *Lomatogonium* (as *Pleurogyne*) because of its placentation and ovule arrangement. Bentham (1876) retained the same position but Gilg (1895) placed *Bartonia* in the Erythraeinae on account of its pollen morphology. The affinities of *Bartonia* with the Swertiinae were substantiated by the flower anatomical studies of Lindsey (1940) and, very recently, by sequence data (Struwe *et al.*, 1998, 2002 Hagen & Kadereit, 2002; I, IV), whereas detailed palynological work (Nilsson & Skvarla, 1969) was of no help in placing this genus. Within the Swertiinae, however, molecular data did not resolve the affinities of *Bartonia* with confidence, although a close relationship with *Obolaria*, was not supported (I; IV). A close relationship of *Bartonia* with *Obolaria*, as suggested by the share imbricate vernation and mycorhizae is also at contradiction with placentation (Lindsey, 1940) and gross morphology (Holm, 1906; Gillett, 1959).

Fig. 9 *Jaeschkea canaliculata* (Redrawn from Ho *et al.* [1988]).

***Jaeschkea* Kurz** This small genus includes 2 to 4 species (see IV). The calyx is divided to the base, but the corolla is campanulate to urceolate. The corolla tube is longer than the lobes, that are basally convolute in the young, buds but soon the aestivation become imbricate to valvate. Each corolla segment bears 2 nectaries without appendages at its base. The main diagnostic character are the filaments, adnate to the corolla tube at one margin of each corolla segment and thus not exactly alternate with the corolla lobes, and becoming free at or shortly below the sinuses of the lobes (Fig. 10). The ovary is sessile or has a short gynophore, and has a distinct, slender style. The seeds may be numerous or only few.

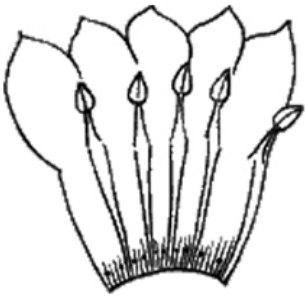

Fig. 10 *Jaeschkea microsperma*. Note the slightly shifted position of filaments (Redrawn from Ho *et al.* [1988]).

The genus *Jaeschkea* was described by Kurz (1870) with as type species *J. gentianoides* (≡ *Gentiana Jaeschkei* Kurz), *nomen illegitimum* (a synonym of *J. oligosperma* (Griseb.) Knobl.). In Grisebach (1895), the epipetalous nectaries of *Jaeschkea* were overlooked. The genus was accepted but placed next to *Gentiana*

from which it was separated mainly by its staminal peculiarity. Since then, the soundness of *Jaeschkea* as a distinct genus has never been challenged, in spite of its now obvious similarities with *Gentianella* s. l., which has been subject to considerable taxonomic work since it began to be recognised as a valid genus (e. g. Schustler, 1923; Smith, 1936). Two species are morphologically similar (*J. oligosperma* and *J. canaliculata* [Fig. 9]) and formed a monophyletic lineage related to *Gentianella* s. str. (Hagen & Kadereit, 2002). Yet the genus was found to be polyphyletic with respect to *J. microsperma* (IV). Except for the vernation and filament insertion, *J. microsperma* is rather different from the other species in habit and flower morphology, and also as regards karyology (see II) and palynology (III). A possible synonym is *Kurramiana* Omer & Kaiser but this is still uncertain (see IV). In sum, *Jaeschkea* seems to bear a phylogenetic and taxonomic significance, but characters delimiting this lineage have not all been brought out correctly yet.

***Gentianella* Moench.** This is the largest genus of the Swertiinae with ca 250 species (Hagen & Kadereit, 2001), that are almost cosmopolitan, but have diversified mostly in South America, Australia and New Zealand. In the circumscription adopted by Struwe *et al.* (2002), *Gentianella* includes a distinct and monophyletic lineage, *Gentianella* s. str. sensu Hagen & Kadereit (2001) comprising a wide majority of the species (e. g. *G. amarella*, Fig. 11), but also a small number of Asian species clearly excluded from this lineage, as *G. moorcroftiana* (Fig. 12) (Hagen & Kadereit, 2001, 2002; I; IV).

Fig. 11 *Gentianella amarella*.

Species of *Gentianella* s. str. exhibit an impressive diversity in flower morphology. The calyx may form a long tube or be divided to the base, or be split to the base on one side only. The corolla may be campanulate, rotate, cylindrical, hypocrateriform or infudibuliform. The corolla segments are variably fused, nearly on their entire length and forming a very long tube, or only at their base, the corolla tube then being inconspicuous. The corolla tube may be provided with fimbriae or naked. The fimbriae are vascularised or not, and may be united in a ring or arranged in a row at the base of the corolla lobes. Each segment of the corolla has at its base a single nectary (e. g. *G. cerastioides*, Fig. 13), that is more or less bilobed in some species (e. g. *G. umbellata*). The filaments are adnate to the

corolla tube, and become free at a variable height, but usually conspicuously below the sinuses of the lobes. The ovary is sessile or borne on a short gynophore, and a style is present or absent. Seeds are rounded in outline and with a more or less smooth testa, rarely winged (*G. tornezyana*).

Fig. 12 *Gentianella moorcroftiana*.

Species not belonging to the *Gentianella* s. str. lineage are only few and all Asian. Their morphology is rather variable but all share the presence of two distinctly separate nectaries at the base of the corolla segments (Figs. 14-16). The corolla tube is always well developed and either naked (e. g. *G. pygmaea, G. moorcroftiana*), or with sparse compound fimbriae (*G. azurea*, Fig. 14), or with small fimbriate scales (*G. gentianoides*, Fig. 16, and *G. anomala*).

As *Gentianella* s. l. (including *Comastoma*, *Gentianopsis* and *Megacodon*) was segregated from *Gentiana*, their history are closely interwoven (see Gillett, 1957 for a detailed historical review). *Gentianella* (*nomen conservandum*) was described by Moench (1794: 482), who assigned no other species to this new genus than *G. tetrandra*, *nomen illegitimum*, (a synonym of *G. campestris* L. Börner). The name was then used by Borkhausen (1796) for the species of *Gentiana* section *Crossopetalum* Froelich (now included in *Gentianopsis*). In 1839 and 1845, Grisebach returned all the species of *Gentianella* s. l. to the umbrella of *Gentiana*, except for a few that were retained in the genus *Eudoxia* Don. Kusnezow (1895), in *Die Natürlichen Pflanzenfamilien*, again included all species of *Gentianella* s. l. in *Gentiana*, but in subgenus *Gentianella*. The distinction from subgenus *Eugentiana* was mainly based on the absence of an intracalycine membrane and of corolla plicae in the species of subgenus *Gentianella*. The position of nectaries on the corolla lobes and not at the base of the ovary was also retained as an important character but probably less so

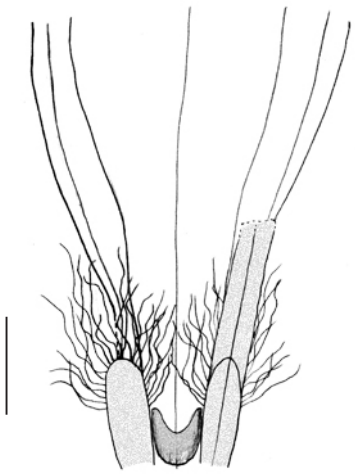

Fig. 13 *Gentianella cerastioides*. Corolla lobe showing a single nectary. Note the barbate base of the filaments. Scale bar 2mm.

than the others, as (in modern nomenclature) *Megacodon* species, which have gynoecial nectaries were included in subgenus *Gentianella*. Moreover, it appears from the generic key of the Gentianinae (Gilg 1895) that it is more the structure of the nectaries (small and naked in *Gentiana* subgenus *Gentianella* versus conspicuous and pouch-shaped in *Swertia*), rather than their position, that was retained as informative character at generic level. In sum, *Gentianella* s. l., as the taxonomic entity adopted by Kusnezow (1895), was no more than a grab-bag for all the species previously described under *Gentiana*, but that did not possess the characteristics of subgenus *Gentiana*. *Gentianella* was returned to the rank of genus by Schulster (1923), who retained the circumscription defined by Kusnezow (1895). There are numerous early proposed genera pertaining to *Gentianella* s. l. (e. g. *Eudoxia* D. Don, *Glyphospermum* D. Don, *Pytigentias* Gilg, *Selatium* D. Don, *Ulostoma* D. Don), none of which were accepted over the time (see Hagen & Kadereit, 2001). Later on, *Comastoma*, *Gentianopsis* and *Megacodon* were segregated from *Gentianella* s. l (see below). These were at first often perceived as superfluous, but are, in present days, generally recognised. A number of segregates were proposed more recently (e. g. *Aliopsis* Omer & Qaiser, *Chionogentias* L. G. Adams, *Kurramiana* Omer & Qaiser) or resurrected (*Aloitis* Rafin.), but these were based on regional floras and were not generally accepted (but see IV). Yet, *Gentianella*, in its present circumscription, has actually never been defined more precisely than by not having the distinguishing characters of other genera.

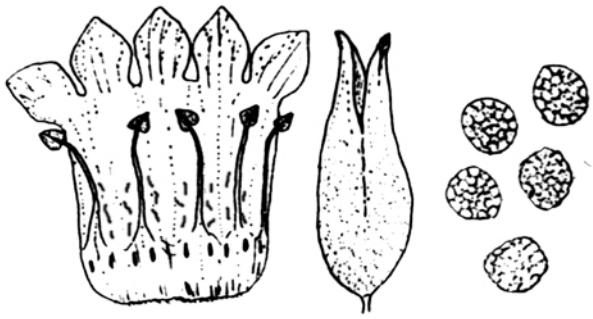

Fig. 14 *Gentianella azurea*. Corolla with 2 nectaries per segment and sparse fimbriae, capsule and seeds (redrawn from Garg [1987]).

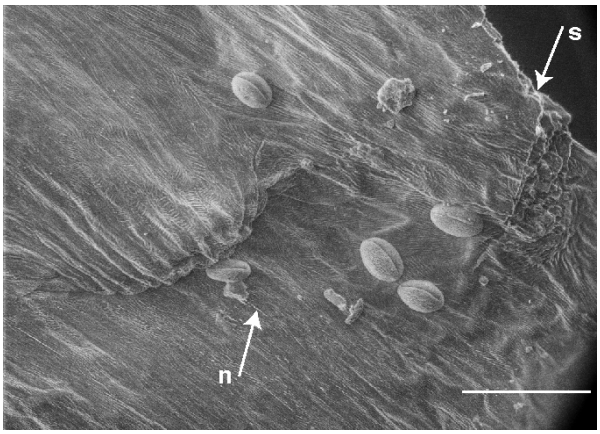

Fig. 15 *Gentianella pygmaea*. SEM image of nectariferous folds (n) just above and on the side of filament insertion on the corolla tube. Scale bar 100 µm.

From a phylogenetic point of view, *Gentianella* s. l. has historically been related to *Gentiana*, and it is only recently that epipetalous nectaries have been stressed as in important phyletic synapomorphy in the Gentianeae delimiting two distinct evolutionary lines (Gillett, 1957; Toyokuni, 1965b). It is probably also for historical reasons that the differences between

the recent segregates of *Gentianella* s. l. are sometimes not considered as sufficient to justify generic rank (e. g. Gillett, 1957; Aeschimann & Burdet, 1989; Aitken, 1999), and that the same segregates were often considered as part of a same evolutionary line (e. g. Gillett, 1957; Toyokuni, 1965b) prior to the first molecular studies (Yuan, 1995). According to more comprehensive molecular phylogenies (Hagen & Kadereit, 2001, 2002; I; IV), all the segregates of *Gentianella* s. l. are only distantly related. Moreover *Gentianella* itself is still polyphyletic. A narrower definition of a *Gentianella* s. str. was proposed in Hagen & Kadereit (2001). Phylogenetic relationships among *Gentianella* species excluded from this lineage are partly well resolved (IV), although a correlation between molecular results and other data, in particular gross morphology, could not be brought out.

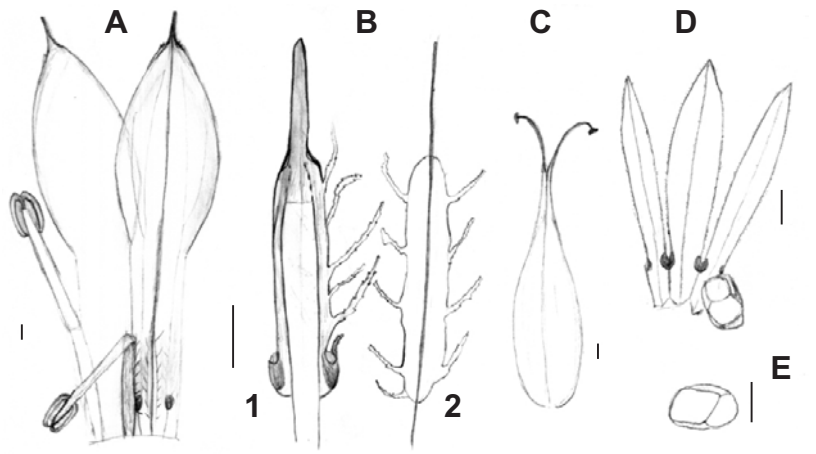

Fig. 16 *Gentianella gentianoides*. A: 2 corolla segments, B: detail of the fimbriate scale attached to the part of the filament adnate to the corolla tube (1) and of the scale covering the central vascular bundle of the corolla segment, C: ovary with recurved stigmata, D: Calyx with thickened sinuses, E: polyhedral seeds. scale bar 1 mm.

***Megacodon* (Hemsl.) Harry Sm.** Only two species belong to this genus, distributed in the Sino-Himalayan region. These are robust, somewhat fleshy, perennial plants with very large pentamerous, nodding flowers (Fig. 17). The calyx is campanulate and divided to shortly below the middle. The corolla is broadly campanulate and divided to below the middle. The stamens are inserted at the sinus of the corolla lobes. The ovary is sessile or has a short gynophore, and has a stout or filiform style. The nectaries are gynoecial. The

Fig. 17 *Megacodon stylophorus* (Photography Y.-M. Yuan).

vernation is convolute. The seeds are warty or corrugate.

The two species of this genus were included in *Gentiana* subgenus *Gentianella* in two different sections by Kusnezow (1895). *Megacodon venosus* was in sect. *Megacodon* Hemsl., and *M. stylophorus* in sect. *Stylophora* Clarke. The genus was erected by Smith (1936) with *M. venosus* as its type species. The genus was often related to the *Gentiana*-lineage (e. g. Gillett, 1957; Ho & Liu, 1990), due to the gynoecial nectaries, but its affinities with taxa having epipetalous nectaries was established by DNA sequence data (Yuan, 1995; Hagen & Kadereit, 2002; I; IV). The two species differ in pollen, seed and ovary traits, and the monophyly of this genus has never been ascertained.

***Gentianopsis* Ma.** The species of *Gentianopsis*, or «fringed« gentians (Fig. 18) are distributed in Eurasia and North America. Plants of this genus have a number of clear-cut diagnostic feature. The flowers are always tetramerous, with two dissimilar pairs of calyx lobes distichously imbricate in aestivation, and showing discontinuous membranes at their base (Fig. 19 A). The corolla has a long tube and usually dentate to erose obovate lobes frequently also fringed towards their base (Fig. 18). The gynophore is often quite long and the stigmas are broadly capitate. The nectaries are epipetalous and often swollen. The seeds are usually spinose (see Yuan, 1993 and Iltis, 1965 for illustrations), but flattened with an elliptic wing in some species (Fig. 19 B). Lindsey (1940) and Ma (1951) have shown that the arrangement of vascular bundles in the ovary, corolla and calyx in *Gentianopsis* is confined to this genus.

Fig. 18 *Gentianopsis ciliata* (Photography Y.-M. Yuan).

Species of *Gentianopsis* had been placed in *Gentiana* section *Imaicola* Griseb (*G. contorta*). and section *Crossopetalum* Froel. by Grisebach (1845) (all other species known then). Kusnezow (1895) retained both sections but under *Gentiana* subgenus *Gentianella*. Ma coined the genus *Gentianopsis* in 1951. He discussed no synonymy, but made a new combinations for *G. contorta*, thus merging both sections in his new genus, and selected *G. barbata* as type species. Gillett (1957) rejected the genus, which he placed in synonymy with

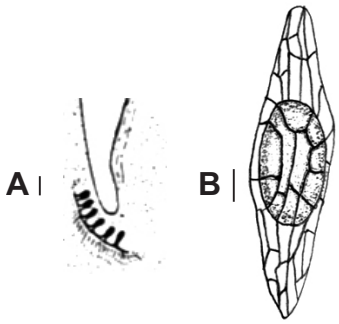

Fig. 19 A: *Gentianopsis procera*, intracalycine membrane (redrawn from Iltis, 1965). B: *Gentianopsis ciliata*, winged seed. Scale bar 2 mm (A), 0.1 mm (B).

Gentianella subgenus *Eublephis* (Raf.) J. M. Gillett. Phylogenetically, *Gentianopsis* appears to be paraphyletic with *Pterygocalyx* (Hagen & Kadereit, 2002; I; IV). It occupies a basal position in the Swertiinae and is distantly related to other taxa of *Gentianella* s. l. (I; IV).

***Pterygocalyx* Maxim.** The only species in this genus, *P. volubilis*, has a broad distribution in eastern Eurasia. It is a twining or creeping plant with tetramerous flowers. The calyx is tubular to campanulate, markedly keeled, and the lobes are much shorter than the tube. The corolla is tubular with a tube longer than the lobes. Each segment bears 2 nectaries at the base of the tube. The ovary has a short gynophore and a cylindrical style. The seeds are minute with an annular to elliptic wing.

The genus was described by Maximowicz (1859: 198-199), but treated as part of *Crawfurdia* (Bentham, 1876; Gilg, 1895) due to its twining habit. Marquand (1931) recognised its similarities with species of *Gentianella* s. l. and included it in this genus. More recently, its affinities with *Gentianopsis* were recognised by Smith (in Nilsson, 1967b) who considered these taxa as congeneric. A detailed embryological and anatomical study aiming at establishing the limits between *Gentianopsis* and *Pterygocalyx* was carried out by Chen & Ho (1998), but they failed to include those species of *Gentianopsis* most resembling *Pterygocalyx* (e. g. *G. ciliata*) in their sample. The affinities of *P. volubilis* with *Gentianopsis* were fully confirmed by molecular results (Yuan, 1996; Hagen & Kadereit, 2002; I; IV), although its rank as genus is questionable (IV).

***Comastoma* Toyok.** Species of *Comastoma* are annual or perennial, and usually of small size. Most species occur in temperate Asia, but *C. tenellum* is circumboreal. The flowers are tetra- or pentamerous, and of convolute aestivation. The calyx is divided nearly to the base and the lobes are often saccate at their base. The corolla is salverform or tubular, with a long tube. Each corolla segment has 2 naked nectaries at the base of the tube. A scale is adnate to the corolla tube, which is fimbriate at the apex, forming a ring of hairs in the corolla throat (Fig. 20) (hence the name *Comastoma*). The fimbriae are non-vascularised and flattened. The ovary is sessile, a style is absent, and the stigmata are somewhat foliaceous (Fig. 21 A). The corolla tube is poorly developed in the young buds (Fig. 21 B) and extends as the flower matures.

Fig. 20 *Comastoma falcatum* (Photography Y.-M. Yuan).

Species of *Comastoma* were grouped in *Gentiana* subgenus *Gentianella* section *Amarella* Griseb. by Kusnezow (1895) due to their fimbriate throat, a trait also present but not homologous in most other species of the section, where the fimbriae are vascularised, contrary to those of *Comastoma* species. Shortly after, Wettstein (1896) described a new section *Comastoma* for *G. tenella* and *G. nana*. This group was much later raised to generic rank by Toyokuni (1961: 198). Wettstein (1896) suggested a close relationship with *Lomatogonium*, and Löve & Löve (1956) transferred sect. *Comastoma* to *Lomatogonium* on the grounds of chromosome numbers (2n = 5 in *C. tenellum* and *L. rotatum* only known for one species in each group!). Liu (1994), on the other hand, carried out detailed embryological studies in *Comastoma* and concluded that its species were very different from those of *Lomatogonium* and closely related to *Gentianella* and *Gentianopsis*. Molecular results suggested a monophyletic *Comastoma* closely related to *Lomatogonium* (Hagen & Kadereit, 2001, 2002; I; IV).

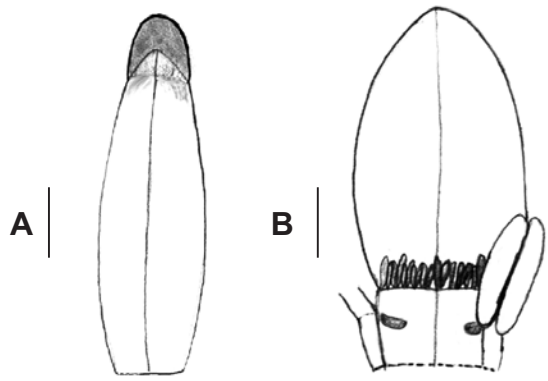

Fig. 21 *Comastoma disepalum*. A: Ovary with characteristic stigma, B: a young corolla lobe showing the short corolla tube. Scale bar 2 mm (A), 1 mm (B).

***Swertia* L.** In its present circumscription (ca. 135-140 species), which is rather broad, the genus *Swertia* is present mainly in Eurasia and Eastern Africa, with a few species in western Africa and one, the type species *S. perennis*, that is circumboreal. The morphological diversity of *Swertia* is relatively high. The plants are annual, biennial or perennial (mono- or polycarpic). Perennials have long or short rhizomes, or have an elongated and fleshy taproot. Annuals and biennials commonly have a few slightly fleshy lateral roots. Occasionally the taproot is well developed or the lateral roots are numerous and fasciculate. The leaves are usually opposite, as in all other genera of the Swertiinae, but occasionally alternate or verticillate. The flowers are tetra- or pentamerous, grouped in large panicles, racemes,

Fig. 22 *Swertia patula* (Photography Y.-M. Yuan).

few flowered cymes or are solitary. The calyx is lobed to the base except in *S. teres*, and the lobes are frequently unequal. The corolla is characteristically rotate, and the lobes bear one or two nectaries at their base, that are partially confluent in some species (Fig. 22). The nectaries may be naked (Fig. 23 A), but are usually enclosed or bordered by a scale. The scale is frequently fimbriate (Fig. 23 B or laciniate (Fig. 23 C & D), or the scale is absent and only fimbriae surround the nectaries (Fig. 23 E). In some species, the nectaries appear inverted, as they open towards the receptacle (Fig. 23 F) (see also Maiti & Banerji, 1976b). The corolla aestivation is convolute. The filaments are normally free and inserted in the sinuses of the corolla lobes, but may be monadelphous. The ovary is sessile or has a short gynophore, and a style may be present, usually short, slender or stout. The stigma is of variable shape, but not decurrent on the carpel suture, or only very shortly. The seeds are generally small, although larger in a few species (e. g. *S. macrosperma*) and very large in *S. swertopsis*. Seeds may be sub-globose to ovoid, flattened, triquetrous, polyhedral or corrugate in shape, and winged or not. The testa is nearly smooth, echinate or finely or conspicuously warted (see Maiti & Banerji, 1976a and IV for illustrations).

The genus *Swertia* was established by Linnaeus (1753: 226). Under *Swertia*, Linnaeus also described a species of the later genus *Lomatogonium*, *S. rotata* (≡ *Lomatogonium rotatum*), and *S. corniculata*, the future type of *Halenia*. *Veratrilla* had been given a manuscript name under *Swertia* and was first described as a section of *Swertia* by Baillon (see Smith, 1970). The absence of convincing differences between *Frasera* and *Swertia* were pointed out as early as 1837 by Don, and finally reduced to a section of *Swertia* in 1891 by Kuntze. All these genera obviously have a close taxonomic history. Additionally, in its present circumscription (Struwe *et al.*, 2002), *Swertia* counts 10 generic synonyms (*Agathotes* Griseb., *Anagallidium* Griseb., *Blepharaden* Dulac, *Henriceae* Lemaire-Lisencourt, *Monobothrium* Hochst., *Ophelia*

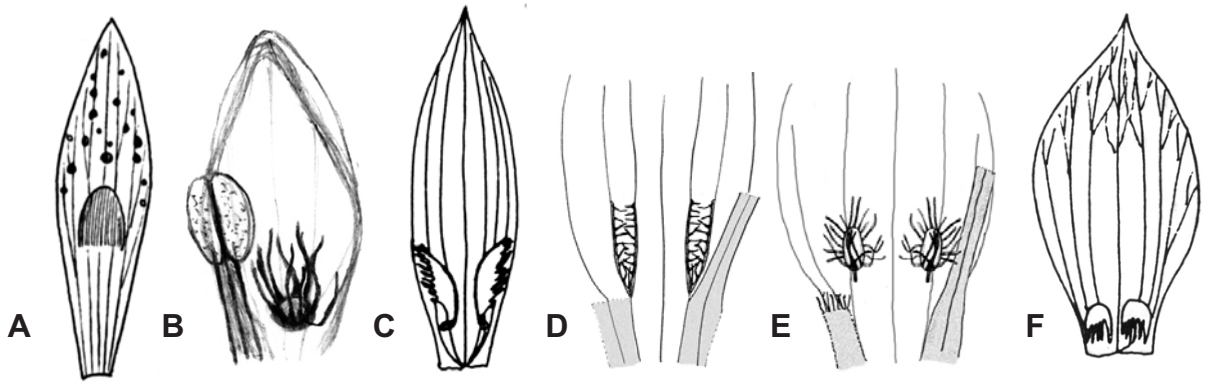

Fig. 23 Some nectary types in *Swertia*. A: *S. tashiroi* (redrawn from Wang & Lu, 1998), B: *S. tenuis*, C: *S. yunnanensis* (redrawn from Shah, 1984), D: *S. mussotii*, E: *S. macrosperma*, F: *S. hispidicalyx* (redrawn from Shah, 1984).

Griseb., *Sczukinia* Turcz., *Stellera* Turcz., *Swertopsis* Makino, *Rellesta* Turcz.), 2 of which (*Ophelia* and *Anagallidium*) are still used in some modern floras (e. g. Czerepanov, 1995). Whereas *Halenia*, *Lomatogonium* and *Veratrilla* were separated from *Swertia* on grounds of relatively conspicuous and constant characters (see below), and were accepted early on in the history of the Swertioid complex, the distinguishing traits of other taxa were never brought out convincingly. Taxonomically, *Swertia* underwent two major phases. In a first step, several genera such as *Agathotes, Anagallidium*, *Frasera*, *Rellesta*, *Sczukinia* were described to accommodate the morphological diversity of newly discovered species. These genera ambiguous, as they were often based on erroneous morphological observations, and were described when only few species of *Swertia* were known. In a second step, they were successively reduced to various subdivisions of *Swertia*, and finally abandoned as the number of described species increased, and as generic boundaries became ever weaker (see Shah, 1984 for a history of the genus). Gilg's (1895) treatment of *Swertia* marked a turning point in the taxonomic history of *Swertia*. He lumped all previous segregates and recognised three section only. Section *Euswertia* C. B. Clarke included the perennial species, section *Ophelia* Griseb. the annuals and biennials, and section *Poephila* C. B. Clarke the single acaulous species known to him, *S. multicaulis*. *Frasera* was included in section *Euswertia*. This broad circumscription of *Swertia* has persisted as such until now, with exception of *Frasera* that is still disputed as a genus. In sum thus, the boundaries of *Swertia* have been greatly widened in the course of time to fit all the taxa of the Swertioid complex that did not have the characteristics of *Halenia* (spurs), *Lomatogonium* (decurrent stigmata) or *Veratrilla* (dioecious, corolla tube and stamens inserted in the sinus of the lobes), and the genus may be viewed as a generalised acknowledgement of failure to correctly accommodate taxonomically its overall diversity.

A number of infrageneric classifications have been proposed later on to try to make up for this situation, but all were based on regional floras (e. g. Satake, 1944, 1947 in Japan; Hedberg, 1957 in Africa; Pissjaukova, 1979 in Russia; Garg, 1987 in Indian Himalaya; Ho *et al.*, 1988 in China), and none intended to transgress Gilg's (1895) concept of the genus. The number of subdivisions varied greatly, from only few (e. g. Garg, 1987) to nearly as many as the number of species treated (e. g. Satake, 1944, 1947), but the convenient distinction between perennial and annual or biennial species was consistently retained. World-wide treatments of *Swertia* (including *Frasera*) were provided only recently. In a monograph of the genus, Shah (1984), recognised 35 informal groups (for 148 species) based mainly on floral morphology. Ho *et al*. (1994) divided *Swertia* into 11 sections and 16 series. Although both

treatments conserved the broad division into two subgroups based on the annual/perennial habit, they are contradictory in many cases (see I, IV). In both cases also only gross morphology was considered, and palynological and karyological data were left aside.

As regards phylogenetic relationships among species of *Swertia*, very few hypotheses have been proposed, that were based on gross morphology mainly (e. g. Ho *et al.* 1994). Shah (1984) discussed affinities and possible evolutionary schemes between his narrow groups based on the structure of the nectaries, seedcoat, and root system. Xue *et al.* (2000) emitted some hypotheses based on characters of leaf epidermis. In all these studies, the interpretation of morphological data was strongly constrained by taxonomic apriorism, in particular the *Swertia*/*Ophelia* subdivision reflecting the annual/perennial habit of the plants. Molecular results (I; IV) corroborated nearly exactly the informal groups of Shah (1984), but showed quite some contradictions with the classification of Ho *et al.* (1994). They also suggested that lineages and/or species shifted from annual to perennial habit and vice versa several times, thereby contradicting all previous phylogenetic inferences.

Relationships between *Swertia* and other genera in the Swertiinae were occasionally addressed using gross morphology (e.g. Liu & Ho, 1992), embryology (e. g. Liu, 1994), karyology (e. g. Favarger, 1962; Toyokuni, 1965b), or palynology (Nilsson, 1967b), but were necessarily biased by a lack of data, and/or the implicit choice of «typical» representative species induced by the heterogeneity of *Swertia*, and were thus not convincing. Molecular results (I; IV) clearly showed that *Swertia* cannot be considered as a single entity for phylogenetic considerations. Species of *Swertia* were found to belong to at least 14 different lineages. Whereas these lineages may be relatively well defined by karyological (II) and palynological (III) data in association with combinations of gross morphological characters (see IV), evidence supporting the relationships between lineages of *Swertia* and/or other genera was generally not found. It appears clearly that gross morphological characters are of little use for inferring phylogenetic relationships in the Swertiinae. Micromorphological and ontogenic studies may be envisaged in the future to better understand the evolutionary history of this group.

***Frasera* Walter.** Species of *Frasera* grow in western North America, except for *F. caroliniensis* that occurs in eastern North America. A majority are desert dwellers (e. g. *F. paniculata*, Fig. 24). All the species are perennial, monocarpic (e. g. *F. albomarginata*, *F. parryi*, *F. speciosa*) or polycarpic (e. g. *F. tubulosa*, *F. gypsicola*; Fig. 25). The leaves are often verticillate, and conduplicate with a white margin in a majority of species. The flowers are tetramerous and form lax or congested paniculate inflorescences. The calyx and corolla

are lobed to the base. There is usually one nectary at the base of the corolla lobes, less often two and distinct or partly fused or confluent. Nectaries are rather variable in shape and type of scale and fimbriae associated. The filaments are often united by a membranous tissue variable in size and often deeply incised (corona). The ovary is sessile and the style long and slender. The seeds are large, ellipsoid, reniform, triquetrous or flattened, winged, nearly smooth or shallowly corrugate.

Fig. 24 *Frasera paniculata*.

Frasera was described by Walter (1788: 87), based on a single species, *F. caroliniensis*. The diagnostic character were its verticillate leaves. It was kept as a distinct genus by Grisebach (1836,1839,1845), D. Don (1837), G. Don (1837) and Bentham (1876) on account of obscure characters, of which only the presence of a distinct style corresponds to a botanical reality (see Shah, 1984). Later synonyms are *Leucocraspedum* Rydb. and *Tesseranthium* Kellog. Shortly after the description in the *Flora of British India* of *Swertia hookeri* (Clarke, 1885), which is extremely similar to the robust species of *Frasera* (see V), Baillon (1889) reduced

Fig. 25 Roots of polycarpic perennial *Frasera* species.
↑ *Frasera gypsicola*.
← *Frasera tubulosa*.

Frasera to *Swertia*, where it stayed until Card (1931) revived the genus in his revision. Since then, the status of *Frasera* has constantly been under debate (St. John, 1941; Post, 1956; Wood & Weaver, 1982; Shah, 1984; Pringle, 1990; Struwe *et al.*, 2002), and Toyokuni (1965b) even made some new combinations for Asian species of *Swertia* under *Frasera*. It is obvious that from a flower morphological point of view (compare Figs. 22 and 24), the two genera barely differ by the occurrence of a corona in some species of *Frasera*. The only feature confined to *Frasera* is the relatively large size of the seeds, comparable only with those of *S. swertopsis*. Yet, although not distinguished by a clear-cut synapomorphy, the species of *Frasera* exhibit combinations of characters that do not co-occur in any other species of *Swertia* (see IV). Molecular results also supported the monophyly and distinctness of this lineage, although its precise affinities with other members of the Swertiinae could not be ascertained (IV).

***Halenia* Borkh.** Three species are present in Asia, one in North America, while the rest of the genus (ca 80 species presently, but possibly only ca 36 [Hagen, pers. com.]) diversified in South America. The species of *Halenia* are annual, biennial or perennial herbs, with a decumbent or erect, occasionally rather robust, stem. Flowers are constantly tetramerous, and the corolla sinistrorsely contorted. Floral dimorphism is frequent. The calyx is lobed nearly to the base, and the corolla may be lobed to the middle or to the base. Each corolla segment bears on the tube a nectary forming a small abaxial protuberance or a spur of variable length. The stamens are inserted at the sinuses of the corolla lobes. The ovary is sessile and has no style. The seeds are globose with a smooth testa.

Halenia was described by Borkhausen (1796: 25). Its type species *H. sibirica* (*nomen illegitimum* ≡ *H. corniculata*, *nomen conservandum*), had previously been described by Linnaeus as *Swertia corniculata*. Several South American species were described under *Swertia* after the genus *Halenia* had been coined, but were correctly placed in *Halenia* by Grisebach (1836, 1839, 1845), who created a new genus, *Exadenus* Griseb., to accommodate the species that had only small pits at the base of the corolla lobes instead of the typical spurs. Since then *Halenia* has stood unchanged, except for the merging of *Exadenus*, and the adjunction of new species. Phylogenetically, *Halenia* is strongly supported monophyletic lineage closely related to species of *Swertia* (Hagen & Kadereit, 2002; I; IV).

***Lomatogonium* A. Braun.** Species of *Lomatogonium* are annual or rhizomatous perennial herbs. The stems are usually decumbent or ascending, less often erect. The flowers

are pentamerous and usually borne on long pedicels. In most species, the young corolla lobes have one longitudinal half of a lighter blue than the other, as they are tightly convolute in the bud. The calyx and corolla are lobed nearly to the base. The corolla lobes usually have darker striae (Fig. 26), and have one or two, sometimes confluent nectaries at their very base. The nectaries may be naked, but usually are enclosed in a tubular, membranous appendix laciniate at the apex (Fig. 27 A), or have a galeate scale (Fig. 27 C). In most species, the nectaries are very close to the filaments. The sessile ovary is oblanceolate to ensiform with a stigma decurrent on the carpelar suture in some species (Fig. 27 B), but ellipsoid to lanceolate with membranous stigmata decurrent only apically in other (Fig. 27 D). The seeds are sub-globose with a smooth testa, and of a light colour.

Fig. 26 *Lomatogonium bellum* (Photography Y.-M. Yuan).

The genus is due to Braun (1830) and the type species is *L. carinthiacum* (Wulfen) Rchb., based on *Swertia carinthiaca*. Grisebach (1839,1845) and Gilg (1895) placed all relevant species under *Pleurogyne* Eschsch. mentioned in Chamisso & Schlechtendal (1826) *pro synonymo*. Several species were thus described under *Pleurogyne*, but a number also under *Swertia*. Satake (1944) reduced *Lomatogonium* to a section of *Swertia*. Ikonnikov (1970) described *Pleurogynella* for the decumbent species that lack the characteristically decurrent stigmata, but Ho (1982) made it a section of *Lomatogonium*. A taxonomic revision of *Lomatogonium* was carried out by Liu & Ho (1992), who divided its species into three

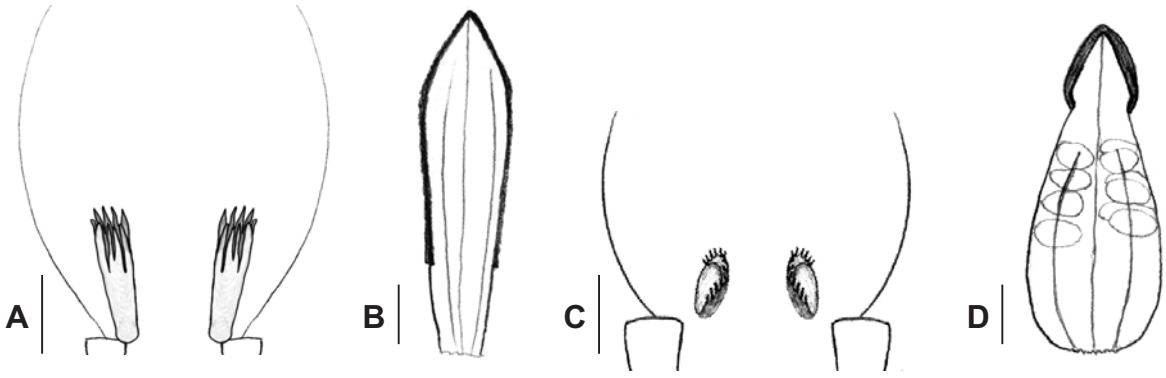

Fig. 27 Types of nectary and ovary in *Lomatogonium*. A & B: *L. macranthum*, C & D: *L. chumbicum*. Scale bar 2 mm.

sections. Smith (in Nilsson, 1967b) noticed a peculiar species (referred to as *Phyllostoma concinna* Harry Sm., ined., and *L. bicoronatum* Harry Sm., *nom. nud.*) with petaloid nectary appendages and a pollen morphology intermediate between *Lomatogonium* and *Comastoma*. This species was later described as the type of the controversial genus *Lomatogoniopsis* by Ho & Liu (1985).

Molecularly (IV), *Lomatogonium* was found to be polyphyletic with respect to 2 species related to species of *Gentianella* and *Swertia*, and paraphyletic with *Comastoma*. Yet, relationships suggested by sequence data are difficult to reconcile with morphology, karyology or palynology (see II, III, IV).

***Veratrilla* Baill. ex Franch.** Both species of *Veratrilla* occur in the Sino-Himalayan region. They are dioecious, erect, perennial herbs. The female flowers form elongated inflorescences, while male flowers are grouped in dense, sessile clusters. The flowers usually are tetramerous and have sterile vestigia of the other sex. The calyx is lobed nearly to the base, and the corolla presents a short tube. The corolla lobes have one or two blue, naked nectariferous patches at their base. The stamens are inserted in the sinuses of the lobes. The ovary is sessile and a style is short or absent. The seeds are rather large, compressed, and with an annular wing.

Veratrilla was first proposed as a section of *Swertia*, but its type species, *Veratrilla baillonii*, was described at the rank of genus by Franchet (1899: 310) (see Smith, 1970). *Veratrilla burkilliana* (W. W. Smith) Harry Sm. was described under *Swertia* and transferred to *Veratrilla* only recently (Smith, 1970). *Veratrilla* plants closely resemble some robust Asian perennials of *Swertia*, from which they differ only by the longer corolla tube, dioecious flowers and larger seeds. Phylogenetically, only the type species has been studied molecularly. It was related as a distinct lineage to a clade including *Swertia* species and *Halenia* (Hagen & Kadereit, 2002; I), but this position was not confirmed when the taxon sample was enlarged (IV). Its affinities with other basal lineages of the Swertiinae are thus still uncertain.

Aims and Context of the Study

Taxon Sample. The investigations carried out in this thesis were initially aimed at exploring the extent of polyphyly in the genus *Swertia*, as suggested by the results of Yuan & Küpfer (1995), but that were based on ITS data only and included relatively few species. Preliminary results (Chassot, 1998) on 27 species of *Swertia* and representatives of 5 other genera of the Swertiinae, based on ITS and *trn*L data, clearly showed that different lineages of *Swertia* were related not only to taxa of the «Swertioid» complex, as floral morphology would have suggested, but also to markedly different genera such as *Gentianella* and *Gentianopsis*. The same results also showed affinities between taxa of the Swertiinae (e. g. *Comastoma* and *Lomatogonium, Gentianella* and *Swertia, Halenia* and *Frasera*) implying an important level of homoplasy in floral macromorphology. Thus, it soon became evident that the taxon sample needed to be expanded to the entire subtribe. Moreover, the lineages of *Swertia* identified, as well as their interrelationships, were apparently at contradiction with the taxonomic subdivisions established by Ho *et al*. (1988), themselves in opposition with the morphological groups recognised by Shah (1984), and it became clear that the only acceptable taxon sample for *Swertia* was one encompassing the entire morphological, geographical and ecological variability of the genus. In addition, the taxon sample for *Gentianella* and *Lomatogonium* was also maximised as a consequence of the results of Hagen & Kadereit (2001 & 2002), revealing polyphyly for these genera.

Plant Collections. In turn, this has led to considerable efforts to collect fresh material suitable for DNA extraction, in particular in the genus *Swertia*, as species accessible from various herbaria were mostly represented by material collected during the first half of the past century or even before, and from which DNA of satisfactory quantity and quality could only rarely be obtained. The abundant material collected by Küpfer, Yuan and others between 1990 and 1995, deposited in the herbarium of Neuchâtel but mostly undetermined, was entirely reviewed and identified. These collections were completed by fieldtrips to Nepal in 1997, China, Japan, Thailand and Taiwan in 1999, and Ecuador and USA in 2000, as well as by material collected by friends and colleagues from parts of the world that could not be visited. All vouchers are deposited at the herbarium of Neuchâtel, and represent a large proportion of the Asian species of Swertiinae (see Appendix).

Genes Sequenced. As regards the regions of the genome sequenced, the rate of evolution of different genes and non-coding fragments, and their adequacy at a given taxonomic level have been extensively reviewed (e. g. Olmstead & Palmer, 1994; Soltis & Soltis, 1998). For this study, only non-coding fragments were analysed, in order to obtain sufficient sequence divergence for the taxonomic level approached. In addition, the poor resolution and the relatively low branch support obtained for phylogenetic trees inferred from the *trn*L intron in the preliminary results required the sequencing of more chloroplast regions. Both nuclear and chloroplast regions were analysed, as this allowed to address question such as hybridisation and chloroplast capture, considering potentially divergent phylogenies inferred from different genomes. The transcribed spacers between the 18S and 5.8S, and between the 5.8S and 25 S genes (ITS1 and ITS2) of the nuclear ribosomal DNA were chosen for three reasons. Firstly, ITS sequences have been used successfully in innumerable studies. Mechanisms of concerted evolution among the multiple tandem repeats of the 18S-5.8S-25S nuclear ribosome encoding genes allows in most cases to consider these as single copy genes (Hillis & Dixon, 1991; Hamby & Zimmer, 1992). The phylogenetic applicability of other genes (e. g. introns of the alcohol dehydrogenase *adh*, phosphoglucose isomerase *Pgi*, H3 histone H3-D locus, starch synthase *waxy*, etc. [see Soltis & Soltis, 1998]) is still experimental, and their number of copies are still not established with certainty, hence the risk of basing a phylogenetic hypothesis on paralogous sequences. Secondly, the evolution of these two regions involves mainly point mutations rather than structural rearrangements, facilitating alignment and homology assessment of characters. And lastly, their widespread use for molecular studies in the Gentianaceae allowed to reuse previously published sequences. Within the chloroplast genome, on the other hand, an important number of genes have been sequenced and used for molecular taxonomy. Among advantages of chloroplast genes, one may mention their relative facility to sequence compared to nuclear genes, and the fact that most of them are single copy genes (Palmer, 1995). Yet chloroplast genes also present disadvantages, such as their monoparental and usually maternal inheritance, with the inherent risk of not detecting relationships biased by chloroplast capture, their rather slow evolutionary rate, and mutations frequently consisting of structural changes often making homology assessment problematic. The *trn*L intron sequences were found to evolve relatively fast compared to other genes (Gielly & Taberlet, 1994a & 1994b)and were used for phylogenetic inference in the genus *Gentiana* (Gielly *et al.*, 1996) with some success. Yet, *trn*L intron sequences did not provide sufficient information to resolve relationships in the Swertiinae, and two additional regions,

the *trn*L-*trn*F and *trn*S-*ycf*9 intergenic spacers, were sequenced to obtain a level of sequence divergence in the chloroplast data set comparable with that of the ITS data.

Phylogenetic Inference. Phylogenetic relationships were initially inferred using the standard unweighted maximum parsimony criterion (i. e. any character state may change freely to another state). This approach yielded topologies that were rather surprising considering affinities suggested by gross morphology, thus legitimating the criticism that the relationships deduced from the sequence data are erroneous and may result from a methodological bias. Especially considering that maximum parsimony retains those phylogenetic trees implying the least number of substitutions, regardless of the evolutionary mechanisms constraining mutations in a given sequence, and does not take in consideration parameters such as unequal base frequencies, different substitution rates between nucleotides, and site-dependant rate heterogeneity. This called for a confirmation of parsimony based results by a different reconstruction method. Among alternatives, distance based methods make use of model parameters, but do not integrate the evolution of character states of ancestral taxa at each phylogenetic node, as the sequence data of extant taxa are converted to pairwise distances prior to phylogenetic inference. Maximum likelihood approaches, on the other hand, combine DNA substitution models and evaluation of character states in putative ancestral sequences. Yet, the maximum likelihood method, as implemented in PAUP* (Swofford, 2002), is prohibitively time-consuming when analysing large data sets, and was necessarily disregarded. Closely related to maximum likelihood inference (Holder & Lewis, 2003), Bayesian inference uses a Markov chain Monte Carlo (MCMC) procedure to sample trees from a posterior probability distribution. The advantages of this method are its time-efficiency and the integration of full substitution models. On the negative side, trees found by MCMC are strongly autocorrelated and, as they may only reflect local optima, the procedure must be repeated several times varying the starting parameters. The method also implies a subjective prior probability of parameters, that must be set as to have a minimal incidence on the likelihood of the trees. In addition, the posterior probability of a branch in the phylogeny, in terms of support for that branch, is sensibly higher than as estimated by resampling techniques (Douady *et al.*, 2003). In spite of these drawbacks, Bayesian inference was used here as an alternative to parsimony in order to verify the consistency of phylogenies inferred from molecular data.

Phytochemical, Embryological and Anatomical Variation. Sources of data other than floral macromorphological characters were also sought, in order to be compared with molecular results. Phytochemical data, in particular xanthone oxidation patterns, have been shown to be useful when considering phylogenetic relationships (e. g. Massias *et al*., 1982; Meszaros, 1994). Chemical constituents of *Swertia* species are rather well documented (reviewed in Inayat-Ur-rahman, 1997), and research is still progressing in the genus, mainly due to the pharmacological properties of several compounds, as attested by the use of a number of species in traditional medicine. Unfortunately, other genera such as *Bartonia*, *Comastoma, Gentianella*, *Latouchea*, *Megacodon, Obolaria* and *Veratrilla* are still not or barely documented. Since investigation of chemical constituents requires important quantities of material, it was not considered as feasible in the context of this study, and was thus left for future studies.

Likewise, embryological data (Stolt, 1921; Guérin, 1926; McCoy, 1949; Maheswari Devi & Lakshminarayana, 1977; Rao & Nagaraj, 1982; Akhalkatsi & Wagner, 1997; Shamrov, 1991; Liu, 1994; Liu & Ho, 1996; Chen *et al*., 1998; Ho *et al*., 1999; Xue *et al.*, 1999, 2002) is available for a reasonable proportion of the genera of Swertiinae, but cover in fact only few species. Taxonomic and evolutionary generalisations (e. g. Liu & Ho, 1996; Xue *et al*., 2002) based on data from two or three species appear to be premature. Variation of available embryological data was reviewed in Liu (1994). A few characters were found to be informative, yet relationships between genera such as *Comastoma* and *Lomatogonium,* as suggested by sequence data, were not supported by embryological characters. Moreover, the restrained studies on species of *Swertia* did not hint at a significant variability, and embryology was thus not considered for further investigation.

Floral anatomy in the Swertiinae was studied in quite some detail by Lindsey (1940), Gopal Krishna & Puri (1962), Xue *et al*. (2002), and was particularly useful for segregating *Gentianopsis* from *Gentianella* s. l. Nevertheless, variability between some other genera of the Swertiinae (reviewed in Liu, 1994) did not seem particularly high. In addition, in the survey of Gentianaceae by Gopal Krishna & Puri (1962), the 5 species of *Swertia* included, that belonged to distantly related lineages according to the preliminary molecular results (Chassot, 1998), were generally not different in their vascularisation. Anatomy was thus not explored in more depth.

Karyology. As a consequence of the increased interest for cytotaxonomy in the Gentianaceae starting in the 1950's, chromosome numbers were readily available for all but 2

genera of the Swertiinae (*Latouchea* and *Veratrilla*), and quite some different numbers had been reported for *Swertia* (see II). Although the total number of species documented was still relatively unimportant in some genera (e. g. *Comastoma*, *Lomatogonium*, *Swertia*), a number of chromosomal evolutionary schemes had been proposed (Löve, 1953; Favarger, 1962 Löve & Löve, 1976), and some taxonomic considerations had been based on chromosome numbers (e. g. Löve & Löve, 1956; Toyokuni, 1965b, Pringle, 1990). It seemed thus interesting to complement the available data in order to gain a more precise idea of the overall karyological variability, and to compare these data with molecular results to verify previous hypotheses such as the parallel evolution of karyotypes (Favarger, 1962). Young flower buds suitable for the determination of chromosome numbers, when available, were systematically fixed in Carnoy on the field. In some cases, counts were realised from seedlings, but successful germinations could not always be obtained from several robust perennials such as *Veratrilla* and *Latouchea*.

Palynology. Pollen morphology in the Swertiinae was studied rather extensively by Nilsson (1967b), who conducted a broad survey of the entire tribe Gentianeae. On a smaller scale, investigations were also carried out on, e. g., *Lomatogonium* (Nilsson, 1964; Liu & Ho, 1992), *Comastoma* (Liu, 1994), *Bartonia* and *Obolaria* (Nilsson & Skvarla, 1969), African (Jonsson, 1973) and Chinese (Ma & Zhao, 1991) species of *Swertia*, selected species of *Gentianella* from Pakistan (Omer & Quaiser, 1991 & 1992), and a few representative species of the genera of Swertiinae (Nilsson, 2002). In spite of a frequent striate-reticulate exine pattern common to sometimes only distantly related taxa, a number of genera/species presented peculiarities in their exine structure such as spines, warts, exine protruding over the ora, or adjacent but not fused muri. Notably in the genus *Swertia*, palynological heterogeneity was pointed out by Nilsson (1997b), but its interpretation was made problematic by the absence of a clear taxonomic and phylogenetic understanding of this group. A taxon sample was thus elaborated based on molecular results, in the aim of exploring the potential congruence of palynological and sequence data. As this approach was only exploratory, no fine scale analyses were carried out, but exine ornamentation for a large number of species was reported using scanning electron microscopy.

Gross Morphology. As detailed above (*Genera of the Swertiinae*), taxonomic ambiguities, in particular in the genus *Swertia*, do not follow from a lack of gross morphological variation, but rather from different emphases of perceived similarities and

differences. Likewise, the occurrence in the Swertiinae of, e. g., such a conspicuous (and taxonomically handy) phenotype as the presence or absence of a well developed corolla tube is often considered as a reliable indicator of phylogenetic affinities, although no study is available about the genetic determinism of this trait, which apparently evolved rather freely and frequently in the subtribe. When placed in a molecular phylogenetic context, flower morphological characters are often deceptive (e. g. Wagenitz, 1997) and this is particularly true for subtribe Swertiinae, which encompasses a majority of genera essentially defined by floral traits. As no micromorphological analyses were carried out other than on pollen, emphasis was laid on the identification of phylogenetically reliable gross morphological characters or combinations of characters. Among these, seeds, root system and life habit were documented in detail in the perspective of their comparison with molecular phylogenetic results.

This study was thus undertaken in the aim of addressing

1. the phylogenetic relationships in subtribe Swertiinae using the non-coding sequences of the nuclear ribosomal internal transcribed spacers 1 and 2 (ITS), and of the chloroplast *trn*L intron, *trnr*L-*trn*F intergenic spacer, and *trn*S-*ycf*9 intergenic spacer;
2. the chromosomal evolution in the subtribe in regard of molecular results, by complementing the existing chromosome number data in the genera of Swertiinae;
3. the potential usefulness of pollen morphology to infer phylogenetic affinities by a survey of exine structure variability using scanning electron microscopy;
4. the generic limits, in particular in the broad *Frasera-Lomatogonium-Swertia-Veratrilla* complex, and the evolution of flower morphological characters commonly used in the taxonomy of the genera of Swertiinae.

Main Results and general Conclusions

Molecular Phylogeny (I, IV)

A total of 128 species representing all the genera of the Swertiinae were sequenced for the ITS, *trn*L-F and *trn*S-*ycf*9 regions (Appendix 2). Analysed individually, and regardless of the reconstruction method, the chloroplast data and, in particular, the nuclear data offered only limited resolution. ITS sequences, more variable and homoplasious than chloroplast ones, contained phylogenetic information adequate for addressing relationships within more terminal parts of the trees, while plastid data brought resolutions for deeper level branches. Considered separately, the nuclear and chloroplast phylogenies were similar in many parts, but also incongruent in some occasions. The extent of character and topological conflict was examined carefully, but was not found to be enough significant to preclude the combination of all sequences for further analyses. Trees inferred from the combined data were in general more resolved and received higher branch support, although in some parts, the phylogenies remained polytomic. Relationships that were found to be in conflict in the separate analyses of the data had diminished branch support in the combined analyses, and usually were similar to the best supported one of the separate analyses.

The most parsimonious and Bayesian phylogenies were generally found to be congruent, regardless of the dataset (i. e. nuclear or chloroplast sequences) used for inference. Some method-dependant differences were noted in the resolution of weakly supported branches only.

At the species level within genera/lineages, relationships were often unresolved, due to a lack of sequence divergence (e. g. *Frasera*, *Swertia* 12, *Swertia* s. str. [IV]). The lack of resolution and/or poor support at deeper levels, however, is not attributable to low sequence divergence between lineages, but probably to a rapid ancient cladogenesis, and thus to the lack of synapomorphies informative at that level. Indeed, the analysis of an expanded sequence set including *mat*K data, in addition to the other genes, for a smaller number of taxa resulted in a single most parsimonious tree showing a hard polytomy for several basal

lineages. Compared to the phylogenies inferred without *mat*K sequences, some additional resolution was gained but without branch support. Resolving the phylogeny of Swertiinae in its deeper parts may thus necessitate a considerable amount of additional sequences.

It was considered that the phylogeny deduced from the analysis of all data combined (summarised in Fig. 28) offered the best approximation of the evolutionary history of the Swertiinae. From this phylogeny, the following conclusions were drawn.

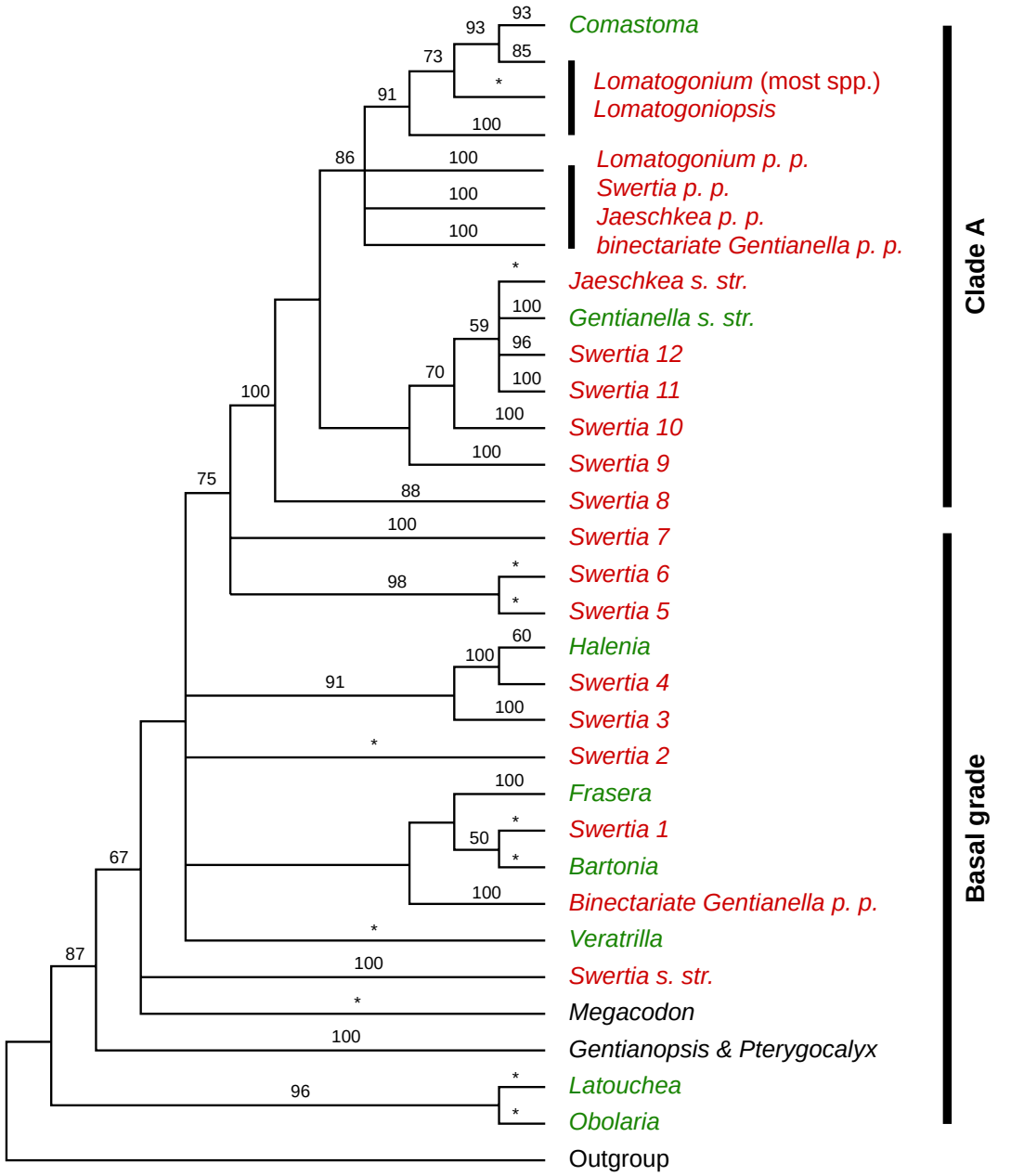

Fig. 28 Simplified strict consensus of the most parsimonious trees inferred from all nuclear and chloroplast DNA sequences combined (data from IV). Above branches: Jackknife values if > 50 %. Asterisks indicate monospecific branches. Monophyletic, unproblematic or monotypic genera or lineages are represented in green. Genera for which monophyly is dubious or has not been verified are in black. Red indicates polyphyletic genera.

1. Subtribe Swertiinae sensu Struwe *et al.* (2002) was confirmed as a monophyletic lineage sister to subtribe Gentianinae. The monophyly of Swertiinae has been demonstrated in recent studies (Yuan, 1995; Struwe *et al.*, 1998; Hagen & Kadereit, 2001, 2002). These results are thus not new in this respect, except for *Latouchea fokienensis*, which is herewith (I) for the first time shown to belong to the Swertiinae also from a molecular point of view. The last common ancestor of the Swertiinae was estimated to have grown in Asia in the early Neogene (IV).

2. Taxa of the Swertiinae formed a broad basal grade and a highly supported derived clade (Fig. 28). The basal grade includes *Bartonia*, *Frasera*, *Gentianopsis*, *Halenia*, *Megacodon*, *Obolaria*, *Pterygocalyx*, *Veratrilla*, and a majority of *Swertia* species distributed in 8 lineages. Two binectariate species of *Gentianella* also belong to this grade, *Gentianella arenaria* and *G. pygmaea*, but this position needs confirmation. This lineage was excluded from clade A, which includes all other binectariate species of *Gentianella*, although the chromosome numbers reported for *G. arenaria* (2n = 16, 18) are more typical of the taxa of clade A, and in particular of *Lomatogonium*, to which other binectariate species of *Gentianella* were related. The remaining taxa (i. e. *Comastoma*, *Gentianella*, *Jaeschkea*, *Lomatogonium* (including *Lomatogoniopsis*), and the other lineages of *Swertia*) all formed a clade very well supported by jackknife (Efron, 1982; Farris *et al.*, 1994) and decay (Bremer, 1988; Donoghue *et al.*, 1992) values. Clade A was identified by both nuclear (ITS) and plastid (*trn*L-F and *trn*S-*ycf*9) sequences, and support for this clade was greatly improved in the analysis of all data combined.

3. Phylogenetic analyses identified several long monospecific branches and well supported clades, between which relationships were often unresolved or only weakly supported. In general, monospecific lineages or lineages that are characterised by high support values in the analyses of combined data represent either unproblematic and probably monophyletic genera such as *Bartonia*, *Comastoma*, *Frasera*, *Halenia*, *Latouchea*, *Obolaria*, and *Veratrilla*, or groups of species of polyphyletic genera, that are coherent from a morphological, karyological and/or palynological point of view (see below). Yet, relationships between these lineages, are often unresolved, or partly resolved but with very low branch support, except in the basalmost part of the tree, and for a larger clade within clade A including *Comastoma*, *Jaeschkea* p. p., *Lomatogonium*, most binectariate species of *Gentianella* and three species of *Swertia*.

4. *Obolaria virginica* and *Latouchea fokienensis* are well supported sister taxa, and form a lineage sister to the remainder of Swertiinae. Both genera are monotypic, and show an eastern North American – Southeast Asian disjunct distribution. It has been argued (Gillet, 1957; Toyokuni, 1965ab; Ho & Liu, 2000;) that taxa with ovary nectaries, and in particular *Megacodon*, may be more closely related to *Gentiana* and other Gentianinae than to taxa of the Swertiinae sharing petal nectaries. Molecular results (Yuan & Küpfer, 1995; Hagen & Kadereit 2002; I) have established their basal position in the subtribe Swertiinae, but without clearly ascertaining their interrelationships. The assumption (Hagen & Kadereit 2002) that all taxa with gynoecial nectaries form a mono- or paraphyletic group from which a single monophyletic lineage with epipetalous nectaries was derived is contradicted by our results, considering the position of *Megacodon* and *Gentianopsis*/*Pterygocalyx*. Although weakly supported, the position of these two taxa suggest that either gynoecial or corolline nectaries may have evolved twice independently in the subtribe. Moreover, the affinities of *Bartonia* with *Obolaria* postulated on morphological grounds (Hagen & Kadereit, 2002) is also not supported by these results (but see IV for a possible bias due to an accelerated substitution rate in *B. virginica*).

5. Species of *Swertia* were divided into 14 distinct lineages scattered across the phylogeny. Based on floral morphology mainly, Shah (1984) recognised 32 informal groups in this genus (35 including *Frasera*), but maintained the historical broad subdivision of the species into a group of annuals or biennials, and a group of perennials. This was clearly not supported by molecular data, as the phylogeny suggests that a shift in habit may have occurred at least 5 times in various lineages of *Swertia*. On the other hand, Shah's (1984) rather narrow groups were almost all supported as monophyletic clades, except for *S. hispidicalyx*, *S. rosularis* and *S. tashiroi* that were not related to species of the groups in which they were placed by Shah (1984), and for the Asian perennials of *Swertia* s. str. for which the number of species sequenced and phylogenetic resolution were too low to allow for comparison. Several of these small groups were connected into larger ones (e. g. 9 in *Swertia* s. str., 3 (or possibly 5) in *Swertia* 8, 5 (6) in *Swertia* 10, 2 in *Swertia* 12), enabling the identification and emphasising of phylogenetically informative morphological character, among which the type of root system and especially the exomorphic seed structure were preponderant. Three of Shah's (1984) monospecific groups and one group of 3 species that was not sequenced could thus be related to the identified lineages, albeit additional palynological and karyological data is necessary to

ascertain their position (see IV). Three species, *S. hispidicalyx*, *S. tenuis* and *S. yunnanensis*, were not related to any of the other lineages of *Swertia*, but showed highly supported affinities with binectariate species of *Gentianella* and species of *Lomatogonium* («abominable» clade in IV). Not a single morphological character was found in support of this clade. However, although nuclear and plastid data resulted in identical topologies for this clade, some karyological (II) and palynological (III) evidence was found suggesting possible hybridisation or introgression.

6. Within clade A, the well supported but heterogeneous clade including *Comastoma*, *Jaeschkea* p. p., *Lomatogonium*, binectariate species of *Gentianella* and three species of *Swertia* is the most problematic in the Swertiinae. Relationships in this clade suggest that *Lomatogonium* is paraphyletic with respect to *Comastoma*, and also polyphyletic as regards *L. oreocharis* and *L. zhongdianense* of the «abominable» clade. To this clade also belong most binectariate species of *Gentianella*. Although included in the same well supported polytomy, these species of *Gentianella* are, however, not monophyletic. *Gentianella azurea* is part of the «abominable» clade, whereas *G. moorcroftiana* is sister to a species of the polyphyletic genus *Jaeschkea*. *Gentianella anomala*, which was considered close to *G. arenaria* and *G. pygmaea* based on morphology (tetramerous flowers, petiolate leaves, minute plant) (Hagen & Kadereit, 2002), is in fact closely related to *G. gentianoides*. The relationships revealed by sequence data in this broad clade suggest an important amount of homoplasy in morphology (corolla tube, insertion point of stamens, type of nectaries, pollen exine ornamentation), which in turn raises questions of a possible hybrid origin for some of the taxa/lineages of this clade (see II; III; IV). This clade doubtless requires more detailed studies to fully understand the speciation processes that resulted in the observed phylogeny.

7. Using a penalised likelihood approach, a rough estimation of divergence times was obtained, suggesting an important role of the rising Himalaya and of the Asian monsoon in the history of the Swertiinae, as well as recent Pleistocene dispersals. Due to the lack of resolution at lower levels of the phylogeny, to heterogeneous substitution rates across branches, and to the absence of reliable fossil material, it was not possible to elaborate a precise historical biogeographical hypothesis for the subtribe. Nevertheless, the penalised likelihood method (Sanderson, 2002) accommodates rate heterogeneity, and thus allowed for a rough approximation of divergence times for some interesting parts of the phylogeny.

The rapid cladogenesis reflected in the phylogeny by polytomies or weakly supported short branches at lower levels of the basal grade and of clad A was situated by this estimation in a time interval (ca4.5 – 15 mya) corresponding to the estimates provided for major orogenic events in the Himalayan region (8 ± 3 mya) (Molnar *et al*., 1993). This orogeny was fast (several thousand meters in a few million years, and as a consequence of this immense physical barrier, wind circulation disruption considerably accentuated the monsoon climatic regime. It seems thus reasonable to argue that the coupled geological and climatic changes in those ages could have had a major incidence in Swertiinae in terms of adaptation and migration. On the other hand, several dispersals to southern India, Indonesia and Melanesia seem to have occurred only recently (Pleistocene).

As regards the origin of the Swertiinae, the estimates for the last common ancestor are vague and ranged from ca 12 to 42.5 mya. Geographically, an Asian origin of the subtribe seems reasonable considering the overwhelming majority of basal species occurring in Asia: But the disjunct distribution of the basalmost clade including *Latouchea* (China) and *Obolaria* (North America) did not allow to ascertain this hypothesis.

Karyology (II)

Chromosome numbers were counted from 60 species of the Swertiinae, with special emphasis on taxa of *Swertia* and *Lomatogonium*. Data available from literature was also reviewed and compiled (II, appendix). In total, chromosome numbers are now available for some 196 species of the Swertiinae. The chromosome number of 25 species was documented here for the first time, while 10 species had a chromosome number different from previous reports. Two numbers, 2n =12 and 2n = 42 were new for *Swertia*. In addition, 2n = 22, which was otherwise only known in this genus in relation with intraspecific dysploidy, was found to occur consistently in *S. tenuis* and *S. yunnanensis*. 2n = 60 in *S. tashiroi*, thought to be a hexaploid number based on x =10, was shown to be probably tetraploid and derived from x = 13. In the genus *Frasera*, 4 species were known to be hexaploid with 2n = 78, while others were diploid with 2n = 26. For 3 of these species, tetraploid numbers were also found. Important descending dysploidy was noted in binectariate species of *Gentianella*. 2n = 10 was recorded for *G. azurea*, which usually has 2n = 22 or 44, while 2n = 12 was found in *G. moorcroftiana*, for which previous data indicate 2n = 18. Cases of ascending dysploidy were noted in *Gentianella* s. str. and *Swertia*, with 2n = 38 in *G. rapunculoides* (2n = 36 in other reports) and 2n =42 in *S. rosulata* from Madagascar, which is related to species of 2n = 20.

Altogether, the Swertiinae exhibit an important karyological variability, with somatic chromosome numbers ranging from 2n = 10 to 2n = 78, and putative basic chromosome numbers covering nearly every number between 5 and 21 (Table 2). Yet, the interpretation of chromosome numbers and their variation in the context of the molecular phylogeny (Fig. 29) shows that this variation is important in certain lineages, but much less so or inexistent in others, and allowed for the following conclusions.

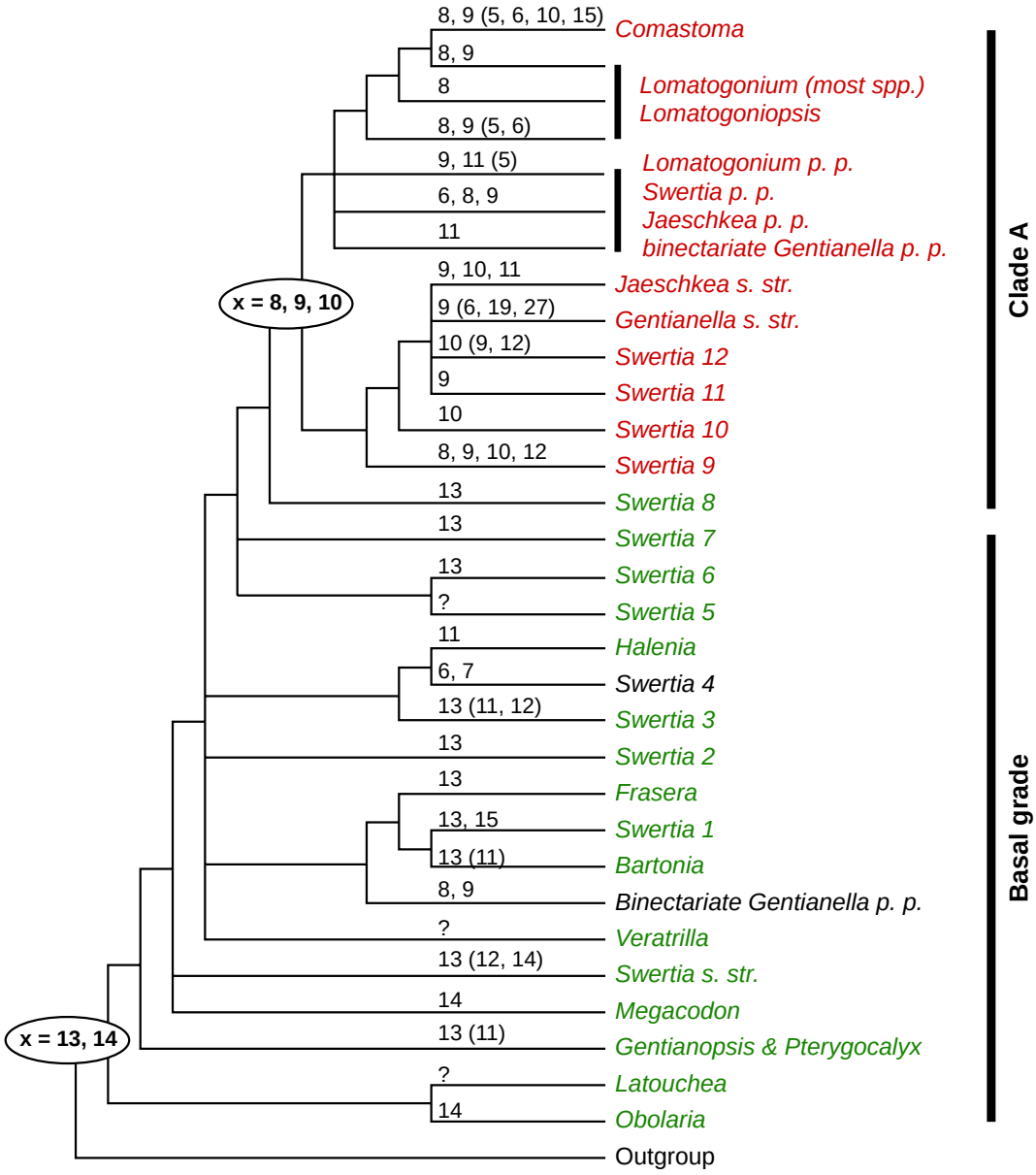

Fig. 29 Distribution of basic chromosome numbers in the lineages of the Swertiinae. Taxa in green have chromosome numbers related to (or clearly derived from in the case of *Halenia*) x = 13 or 14. Taxa in red have numbers mostly related to x = 8, 9 or 10 (see II and IV for details), but never to x = 13 or 14. Taxa in black have basic chromosome numbers markedly different from x =13. Tree identical to Fig. 28.

1. The distribution of basic chromosome numbers agrees with the subdivision of the Swertiinae into a basal grade and clade A suggested by molecular results. Most taxa of the basal grade have basic chromosome numbers of x = 13 and less frequently x = 14. Some variation implying the parallel evolution of a stabilised x = 11 occurs in *Bartonia*, *Gentianopsis* and *Halenia*, while other different chromosome numbers in lineages with x = 13 or 14 are all attributable to intraspecific dysploidy. In this respect, taxa of the basal grade show a remarkable chromosomal homogeneity. Only 2 exceptions blemish this generalised scheme. One occurs in *S. tetraptera*, the only documented species of *Swertia* 4, with 2n = 12 and 14. the second one is found in *Gentianella arenaria*, a binectariate species of *Gentianella* placed in the basal grade in all analyses, along with *G. pygmaea*. Whether 2n = 16 and 18 in this species resulted from descending dysploidy from x = 13 (or 14), or if on the contrary, this number indicates an erroneous phylogenetic position for this lineage is not clear.

Within clade A, the documented species of *Swertia* 8 all have 2n = 26, whereas this number, or any number related to x = 13 or 14 was never found for other taxa of clade A. The basal position of *Swertia* 8 in clade A, that was only weakly supported by molecular data (Fig. 28), is thus well supported from a karyological point of view. Disregarding clade *Swertia* 8, the lineages of clade A mostly have chromosome numbers related to x = 8, 9 or 10, although variation in this clade is manifestly more pronounced than in the basal grade.

Genus	Basic number	Ploidy level	Spp. documented / spp. in genus
Bartonia	11, **13**	4x	3 / 4 [(1)]
Comastoma	5, 6, 8, **9**, 10	**2x**, 4x, 6x	11 / 7-25 [(1)]
Frasera	13	**2x**, 4x, **6x**	13 / 14 [(2)]
Gentianella s. str.	8, **9**, 19	2x, **4x**, 6x	60 / ca 250 [(1)]
Gentianella binectariate	5, 6, 8, 9, 11	2x, 4x	
Gentianopsis	11, **13**	**2x**, 4x, **6x**	11 / 16-24 [(1)]
Halenia	11	2x	16 / 80 [(1)]
Jaeschkea	8, 9, 11	2x	3 / 4 [(1)]
Latouchea	—	—	— / 1 [(1)]
Lomatogoniopsis	6	2x	1 / 3 [(3)]
Lomatogonium	5, **8**, 9	**2x**, 4x, 5x	11 / 18 [(4)]
Megacodon	14	2x	1 / 2 [(1)]
Obolaria	14	4x	1 / 1 [(1)]
Pterygocalyx	12, **13**	2x	1 / 1 [(1)]
Swertia	6, 7, 8, 9, **10**, 11, 12, **13**, 14, 15, 21	**2x**, 4x	64 / 138 [(5)]
Veratrilla	—	—	— / 2 [(1)]
			196 / 541-567

Table 2 Basic chromosome numbers of the genera of the Swertiinae. Most frequent numbers in boldface. The last column shows the number of species with documented chromosome numbers out of the total number of species in each genus. [(1)] Struwe *et al.* (2002), [(2)] Shah (1984), [(3)] Ho & Pringle (1995), [(4)] Liu & Ho (1992), [(5)] own estimation.

2. On a finer scale, chromosome numbers have little intrinsic phylogenetic value, but were found to be most useful to delimitate lineages of *Swertia* when combined with other data. The relative constancy of x =13 and 14, and the homoplasious occurrence of x = 11 in the basal grade generally precludes the use of karyology for the delimitation of genera or lineages. The higher variation of chromosome numbers in clade A also looses much of its significance when considering the multiple occurrences of similar numbers in different lineages (e. g. x = 9 in *Comastoma*, *Gentianella* s. str., *Lomatogonium*, *Swertia* 9, 11 and 12). However, chromosome numbers brought considerable support to the molecular relationships revealed for *Swertia*, particularly as regards the distinction between the species of the basal grade or of *Swertia* 8 with x = 13 from those of clade A with x = 9 or 10. For example, the Asian perennial *S. cuneata* (2n = 20) was related to other Asian perennial species of *Swertia* s. str. (x =13, 14) by Ho *et al.* (1988), but its affinities with African species of *Swertia* 10 suggested by molecular data is also supported by its chromosome number. Similarly, karyology supports the phylogenetic position of *S. chirayita* and akin species in *Swertia* 8 (x = 13), although they are morphologically very similar to other annuals of *Swertia* 12 (x = 10), with which they were grouped in a single section (Ho *et al.*, 1988, 1994).

3. Chromosomal evolution in the Swertiinae follows gradual but also occasionally saltatory descending dysploidy (Fig. 30). In general, the evolutionary trend is from x = 13 to x = 11 in lineages of the basal grade, and from x = 13 to x = 8, 9 or 10 in clade A. For some species, however, molecular phylogenetic relationships suggest a drastic reduction of the number of chromosomes. These saltatory decreases happened in parallel in different lineages, and may or may not be accompanied by notable morphological changes. For example, 2n = 10 is found in *Comastoma tenellum* and *Lomatogonium rotatum*, that have all the characteristics of the genera to which they belong. Such dysploidy may also be intraspecific, as in the case of *Gentianella azurea* with 2n = 10 and 22/44. On the other hand, 2n = 12 in *Lomatogoniopsis alpina*, which is closely related to species of *Lomatogonium* with 2n = 16 and 18, comes with a type of nectary

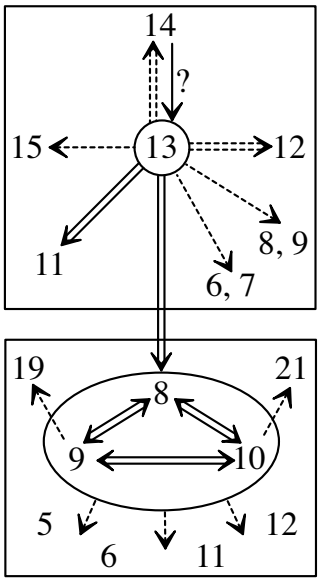

Figure 30. Evolution of basic chromosome numbers in the Swertiinae according to relationships inferred from DNA sequence data. Taxa related to x = 13 and x = 8, 9, 10 in upper and lower boxes respectively. Dashed and double arrows represent rare and multiple occurrences respectively.

appendages unique in the subtribe. Likewise, *Swertia tetraptera* with 2n = 12 and 14, and which was derived from a lineage of x = 13 (*Swertia* 3), has nectaries of a type unique in the genus.

Evidence of ascending dysploidy is less frequent, as in the tetraploid *S. rosulata* with 2n = 42 and in the diploid *Gentianella gentianoides*, *S. tenuis* and *S. yunnanensis* with 2n = 22, or may occur as intraspecific variation, as in *G. rapunculoides* with 2n = 36 and 38 and *S. tashiroi* with 2n = 52 and 60.

4. Polyploidy affects mainly extra-Asian or genetically isolated taxa, and is concentrated in *Gentianella, Gentianopsis* and *Frasera*. Among exclusively North American genera, *Bartonia* and *Obolaria* are tetraploid, while species of *Frasera* are solely diploid or hexaploid, or diploid and tetraploid. In *Gentianella*, which diversified mainly in the southern hemisphere, the overwhelming majority of species are tetraploid. In *Gentianopsis*, Yuan & Küpfer (1993) noted a correlation between increasing distance from the probably Asian centre of origin and higher ploidy levels. Polyploidy thus seems to correspond well with migrations from Asia to other continents, and may have played a facilitating role in the impressive radiation of *Gentianella*.

Palynology (III)

Pollen grains of 65 representative species of all but 2 (*Megacodon* and *Veratrilla*) genera of the Swertiinae were examined by scanning electron microscopy as a complement of Nilsson's (1967b) survey of tribe Gentianeae using light microscopy. For the genus *Swertia*, 24 of the 32 morphological groups defined by Shah (1984) were investigated. Five of the groups not sampled represent African species that have been studied extensively (Jonsson, 1973), whereas the remaining 4 groups are monospecific and thus not likely to have an important incidence on the pollen variation in this genus. The main exine patterns found in the Swertiinae are striate, striato-reticulate, rugulate, reticulate, punctate, perforate, verrucate, spinose, shortly ridged or scabrate to smooth. A comparison of pollen morphological data obtained here and also from other publications (see III and *Aims and Context* above) with molecular phylogenetic results (Fig. 31) led to the following observations.

1. Many distantly related genera share a common striato-reticulate to reticulate exine pattern. This type of exine sculpture is present in *Frasera*, *Halenia*, *Gentianella* s. str.

Lomatogonium p. p., *Veratrilla*, and several lineages of *Swertia* (*Swertia* s. str., 3, 4, 9, 12). For these taxa, pollen morphology did not allow to reliably characterise genera or lineages.

2. Relationships between genera/lineages are generally not well supported by pollen data. The occurrence of similar pollen types, or, on the contrary, of sensibly different exine patterns in sister or closely related lineages or genera often did not allow to verify molecular hypotheses. Thus, the affinities of those taxa sharing a common striato-reticulate to reticulate exine with other members of the Swertiinae could not be addressed precisely. Likewise, the close relationship revealed by sequence data between, e. g., *Comastoma* with striato-reticulate to reticulate pollen grains and species of *Lomatogonium* with spinose punctate pollen, or between *Obolaria* where the exine is reticulate with wide lumina, and *Latouchea* with a densely spinose and nearly imperforate tectum, could not be addressed reliably. Whether similar pollen in different lineages of the broad *Comastoma-Lomatogonium*-binectariate *Gentianella-Swertia* p. p. clade resulted from parallel evolution or from possible hybridisation remains to be investigated in more detail. An exception is the close palynological similarity between *Pterygocalyx* and *Gentianopsis blepharophora*, also suggested by molecular results (Hagen & Kadereit, 2002) and supported by other morphological characters (III).

3. Considered individually, several genera and in particular lineages of *Swertia* are well characterised by pollen morphology. Palynological features confined to *Comastoma, Lomatogonium* and *Gentianopsis*/*Pterygocalyx* were pointed out by Nilsson (1967b). The pollen of *Latouchea* is of a type unique in the Gentianeae. Within the genus *Swertia*, variation in exine patterns agreed well with the lineages identified by sequence data (Fig. 31). Spinulous pollen was found in *S. tashiroi* (*Swertia* 1), *S. cuneata* (*Swertia* 10) and *S. minor* (possibly also *Swertia* 10, see III & IV). The exine of *S. swertopsis* (*Swertia* 2) has very short ridge-shaped processes not found in any other species. In *S. macrosperma* (*Swertia* 11), pollen grains are verrucate-punctate with very short colpi, somewhat similar to the pollen of some species of *Lomatogonium* (e. g. *L. gamosepalum*, *L. oreocharis*). *Swertia piloglandulosa* has similar pollen and probably also belongs to this lineage (see IV). In *S. hookeri*, *S. multicaulis* and *S. membranifolia* (*Swertia* 8), the muri/lirae are comparatively low and wide, with a punctate to perforate tectum. *Swertia decora* (*Swertia* 7) and *S. patula* (*Swertia* 6) share a similar thickening and dense striation at the colpus margin. *Swertia rosularis* and *S. tenuis*, which are remarkably similar in floral morphology, have distinct

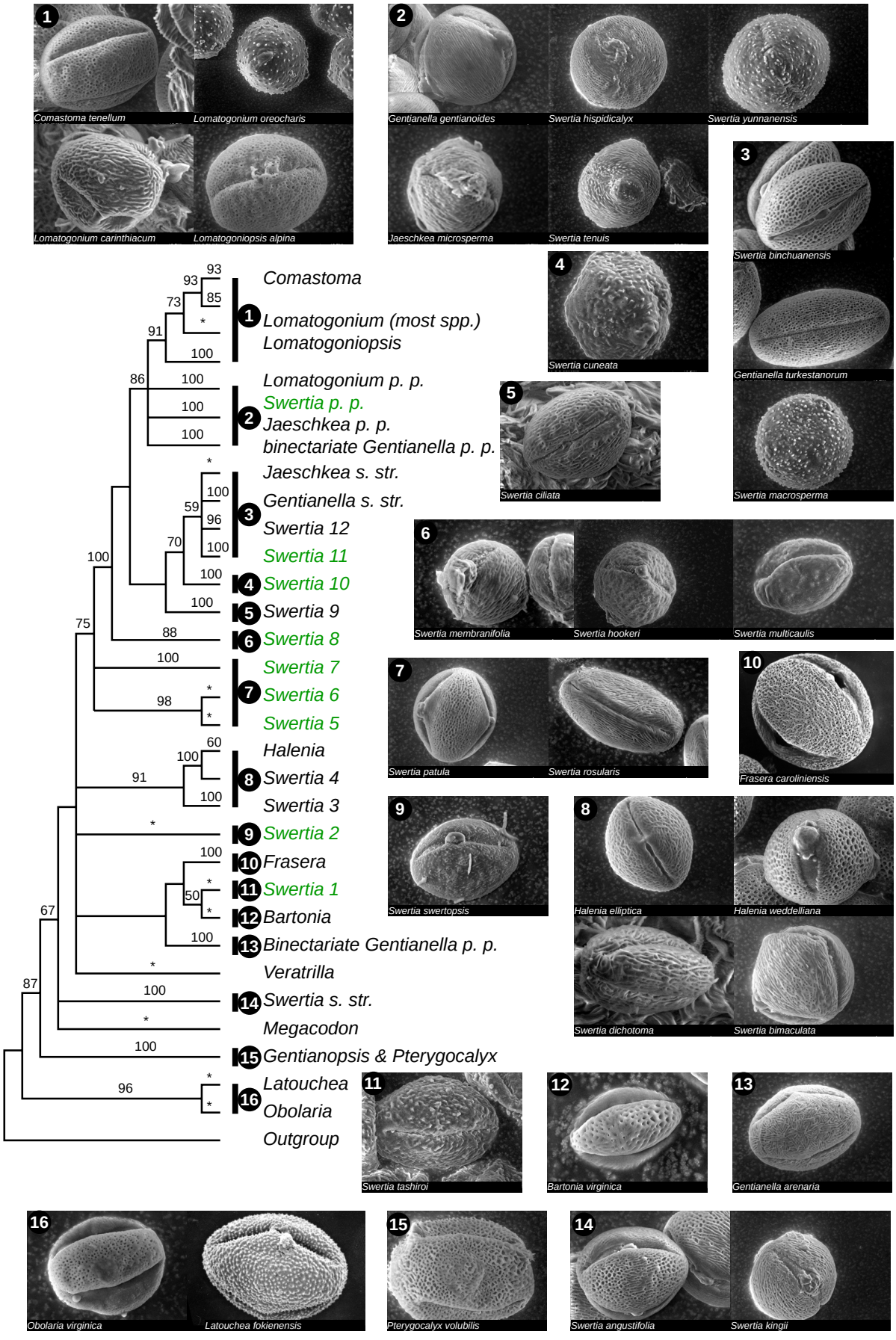

Fig. 31 Distribution of pollen morphological variation in the Swertiinae. Lineages of *Swertia* in green indicate those for which exine ornamentation is characteristic. Tree identical to Fig. 28.

pollen types. Species of *Swertia* of the «abominable» clade (see IV) all have very different pollen. *Swertia yunnanensis* clearly has affinities with species of *Lomatogonium*. *Swertia hispidicalyx* has morphological similarities with species of *Swertia* 9, and comparable pollen. *Swertia tenuis* has an exine with short ridges observed in no other species of the genus. Comparable pollen was reported for *L. forrestii* (Liu & Ho, 1992). In other lineages of *Swertia*, the exine structure is striate-perforate, striato-reticulate, or reticulate. Although some variation was observed in the size of perforations and shape of muri/lirae, it was not possible to distinguish sub-types correlated with molecular phylogenetic results. Yet, considering the good palynological support for several lineages, further studies of cross sections may bring additional supporting evidence for molecular results.

Systematic Implications

Botanical Collections and New Species. The voluminous collections realised by P. Küpfer, Y.-M. Yuan and myself mainly from China, but also from Ecuador, Japan, Nepal, Taiwan, Thailand and the USA (see Appendix) were carefully examined and determined. They constitute an important complement to Asian and Chinese collections present in other herbaria, where taxa of the Swertiinae, and in particular of *Swertia*, are often represented by older material collected by, among other, Cavalerie, Clarke, Cooper, Delavay, Forrest, Handel-Mazzetti, Hooker, Ludlow & Sheriff, Smith, and Stainton & Sykes. Yet floristically, these regions were explored rather extensively, and detailed revisions of herbarium material in relation with often recently published taxonomic works have left very few unknown species (e. g. Smith, 1926, 1936, 1965, 1970, 1981; Shah, 1984; Garg, 1988; Liu & Ho, 1992; Ho & Pringle, 1995). New taxa are discovered only rarely (e. g. Chen, 2000; Suksathan, 2001), and in spite of the ca 850 specimens collected, only one species, *S. barunensis*, was new (V).

Phylogeny of the Swertiinae and Systematics. Many genera in the Swertiinae are now defined as to be monophyletic taxa according to molecular results. The segregates of *Gentianella* s. l. (*Comastoma*, *Gentianopsis*, *Megacodon*,) are particularly well supported at generic rank, as they are clearly not closely related (but see *Genera of the Swertiinae* above for *Megacodon*), in spite of a shared but homoplasious long corolla tube. *Bartonia*, *Halenia*, *Latouchea*, *Obolaria* and *Veratrilla* are morphologically unambiguous and also formed distinct lineages according to molecular data. For the remaining genera, our results support the following taxonomic implication.

1. ***Gentianella*.** Of the ca 250 species in *Gentianella*, a majority were shown to be monophyletic, and were accommodated under *Gentianella* s. str. (Hagen & Kadereit, 2001). But our knowledge of the species diversity and morphological variation of the remaining binectariate species of *Gentianella* is not yet satisfactory (IV). The segregation of *G. pygmaea* and *G. arenaria* under *Aliopsis* has molecular and palynological support (III;IV), but not considering morphology and karyology (II). Other binectariate species are not likely to form a monophyletic group (IV), but their origin and affinities with other taxa in the broad *Comastoma-Lomatogonium-Swertia* p. p. clade (Fig. 28) need to be understood better before taking a nomenclatural step.

2. ***Jaeschkea*.** Although based mainly on the height of filament insertion on the corolla tube, the genus *Jaeschkea* represents a lineage of its own (Hagen & Kadereit, 2002; IV), at the same phylogenetic level as e. g. *Gentianella* s. str. Its status is thus supported by our results. Yet, *J. microsperma* was not related to other species of *Jaeschkea*, from which it also differs in pollen morphology (III) and chromosome number (II), but to *G. moorcroftiana*, a binectariate species of *Gentianella*. The exclusion of *J. microsperma* from *Jaeschkea* is thus well supported, but its ascription to another genus is problematic, as *G. moorcroftiana* is itself of uncertain status.

3. ***Lomatogonium* and *Lomatogoniopsis*.** Molecular results (IV, Fig. 2) do not support *Lomatogonium* as a monophyletic genus, nor do they agree with the sectional classification of Liu & Ho (1992). While a majority of species (including the type species *L. carinthiacum*) formed a well supported clade, others were related to *Comastoma*, or to species of *Swertia* and *Gentianella* in the «abominable» clade. Expanding the definition of *Lomatogonium* to include all related species of other genera would result in an unnatural genus, whereas splitting it would imply at least three different genera. Moreover, these genera would still be heterogeneous, as some species of one lineage are morphologically an palynologically very close to those of other lineages (e. g. *L. micranthum*, *L. oreocharis*, *L. sikkimense*, *L. zhongdianense*) (III). In addition, the apparently highly homoplasious distribution of floral and palynological characters across the lineages of *Lomatogonium* may suggest that introgression and/or hybridisation have biased phylogenetic inference, although nuclear and plastid topologies were congruent for this group. As regards *Lomatogoniopsis*, the genus was delimited from *Lomatogonium* chiefly on grounds of a single nectary per corolla lobe (Ho &

Liu, 1985), but *L. alpina* clearly has two nectaries (Hagen & Kadereit, 2002, pers. obs.), and one species retained in *Lomatogonium, L. graciliflorum*, also has a single nectary. The actual circumscription of *Lomatogoniopsis* does not seem to be satisfactory, and our results concerning *L. alpina* suggest that it should be merged with *Lomatogonium*. Globally, our understanding of the processes of speciation and relationships in the entire complex of *Comastoma, Lomatogoniopsis, Lomatogonium*, binectariate *Gentianella* p.p. and *Swertia* p. p. are still too rudimentary to support taxonomic changes.

4. *Gentianopsis* and *Pterygocalyx*. *Pterygocalyx volubilis* was believed to differ from *Gentianopsis* in having, i. a., nectaries on the ovary and not on the petals (Ho & Pringle, 1995), but detailed studies revealed that this was not the case (Chen & Ho, 1998). Moreover, the affinities of *P. volubilis* with *G. ciliata*, as suggested by ITS data (IV, Fig. 1 B) and with *G. blepharophora* (Hagen & Kadereit, 2002), is also supported by the shared flattened seeds with an annular wing and pollen morphology (Nilsson, 1967b; III). In view of the very high support for the *Gentianopsis* - *Pterygocalyx* clade (Fig. 2), and of the weak morphological and molecular differences between both taxa, it may be better to consider *Pterygocalyx* as part of *Gentianopsis*, as advocated by Smith (1965, and in Nilsson, 1967b).

5. The status of *Frasera*. The monophyly of *Frasera* was highly supported in all analyses (IV, Figs. 1, 2 & 3) but the affinities of this exclusively North American genus with other, mostly Asian, lineages of the basal grade remained essentially unresolved. Considering the large sample of *Swertia* species and the exhaustive one of *Frasera*, the monophyly of *Frasera* in its current circumscription is quasi-certain. Distinguishing features of this genus were never brought out convincingly, and no palynological or karyological characteristics were found to confine to this clade. From a flower morphological point of view, species of *Frasera* conform to the general bauplan observed in most species of *Swertia*, except for the presence of a variably dissected coronal scale (membrane between filaments) present in some species (e. g. *F. caroliniensis*, *F. albicaulis*) but absent in others. Vegetatively, xerophilous species have conduplicate papillose leaves with a silvery-white margin unknown from any species of *Swertia*, while more mesophilous species (*F. caroliniensis*, *F. fastigiata*, *F. speciosa*), which have flat, verticillate leaves with a green margin, and a thick fleshy taproot, bear a resemblance only with some perennial species of *Swertia* 8, to which they were, however, not related. Additionally, only one species of *Swertia*, *S. swertopsis* was found to have seeds comparable to those of *Frasera* (IV). Thus, in view of molecular results, of the distribution,

and of the seed characteristics of *Frasera*, we believe that it should be kept as a separate genus.

6. The lineages of Swertia. Disregarding the 3 species of *Swertia* (*S. hispidicalyx*, *S. tenuis* and *S. yunnanensis*) that were related to the «abominable» clade (see *Molecular Phylogeny* above), the genus *Swertia* may be viewed as a much diversified stem group, of which some lineages have given rise to others in which important morphological changes occurred, leading to their inevitable recognition as separate genera (e. g. *Gentianella*, *Halenia*, and possibly *Bartonia* and *Veratrilla*). Such lack of agreement between molecular phylogenetic results and traditional taxonomic systems implying supra-specific taxa based on gross morphology, and usually defined as lacking the distinctive features of related taxa, occurs in several families (e. g. Hyacinthoideae in Hyacinthaceae [Pfosser & Speta, 1999, and references therein], Lobelioideae in Campanulaceae [Buss *et al.*, 2001, and references therein], Psychotrieae in Rubiaceae [Nepokroeff *et al.*, 1999], Veroniceae in Scrophulariaceae [Albach & Chase, 2001]). The major difficulty in such cases is usually to identify new characters (micromorphological, phytochemical, karyological etc.) that are distinctive synapomorphies at the appropriate taxonomic level, in order to elaborate a new system reconciling taxonomy and phylogeny.

Using the molecular phylogeny as a reference, the significance of morphological (Shah, 1984; IV), karyological (II) and palynological (Nilsson, 1967b, Jonsson, 1973; III) characters was re-evaluated, and 13 of the 14 lineages of *Swertia* could be delimited by different combinations of these characters (IV). Disregarding the non-monophyly of *Swertia*, the lineages identified here did, for several of them, not match any infrageneric taxon or synonymous genus. In this respect, the characters emphasised by Shah (1984) (seedcoat, shape of ovary and stigma, structure of nectaries and appendages) seemed more pertinent than the more trivial ones used by Ho *et al.*, (1988, 1994), in particular for the classification of annual and biennial plants (type of ramification, naked vs. appendaged nectaries, corolla and calyx tube length, sub-perennial habit, floral dimorphism). The question is thus not whether our results support one or the other of the numerous segregates proposed for *Swertia*, as none were found to be congruent with our results excepted for *Frasera* and partly for *Anagallidium*, but whether or not this large group of species should continue to be regarded as a single nomenclatural entity. Because modifications are an intrinsic component of evolution, and also the basis of taxonomic classifications, it has been argued that paraphyly is inescapable, and that recognising monophyletic taxa only may lead to bad taxonomy

(Brummitt, 2002). If the idea of splitting *Swertia* into some 13 smaller genera may thus seem daring, one should nevertheless consider that each of these lineages can readily be characterised by unambiguous combinations of characters (IV), and it can be assumed that more detailed micromorphological studies will uncover additional phylogenetically informative characters. Moreover all these lineages may already be keyed out relatively easily (see key in IV). In addition to achieving a more coherent taxonomic system, the splitting of *Swertia* would contribute much to better grasp the diversity in the *Swertiinae*. In turn, the recognition at generic level of some endemic (and for some of them endangered) monospecific lineages such as *S. rosularis*, *S. swertopsis* and *S. tashiroi* could better reflect their value and facilitate their protection and/or conservation.

Existing names are available for *Swertia* 2 (*Swertopsis* Makino; type: *S. umbellata* Makino), *Swertia* 4 (*Anagallidium* Griseb.; type: *A. dichotomum* (L.) Griseb.), *Swertia* 8 (*Agathotes* Griseb.; no type designated, but *A. chirayita* G. Don, listed under *Agathotes* by Don [1937] is eligible as lectotype), *Swertia* 9 (*Ophelia* Griseb.; lectotype: *O. ciliata* G. Don, selected by Garg [1987]), and *Swertia* 12 (*Sczukinia* Turcz.; type: *S. diluta* (Turcz.) Turcz.). Seven genera would have to be created de novo to account for *Swertia* 1, *Swertia* 3, *Swertia* 5, *Swertia* 6, *Swertia* 7, *Swertia* 10, and *Swertia* 11. Yet, a formal taxonomic treatment aimed at splitting the genus *Swertia* was not undertaken at this stage, as it seems that our understanding of morphological evolution and of the evolutionary processes underlying the phylogeny of Swertiinae can still be improved, and would result in a better appreciation and more precise descriptions of new genera. This applies in particular to the three species of the «abominable» clade (*S. hispidicalyx*, *S. tenuis* and *S. yunnanensis*), for which the taxonomic status under which they could be placed is far from clear.

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I

HIGH PARAPHYLY OF *SWERTIA* L. (GENTIANACEAE) IN THE *GENTIANELLA*-LINEAGE AS REVEALED BY NUCLEAR AND CHLOROPLAST DNA SEQUENCE VARIATION

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High paraphyly of *Swertia* L. (Gentianaceae) in the *Gentianella*-lineage as revealed by nuclear and chloroplast DNA sequence variation

P. Chassot[1], **S. Nemomissa**[2], **Y.-M. Yuan**[1], and **P. Küpfer**[1]

[1]Institut de Botanique, Laboratoire de botanique évolutive, Université de Neuchâtel, Suisse
[2]The National Herbarium, Biology Department, Addis Ababa University, Ethiopia

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Abstract. The genus *Swertia* L., as currently defined, is polymorphic and mainly distributed in temperate regions of the northern hemisphere. Phylogenetic relationships between *Swertia* and the other genera of the Swertiinae sensu Struwe et al. (unpubl. data) are discussed based on cladistic analyses of DNA sequence data. The sequences used for this purpose include the *trn*L (UAA) intron, the intergenic spacers (IGS) between *trn*L (UAA) and *trn*F (GAA) exons, and between *trn*S (UGA) and *ycf*9 exons of cpDNA, as well as the ITS region of nrDNA. Although moderately resolved, the phylogenies resulting from the separate analyses of nuclear and chloroplast data are congruent, and the incongruence length difference test (Farris et al. 1995) detected no character incongruence. The phylogeny suggested by the analysis of combined data sets defines *Swertia* as strongly paraphyletic in relation to the other genera. This taxon may have acted as a stem group, giving rise to diverse lineages, some of which are morphologically distinct and have been recognised at the generic level. *Latouchea* and *Obolaria* are closely related and occupy the basalmost position in the molecular tree. *Swertia* species are distributed in 9 different clades, three of which share a basal polytomy with *Bartonia*, *Frasera*, *Gentianopsis*, *Halenia*, *Megacodon*, *Pterygocalyx* and *Veratrilla*. Two lineages have an intermediate position. The remaining 4 clades occupy a more derived position. Two of the latter clades show a close relation with species of *Gentianella* s. str., and one is included in a large clade comprising *Comastoma*, *Jaeschkea* and *Lomatogonium*. Selected character states and their proposed polarity, such as number and structure of nectaries, stylar and seedcoat characteristics, pollen morphology, fusion of floral parts and chromosome number are discussed in the context of molecular data. Rugose, spinose, or winged seeds are found mainly in basal lineages, while smooth ones are typical for derived species. Chromosome numbers follow a similar pattern with $x = 13$ restricted to basal lineages, while in more derived clades, x is always smaller than 13. With respect to the molecular phylogeny, taxonomic circumscriptions in the Swertiinae sensu Struwe et al. (unpubl. data) does not seem to reflect phyletic relationships.

Key words: Gentianaceae, *Swertia*, phylogeny, paraphyly, nectary, ITS, *trn*L, *trn*L-F, *trn*S, *ycf*9.

Introduction

Since the last infrafamilial classification of Gentianaceae published by Gilg (1895), the circumscription of the subtribe Gentianinae has remained quite stable. *Obolaria* and

Bartonia were included in Grisebach's (1845) tribe Swertieae by Bentham and Hooker (1876) but were subsequently removed from the Gentianinae by Gilg (1895). Both genera were reincluded therein following the recent molecular results of Struwe et al. (1998). The *Gentianella*-lineage (Gillett 1957; Toyokuni 1962, 1963; Ho and Liu 1990), in opposition to the *Gentiana*-lineage, was defined to include those genera of the subtribe Gentianinae sensu Gilg (1895) that have corolline nectaries, corolla lobes with 5–9 vascular bundles, corollas without plicae or folds and calyces without an intracalycular membrane (present in *Gentianopsis* but of a different nature than in *Gentiana* (Gillett 1957)). The genera that fall into this category and that are dealt with in this study are *Comastoma*, *Frasera*, *Gentianella*, *Gentianopsis*, *Halenia*, *Jaeschkea*, *Lomatogonium*, *Swertia* and *Veratrilla*. The description of the *Gentianella*-lineage was a first step towards the recognition of two distinct evolutionary lineages in the Gentianinae Gilg. Previous molecular studies have confirmed these two lineages (Yuan and Küpfer 1995, Struwe et al. 1998). *Pterygocalyx* and *Megacodon* have whorls of nectaries at the base of the ovary (Ho and Pringle 1995) but were found to be distinct from *Gentiana* and its allied genera by the molecular studies of Yuan and Küpfer (1995) and therefore also included in the *Gentianella*-lineage. *Latouchea* also has whorled nectaries at the base of the ovary but has not been sequenced prior to this study. Struwe et al. (unpubl. data) consider that Gilg's subtribe Gentianinae should be raised to a tribal status and further divided into two subtribes. These are subtribe Gentianinae (*Crawfurdia*, *Gentiana*, *Tripterospermum*) and subtribe Swertiinae (*Bartonia*, *Comastoma*, *Frasera*, *Gentianella*, *Gentianopsis*, *Halenia*, *Jaeschkea*, *Latouchea*, *Lomatogonium*, *Megacodon*, *Obolaria*, *Pterygocalyx*, *Swertia*, *Veratrilla*). Therefore, the *Gentianella*-lineage, as used in the text and figures, is herewith defined to include all the aforementioned genera and corresponds to the subtribe Swertiinae sensu Struwe et al.

The genus *Swertia* L. is cosmopolitan in distribution, although its ca. 150 species mainly occur in temperate regions of the northern hemisphere. The genus is, however, represented in tropical regions and in the southern hemisphere, and its highest species diversity is in the Himalayas and in south-western China (Meusel et al. 1978). Owing to the highest species diversity and the occurrence of taxa with the presumed ancestral characters (i.e. tall perennial plants, pentamery, few-flowered inflorescences, rugose seeds), Ho et al. (1994) argued that south-western China is the centre of origin of the genus. From there, *Swertia* has perhaps diversified and dispersed to south-east Asia as well to Africa and North America, where they have formed two secondary centres of diversification. There are two taxa in the Arabian Peninsula and one species in Madagascar. The genus is absent from Australia, New Zealand and South and Central America.

Swertia was described by Linnaeus (1753), in honour of Emanuel Swert, a botanical author of the 17th century. The circumscription of the genus has often been debated, resulting in disagreements among researchers of the family. Part of this debate is due to the morphological similarities (i.e. nectariferous and rotate corolla lobes) of the species of *Swertia* and related genera to one another, namely, *Halenia*, *Lomatogonium* and *Veratrilla*. Many species of these genera were described under *Swertia*, e.g. *Lomatogonium gamosepalum* (as *S. gamosepala*), *Veratrilla baillonii* (as *S. mekongensis*), *Halenia corniculata* (as *S. corniculata*). These genera are now widely accepted as distinct from and perhaps more advanced in evolution than *Swertia* (Allen 1933, Liu and Ho 1992). *Lomatogonium* was established as a distinct genus by Braun (1830) based on *Gentiana carinthiaca* Froehl. on grounds of its decurrent stigma. *Veratrilla* is dioecious and *Halenia* has spurred corolla lobes except in sect. *Swertiella* (Allen 1933) where the corolla lobes have short protuberances.

In addition to the similarities with the aforementioned genera, the definition of

Swertia proper has varied significantly since it was first described by Linnaeus. Many other genera were segregated from it, while others were redundantly described from Asia, North America, Europe and Africa. The following taxa are now recognised as synonyms of *Swertia* (Shah 1990, 1992; Pringle 1993; Ho and Pringle 1995; Garg 1987): *Frasera* Walter (1788), *Tesseranthium* Kellogg (1862) and *Leucocraspedum* Rydb. (1917) from North America; *Anagallidium*, *Ophelia*, *Agathotes* Griseb. (1839, 1845) and *Kingdon-Wardia* C. Marquand (1929) from the Himalayas; *Sczukinia*, *Stellera* and *Rellesta* Turcz. (1840, 1849) from eastern Asia; *Monobothrium* Hochst. (1844) from Africa; *Swertopsis* Makino (1891) from Japan; *Blepharaden* Dulac (1867) from the Pyrénées. *Henricea* Lem. (1824) is not valid due to its earlier use in Asteraceae. For Grisebach (1845), the generic concept of *Swertia* was narrow, that is, *Anagallidium*, *Stellera* and *Ophelia* were segregated from *Swertia*, and *Frasera* was accepted as a distinct genus. Bentham and Hooker's (1876) generic concept of *Swertia* was basically similar to that of Grisebach. Gilg (1895) listed the species of *Frasera* under his section *Euswertia*. *Frasera* is perhaps the most controversial of the variously described synonyms of *Swertia*. Kuntze (1891) discussed its generic relationships and reduced the taxon under *Swertia*. Card (1931) revised *Frasera* and recognised it as a distinct genus. *Frasera* was restored to *Swertia* by St. John (1941) and extensively dealt with by Pringle (1979, 1990) where evidence was presented in favour of its inclusion in *Swertia*. On the other hand, Toyokuni (1965) even went beyond recognising *Frasera* as a distinct genus and has moved some species of *Swertia*, e.g. *S. tashiroi*, *S. pseudochinensis* and *S. bimaculata* to *Frasera* and made new combinations of names accordingly. However, Toyokuni's systematic treatment of *Frasera* has not been widely accepted. It is evident from this brief account that the generic concept of *Swertia* has never been stable, mainly because macromorphological characters do not exhibit clear enough patterns to unambiguously justify taxonomic units.

Nilsson (1964, 1967, 1970) initiated extensive pollen micromorphological studies in the Gentianinae to shed a new light on possible relationships. *Bartonia* and *Obolaria* were examined by Nilsson and Skvarla (1969). African species of *Swertia* were extensively investigated by Jonsson (1973). Pollen morphology in the subtribe was found to be variable although its utility for taxonomic purpose seems limited. A few genera or sections are clearly discriminated by their palynological features, but the authors also report that similarities between other genera and sections are too confusing to allow a comparison. In the case of *Obolaria*, pollen morphology does not even support its inclusion in the Gentianinae preferably to the Chironiinae (as Erythraeinae in Nilsson and Skvarla 1969). Nevertheless, Nilsson (1967) described palynological affinities between *Frasera* and *Halenia*, and between *Lomatogonium* and *Comastoma*, and also noted that the pollen of these four genera shares common aspects. The pollen of *Swertia* is morphologically heterogeneous and variable. African species could be categorised into three groups, but no such clear distinction could be made for Asiatic species. The genera of the subtribe, thus, exhibit reticulate relationships with regard to their palynological data.

Some authors have used karyological data to attempt to establish natural genera and phyletic hypotheses in the *Gentianella*-lineage. Löve and Löve (1956) for example reduced *Comastoma* to a section of *Lomatogonium* on the grounds of shared base numbers ($x = 5$). Toyokuni (1965) on the other hand recognised three sub-groups based on morphological similarities in the *Gentianella*-lineage and, following Favarger (1962), favoured the hypothesis of parallel evolution of karyotypes to account for the co-occurrence of identical base numbers in the three sub-lineages (*Halenia* with $x = 11$, *Swertia-Frasera* with $x = 5, 12, 13$, *Gentianella*, *Gentianopsis*, *Comastoma*, *Lomatogonium*, *Jaeschkea* with $x = 5, 9, 11, 13$). Karyological considerations were also brought up by Post (1956) to justify the generic rank of *Frasera*. Unfortunately, relatively few species were cytologically documented at that time

(i.e. 6 for *Swertia* s. l., and even less for the other genera), and these views were not widely adopted.

Previous molecular results (Yuan and Küpfer 1995, von Hagen and Kadereit 2001) revealed possible polyphyly or paraphyly in *Swertia*, but the limited number of species sampled prohibited estimating to which extent. In view of the summary of the systematic problems involved with regard to the generic concept of *Swertia* and the lack of a rigorous molecular phylogeny of the members of the *Gentianella*-lineage, the present study mainly addresses 1) the circumscription of *Swertia*, i.e., to test the monophyly of *Swertia* in regard to its current taxonomic definition, 2) the systematic position and affinities of *Swertia* to related genera in the *Gentianella*-lineage and 3) the phylogeny of the genera of the *Gentianella*-lineage.

Materials and methods

Taxon sampling. The species included in this study are listed in Table 1. Those sequences retrieved from Genbank are indicated with an asterisk following their accession numbers. All the genera included in the Swertiinae sensu Struwe et al. (unpubl. data) are represented. Sampling of *Swertia* species has taken the following parameters into consideration: geographical distribution, morphological variation such as number and features of nectaries on corolla lobes, seed surface morphology (smooth and rugose), palynology, habit (annual, monocarpic and polycarpic perennials) and existing infrageneric classifications (Ho et al. 1994; Shah 1990, 1992). All the geographical and morphological groups in this taxon are sampled, except for a few monotypic groups for which material was not available. A total of 48 operational taxonomic units (OTUs) are included in this study as representatives of the ingroup taxa. Three *Gentiana* species were used as outgroup, following the latest molecular studies of the Gentianaceae – Gentianinae (Yuan and Küpfer 1995, Struwe et al. 1998). They were selected from 26 other species of the *Gentiana*-lineage (including *Crawfurdia* and *Tripterospermum*) following a preliminary analysis of ITS sequences such as to minimise long-branch attraction between ingroup and outgroup taxa.

Molecular markers. Five molecular markers were used in this study, i.e. internal transcribed spacers of nrDNA (ITS1 and ITS2), and noncoding regions of chloroplast DNA (*trn*L intron, *trn*L-F and *trn*S-*ycf*9 intergenic spacers). Primers ITS5 (5′-GGAAGTAGAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990) were used for the amplification of the internal transcribed spacers of the nuclear ribosomal DNA. The name and sequences of primers used for the amplification of *trn*L intron and IGS *trn*L-F follow Taberlet et al. (1991). Primers "c" (5′-CGAAATCGGTAGACGCTACG-3′), "d" (5′-GGGGATAGAGGGGACTTGAAC-3′) were used for the amplification of *trn*L (UAA) intron and "e" (5′-GGTTCAAGTCCCTCTATCCCC-3′) and "f" (5′-ATTTGAACTGGTGACACGAG-3′) were used for the spacer between *trn*L- (UAA)- 3′-exon and *trn*F-(GAA) gene. The spacer between *trn*S- (UGA) and *ycf*9 genes was amplified with primers *trn*S (5′-GAGAGAGAGGGATTCGAACC-3′) and *trn*fM (5′-CATAACCTTGAGGTCACGGG-3′) (Demesure et al. 1995).

DNA extraction. DNA was extracted from silica gel dried leaf tissue (Chase and Hills 1991). Total DNA extraction was made using the CTAB procedure of Doyle and Doyle (1987).

Polymerase chain reaction (PCR). Double-stranded DNA was directly amplified by PCR of all markers. Reaction volumes were 25 µl and contained 2.5 µl 10X PCR buffer, 1 µl 25mM Mg^{++}, 0.5 µl 10mM dNTPs, 0.5 µl of 10mM primers, 0.2 µl HotstarTaq polymerase (5u/µl) (QIAGEN, Basel), and 17.05 µl ddH_2O. About 10–20 ng genomic DNA was added to the PCR cocktail. PCR was performed in a Biometra® Tgradient thermal cycler and consisted of 15 min at 95 °C for the activation of the Hotstar polymerase, followed by 30 cycles of 30 sec at 94 °C, 30 sec at 55 °C, 1 min 30 sec at 72 °C with a final extension period of 4 min at 72 °C.

PCR purification and sequencing. The quality and quantity of the PCR products were checked on 0.8% agarose gel using a mini-gel applying a low voltage. There was always a single sharp band resolved on the gel for ITS, *trn*L intron and IGS *trn*L-F after each polymerase chain reaction. PCR products were purified using QIAquick™

Table 1. Origin of plant material, voucher information and Genbank accession numbers. Sequences retrieved from Genbank for the purpose of this study are marked with an asterisk

Taxon	Collector	Voucher	Locality	Genbank number *trn*L(UAA) intron	*trn*S(UGA)-*ycf*9 spacer	*trn*L(UAA)-F(GAA) spacer	ITS1	ITS2
Bartonia virginica (L.) Britton Stearns & Poggenb.	Strong	2394	USA, Virginia, Sussex country	AJ315185	AJ315279	AJ315231	AJ318533	AJ410312
Comastoma pulmonarium (Turcz.) Toyok.	Yuan & Küpfer	NEU 92-279	China, Sichuan, Ganzi	AJ315225	AJ315319	AJ315271	Z48108*	Z48121*
Comastoma traillianum (Forrest) Holub	Yuan & Küpfer	NEU 92-197	China, Yunnan, Zhongdian	AJ315186	AJ315280	AJ315232	AJ318534	AJ410313
Frasera albomarginata S. Watson	Schweich	NEU 00-23	USA, California, San Bernardino county	AJ315187	AJ315281	AJ315233	AJ318535	AJ410314
Frasera speciosa Griseb.	Yuan	NEU 91-52	USA, Colorado, Boulder	AJ315230	AJ315324	AJ315276	Z48146*	Z48124*
Gentiana frigida Haenke	Yuan	NEU 93-17	Bulgaria, Mt. Rila	X77883*	AJ315325	AJ315277	Z48063*	Z48084*
Gentiana lutea L.	Yuan	NEU Y91-S5	Switzerland, Neuchâtel	X75702*	AJ315326	AJ315278	Z48122*	Z48119*
Gentiana phyllocalyx C. B. Clarke	Chassot	NEU 97-30	Nepal, Makalu	AJ315189	AJ315283	AJ315235	AJ318537	AJ410316
Gentianella foliosa (Kunth.) Fabris	Chassot	NEU 00-3	Ecuador, Pichincha. Volcán Illiniza	AJ315190	AJ315284	AJ315236	AJ318538	AJ410317
Gentianella umbellata (M. Bieb.) Holub	Küpfer	NEU 91-G3	Georgia, Mt. Caucasus	AJ315226	AJ315320	AJ315272	Z48102*	Z48132*
Gentianopsis contorta (Royle) Ma	Chassot	NEU 97-69	Nepal, Rara lake	AJ315191	AJ315285	AJ315237	AJ318539	AJ410318
Gentianopsis grandis (Harry Sm.) Ma	Yuan & Küpfer	NEU 92-222	China, Yunnan, Lijiang	AJ315227	AJ315321	AJ315273	Z48105*	Z48130*
Halenia brevicornis G. Don	Mansion & Zeltner	NEU 990115	Mexico, Las Piedracitas	AJ315192	AJ315286	AJ315238	AJ318540	AJ410319

Table 1 (continued)

Taxon	Collector	Voucher	Locality	Genbank number *trn*L(UAA) intron	*trn*S(UGA)-*ycf*9 spacer	*trn*L(UAA)-F(GAA) spacer	ITS1	ITS2
Halenia elliptica D. Don	Yuan & Küpfer	NEU 93-52	China, Sichuan, Shiqü	AJ315193	AJ315287	AJ315239	AJ318541	AJ410320
Halenia weddeliana Gilg	Chassot	NEU 00-5	Ecuador, Pichincha. Volcán Illiniza	AJ315194	AJ315288	AJ315240	AJ318542	AJ410321
Jaeschkea microsperma C. B. Clarke	Yuan & Küpfer	NEU 92-107	China, Tibet, Nyalam	AJ315195	AJ315289	AJ315241	AJ318543	AJ410322
Latouchea fokienensis Franch.	Yuan	NEU cn2k-14	China, Fujian, Wuyishan	AJ315196	AJ315290	AJ315242	AJ318544	AJ410323
Lomatogonium bellum (Hemsl.) H. Smith	Yuan & Küpfer	NEU 92-236	China, Yunnan, Lijiang	AJ315197	AJ315291	AJ315243	AJ318545	AJ410324
Lomatogonium brachyantherum (C. B. Clarke) Fernald	Chassot	NEU 97-13	Nepal, Langtang	AJ315198	AJ315292	AJ315244	AJ318546	AJ410325
Lomatogonium macranthum (Diels & Gilg) Fernald	Yuan & Küpfer	NEU 93-91	China, Sichuan, Ganzi	AJ315228	AJ315322	AJ315274	Z48108*	Z48135*
Lomatogonium perenne T. N. Ho & S. W. Liu	Chassot & Yuan	NEU 99-78	China, Yunnan, Deqin	AJ315199	AJ315293	AJ315245	AJ318547	AJ410326
Megacodon stylophorus (C. B. Clarke) Harry Sm.	Chassot & Yuan	NEU 99-36	China, Yunnan, Zhongdian	AJ315200	AJ315294	AJ315246	AJ318548	AJ410327
Obolaria virginica L.	Nicolson	24-IV-00	USA, Virginia	AJ315201	AJ315295	AJ315247	AJ318549	AJ410328
Pterygocalyx volubilis Maxim.	Chassot & Yuan	NEU 99-100	China, Yunnan, Deqin	AJ315202	AJ315296	AJ315248	AJ318550	AJ410329
Swertia angustifolia Ham. ex D. Don	Chassot & Yuan	NEU 99-172	China, Yunnan, Binchan	AJ315203	AJ315297	AJ315249	AJ318551	AJ410330
Swertia bimaculata Hook. f. & Thomas. ex C. B. Clarke	Chassot	NEU 97Z-22	Nepal, Makalu	AJ315204	AJ315298	AJ315250	AJ318552	AJ410331
Swertia binchuanensis T. N. Ho & S. W. Liu	Chassot & Yuan	NEU 99-160	China, Yunnan, Binchan	AJ315205	AJ315299	AJ315251	AJ318553	AJ410332
Swertia calycina Franch.	Yuan & Küpfer	NEU 92-232	China, Yunnan, Lijiang	AJ315206	AJ315300	AJ315252	AJ318554	AJ410333

Swertia chirayita Karst.	Chassot	NEU 97-2	Nepal, Langtang	AJ315207	AJ315301	AJ315253	AJ318555	AJ410334
Swertia ciliata (D. Don ex G. Don) B. L. Burtt	Chassot	NEU 97-6	Nepal, Langtang	AJ315208	AJ315302	AJ315254	AJ318556	AJ410335
Swertia cordata (Wall. ex D. Don) C. B. Clarke	Chassot	NEU 97-17	Nepal, Langtang	AJ315209	AJ315303	AJ315255	AJ318557	AJ410336
Swertia crassiuscula Gilg	Wohlhauser	NEU 98-5	Kenya, Mt. Kenya	AJ315210	AJ315304	AJ315256	AJ318558	AJ410337
Swertia cuneata Wall. ex D. Don	Chassot	NEU 97-14	Nepal, Langtang	AJ315211	AJ315305	AJ315257	AJ318559	AJ410338
Swertia decora Franch.	Chassot & Yuan	NEU 99-176	China, Yunnan, Dali	AJ315212	AJ315306	AJ315258	AJ318559	AJ410339
Swertia delavayi Franch.	Chassot & Yuan	NEU 99-66	China, Yunnan, Zhongdian	AJ315213	AJ315307	AJ315259	AJ318561	AJ410340
Swertia engleri Gilg	Sileshi Nemomissa	ETH 990517-8/3	Ethiopia, Simen mts.	AJ315214	AJ315308	AJ315260	AJ318562	AJ410341
Swertia hispidicalyx Burkill	Yuan & Küpfer	NEU 92-72	China, Tibet, Nagarze	AJ315215	AJ315309	AJ315261	AJ318563	AJ410342
Swertia kilimandscharica Engl.	Sileshi Nemomissa	ETH 990725-2/1	Ethiopia, Bale mts.	AJ315216	AJ315310	AJ315262	AJ318564	AJ410343
Swertia macrosperma C. B. Clarke	Yuan & Küpfer	NEU 92-213	China, Yunnan, Lijiang	AJ315217	AJ315311	AJ315263	AJ318565	AJ410344
Swertia perennis L.	Küpfer	NEU 98-10	Switzerland	AJ315218	AJ315312	AJ315264	AJ318566	AJ410345
Swertia aff. *pseudohookeri* Harry Sm.	Chassot	NEU 97-28	Nepal, Makalu	AJ315219	AJ315313	AJ315265	AJ318567	AJ410346
Swertia pubescens Franch.	Yuan & Küpfer	NEU 92-224	China, Yunnan, Lijiang	AJ315220	AJ315314	AJ315266	AJ318568	AJ410347
Swertia punicea Hemsl.	Chassot & Yuan	NEU 99-63	China, Yunnan, Zhongdian	AJ315221	AJ315315	AJ315267	AJ318569	AJ410348
Swertia tashiroi (Maxim.) Makino	Chassot	NEU 99-7b	Taiwan, Hsinch	AJ315222	AJ315316	AJ315268	AJ318570	AJ410349
Swertia tetraptera Maxim.	Yuan & Küpfer	NEU 92-315	China, Gansu, Magu	AJ315229	AJ315323	AJ315275	Z48115*	Z48139*
Swertia volkensii Gilg	Wohlhauser	NEU 98-4	Kenya, Mt. Kenya	AJ315223	AJ315317	AJ315269	AJ318571	AJ410350
Swertia yunnanensis Burkill	Chassot & Yuan	NEU 99-200	China, Yunnan, Lijiang	AJ315224	AJ315318	AJ315270	AJ318572	AJ410351
Veratrilla baillonii Franch.	Chassot & Yuan	NEU 99-37	China, Yunnan, Zhongdian	AJ315188	AJ315282	AJ315234	AJ318536	AJ410315

purification kit (QIAGEN AG, Base1) following the manufacturer's protocol prior to sequencing.

Cycle sequencing was performed using the dideoxy chain termination method using an ABI PRISM™ BigDye™ Terminator cycle sequencing kit with AmpliTaq DNA polymerase FS (Applied Biosystems) in a Biometra® Tgradient thermal cycler (5 µl reaction volumes). Cycling parameters were 25 cycles of 20 sec at 96 °C for denaturation, 10 sec at 54 °C for primer annealing and 4 min at 60 °C for primer extension. The cycle sequencing products were cleaned by ethanol precipitation and applied to the ABI 310 automated sequencer (Applied Biosystems). Basecalling was checked on the chromatograms using Sequence Navigator (Applied Biosystems) and edited manually when necessary. Sequences have been deposited in Genbank with their accession numbers indicated in Table 1.

Sequence alignment. The boundaries of ITS and *trn*L intron in the study material were determined by comparison with *Gentiana* sequences (Yuan and Küpfer 1995, Gielly et al. 1996). The limits of IGS *trn*S-*ycf*9 and IGS *trn*L-F were established by comparison with sequences of *Arabidopsis thaliana*, *Nicotiana tabacum* and *Zea mais* (Genbank numbers: AP000423, ZOOO44, X86563). ITS sequences were preliminarily aligned with ClustalX (Tompson et al. 1997) and subsequently hand-adjusted. Chloroplast sequences were aligned manually in order to respect indel events otherwise not recognised. Potentially informative indels of more than 1 base were recoded (1 for presence, 0 for absence) and added to the data matrix. The data matrix is available from the authors on request.

Phylogenetic analyses. All data sets were analysed with heuristic parsimony searches using PAUP*, v.4.0b4 (Swofford 2000). In all analyses characters were equally weighted and unordered (Fitch 1971). Gaps were treated as missing data. All searches consisted of 1000 random taxon addition sequences with TBR branch swapping, MULPARS and ACCTRAN options on; branches with minimum lengths of zero ("amb-") were collapsed to form polytomies.

For the results based on both separate and combined data, the robustness of individual clades was evaluated using jackknife with 37% deletion (Farris 1996) as implemented in PAUP*, and decay index (Bremer 1988, Donoghue et al. 1992). Decay analyses were performed with AutoDecay (Eriksson and Wikström 1996) and trees were viewed with TREEVIEW (Page 1996).

A monophyletic *Swertia* topology was used as constraint tree in PAUP*. Most parsimonious trees found under this condition were compared with the unconstrained topologies using Templeton's significantly less parsimonious (SLP) test (Templeton 1983).

Data combination and congruence testing. By increasing the number of characters in an analysis, phylogenetic signal may assert itself over the noise (coincidental similarity due to homoplasy) from each individual data set, resulting in a more accurate estimate of the true phylogeny (Barrett et al. 1991, Mishler 1994, Olmstead and Sweere 1994). However, heterogeneity among data sets other than sampling error, such as different stochastic processes acting on the characters or different branching histories, can lead to erroneous phylogenetic inferences. Under such a circumstance it is justified to keep the data sets separate or to use a consensus method (Bull et al. 1993, de Queiroz et al. 1995). Following the conditional data combination approach (Huelsenbeck et al. 1996), we have used the incongruence length difference (ILD) test of Farris et al. (1995) (with 100 replicates and same settings as for other analyses) as implemented in PAUP* to assess the character congruence between the different data partitions (ITS1, ITS2, *trn*L, IGS *trn*L-F, IGS *trn*S-*ycf*9).

Results

Characteristics of ITS sequences. The spacer length ranges from 173 to 237 bases for ITS1 and from 226 to 232 bases for ITS2. When aligned, the consensus sequences have 268 and 267 sites for ITS1 and ITS2, respectively. Out of these, 6 were excluded from ITS1 and 16 from ITS2 because of alignment ambiguities, leaving respectively 116 and 113 informative sites. ITS1 sequences were found to be slightly less divergent (1%–25%) than ITS2 (1%–28%). Among species of *Swertia*, sequence divergence ranges from 0.5% up to 16%, whereas this difference can be much lower than between some *Swertia* species and species of markedly different genera like *Gentianella* and *Megacodon*. At 152 sites, gaps were needed to accommodate this relatively high

divergence, and only one potentially informative indel larger than 1 nucleotide was found.

ITS phylogeny. The parsimony analysis carried out using PAUP* resulted in 70 equally parsimonious phylogenetic trees of 980 steps (CI = 0.50, RI = 0.53 including autapomorphies). Their strict consensus is presented in Fig. 1a. The single possibly informative indel situated near the end of ITS2 has no influence on the resulting trees. Although relatively low, the ILD test value (p = 0.1) indicates that ITS1 and ITS2 are not significantly incongruent. Separate analysis of ITS1 and ITS2 offered a very poor resolution, high homoplasy indices and received no jackknife support. Some branches are exclusively found in the analysis of one or the other data set only and, in view of their lack of support, are likely to be due to

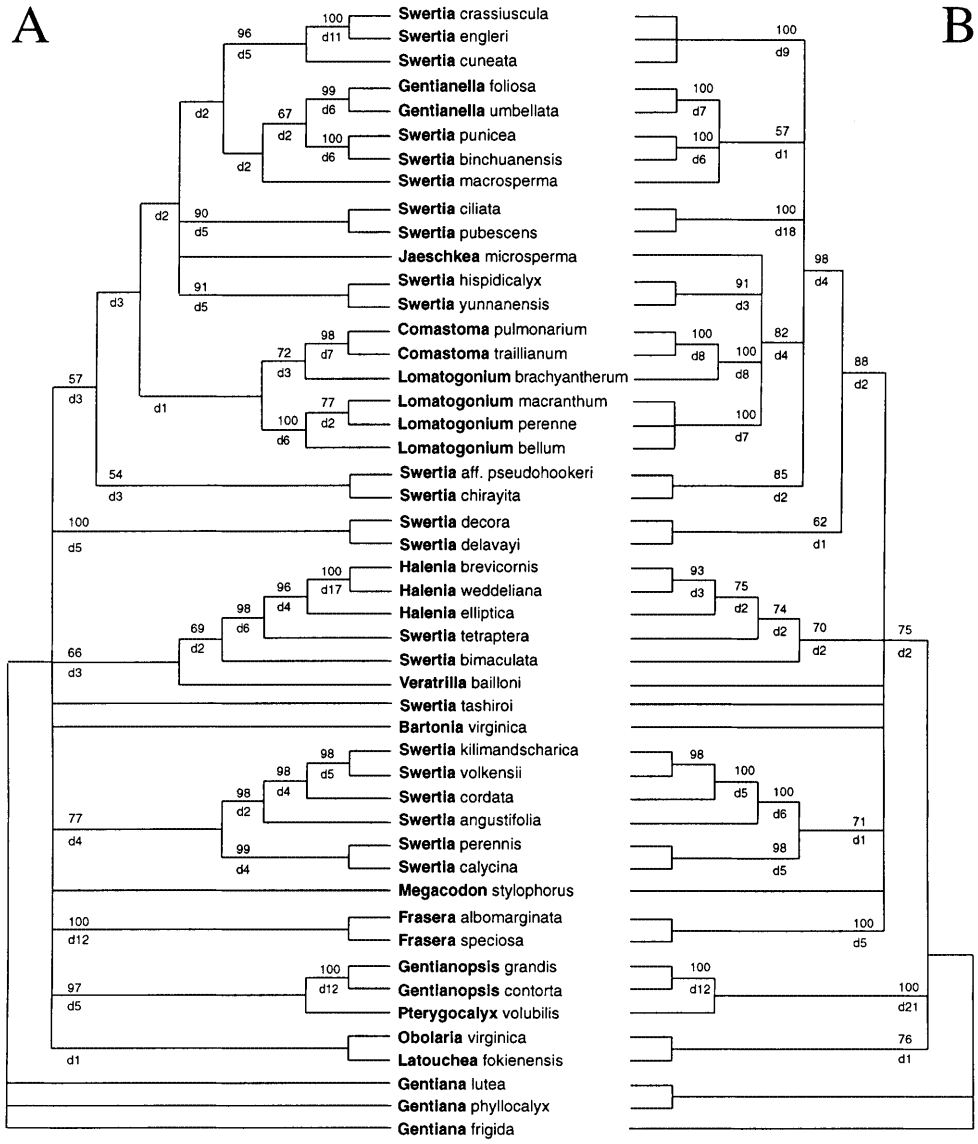

Fig. 1. Strict consensus trees of **A** 70 most parsimonious trees of 980 steps, CI = 0.50, RI = 0.53 (including autapomorphies) from nuclear DNA analysis; **B** 24 most parsimonious trees of 659 steps, CI = 0.75, RI = 0.77 (including autapomorphies) from chloroplast DNA analysis. Above branches: jackknife values if greater than 50%. Below branches: decay index

random error and may in turn account for the low ILD test value.

The ITS phylogeny presents an important polytomy at its base, that consists of several well supported clades. *Latouchea* forms a sister-group relationship with *Obolaria*. *Gentianopsis* and *Pterygocalyx* are strongly associated, as well as the two sampled species of *Frasera*, that join in an independent clade with high support. *Megacodon* forms an isolated branch, as does *Bartonia*. *Veratrilla* shows a sister group relationship to the *Halenia – Swertia* clade with moderate jackknife value (66%) and decay index (d = 3). *Swertia* is present pro parte in this basal polytomy, notably in the well-supported clade containing the type of the genus, *S. perennis*. Two species (*S. bimaculata* and *S. tetraptera*) show a close link with *Halenia*. *Swertia tashiroi* does not group with any other *Swertia* species present at the base of the tree. *Swertia decora* and *S. delavayi* also form a highly supported clade.

The upper part of the tree is better resolved, although jackknife support is below 50% for deeper nodes, with at its base the moderately supported clade of *S.* aff. *pseudohookeri – S. chirayita*. The *Lomatogonium – Comastoma* clade lacks support as a whole, but *L. brachyantherum* is associated to *Comastoma* with good jackknife and decay values. *Jaeschkea* is placed in a polytomy with the *S. ciliata – S. pubescens* clade and the *S. yunnanensis – S. hispidicalyx* clade. *Gentianella* species are more closely related to *Swertia* than to *Comastoma*, *Lomatogonium* or *Gentianopsis*. Within the uppermost *Swertia* clade, they formed a monophyletic sister taxon to *S. punicea* and *S. binchuanensis* with a jackknife and decay index support of 67% and 2, respectively.

Characteristics of chloroplast sequences. The chloroplast sequence length ranges from 361 to 393 bases for the *trn*L intron, from 234 to 403 bases for the IGS *trn*L-F and from 271 to 350 bases for the IGS *trn*S-*ycf*9. This variation is mainly due to long A/T repeats in *trn*L, and to indels in the two IGS's. When aligned, the sequences have 496, 678 and 474 sites for *trn*L, *trn*L-F and *trn*S-*ycf*9, respectively. Out of these, 90 were excluded from *trn*L, 245 from *trn*L-F and 21 from *trn*S-*ycf*9 because of alignment ambiguities, leaving respectively 59, 83 and 76 informative sites. *trn*L-F sequences were found to be slightly more divergent (0%–19%) than *trn*L (0%–12%) and *trn*S-*ycf*9 (0%–11%). When the three data sets are combined, the sequence divergence ranges from 0% to 14%. As noticed in ITS, chloroplast sequence divergence between *Swertia* species and other genera can be much lower than between two different species of *Swertia*. 32 potentially informative indels of more than 1 nucleotide were recoded and added to the data matrix (5 from *trn*L, 14 from IGS *trn*L-F and 13 from IGS *trn*S-*ycf*9).

***Trn*L intron, *trn*L-F and *trn*S-*ycf*9 intergenic spacer phylogeny.** The parsimony analysis of the chloroplast data resulted in 24 trees of 659 steps (CI = 0.75, RI = 0.77 including autapomorphies). The three chloroplast partitions were found to be congruent with p = 0.42. Figure 1b presents the strict consensus of the 24 most parsimonious trees, which offers a moderate degree of resolution. The basal polytomy suggested by the ITS data is partly resolved by the chloroplast sequences. The *Gentianopsis – Pterygocalyx* clade and the *Obolaria – Latouchea* clade appear at the base of the tree, with a jackknife value of 75% and a decay index of 2 supporting their segregation from the remaining taxa. Also, the *Swertia decora – S. delavayi* clade is excluded from this polytomy with good support. For the remaining taxa, the branching is identical to the one found by the analysis of ITS data, with the exception of *Veratrilla*, that is not placed inside the *Swertia – Halenia* clade.

The upper part of the tree consists of a large pentatomy, in which the individual clades are well supported. Here again, but with less resolution, the branching is similar to the ITS phylogeny, with the exception of *Jaeschkea* and the *S. yunnanensis – S. hispidicalyx* clade, that are included in a well supported clade along with *Lomatogonium* and *Comastoma*. As in the

ITS phylogeny, *L. brachyantherum* forms a sister group relationship with *Comastoma* species, although this clade does not group with the remainder of *Lomatogonium* species.

Combined nuclear and chloroplast DNA phylogeny. The ILD test probability was 0.54 in favour of homogeneity between nuclear and chloroplast data. All branches whose position differs across the trees received low statistical support in at least one tree, or formed a polytomy.

The analysis of equally weighted characters yielded 48 trees of 1649 steps (CI = 0.60, RI = 0.62). This represents only 10 more steps than the sum of the most parsimonious trees obtained by separate analysis of nuclear and chloroplast data. Figure 2 presents their strict consensus.

Statistical support is globally increased, not only for clades recognised independently from each data set, but also for the deeper nodes in the upper half of the tree. The phylogeny inferred from the combined data sets presents a different branching pattern than the ones obtained from separate analysis. In the combined tree, *Obolaria* and *Latouchea* are the basalmost taxa, as suggested by the chloroplast tree, whereas *Gentianopsis* and *Pterygocalyx* are placed in the large basal polytomy. Data combination has brought no further resolution of this polytomy, except for the grouping of *Bartonia* and *S. tashiroi*. The position retained for *Veratrilla* is the same as in the ITS tree. *Swertia decora* and *S. delavayi* are placed according to the chloroplast tree, whereas the position of *S. chirayita* and *S.* aff. *pseudohookeri* follows the ITS tree. *Jaeschkea* and the *S. hispidicalyx – S. yunnanensis* clade group with *Comastoma* and *Lomatogonium* as it is the case in the chloroplast phylogeny. The position of *Gentianella* species as sister group to *S. binchuanensis S. punicea* remains unchanged. The *S. crassiuscula – S. macrosperma* clade, present but poorly supported in the ITS tree, and not detected in the chloroplast tree, has a good jackknife support in the combined phylogeny.

The phylogenetic relationships resolved in these analyses confirm previous molecular studies suggesting the paraphyly of *Swertia* (Yuan and Küpfer 1995, von Hagen and Kadereit 2001). This idea has been further investigated by searching for trees compatible with a constrained monophyletic *Swertia* clade. Such trees are 31 steps longer (ITS), 16 steps longer (chloroplast data), and 46 steps longer (combined data) than the unconstrained most parsimonious trees, clearly departing from the notion of sub-optimality ($p < 0.1$ in SLP test for all data sets).

Discussion

Phylogeny of Swertiinae and *Swertia*. The potential difficulties inherent to the distinction between a gene tree and species phylogeny were explored in detail (e.g. Doyle 1992). Combining data sets increases the phylogenetic signal, which may in turn increase support for the "correct" phylogenetic tree (Bremer et al. 1999, Hardig et al. 2000, von Hagen and Kadereit 2001). The resolution of the internal clades and high jackknife values due to the analyses of the combined data sets in the *Gentianella*-lineage suggests that this is also the case in our study. The tree derived from the analysis of combined data sets was thus used as final phylogenetic hypothesis.

Swertia, as currently defined in regional floras or otherwise (Pringle 1990, 1993; Shah 1990, 1992; Ho and Pringle 1995), is not a monophyletic taxon when considering the molecular data. It is a strongly paraphyletic stem group, and the data suggest that different ancestral taxa or lineages of *Swertia* account for the origin of several morphologically different genera of the *Gentianella*-lineage. Nevertheless, despite the use of 5 different markers, parsimony analysis of our data still leaves two major polytomies unresolved.

Phylogenetic relationships in the *Gentianella*-lineage have been addressed previously (Yuan and Küpfer 1995, Struwe et al. 1998, von Hagen and Kadereit 2001), yet, only few of the concerned taxa were included, and the phylogenies were based on fewer markers. These studies differ from our results notably

by proposing additional resolution for some branches included in the polytomies of our tree, although these relationships receive only low statistical support. Whether the lack of synapomorphies that would allow resolving these polytomies is due to a rapid geographic isolation of these lineages is not clear. Nevertheless, it can be noted that many of the basal

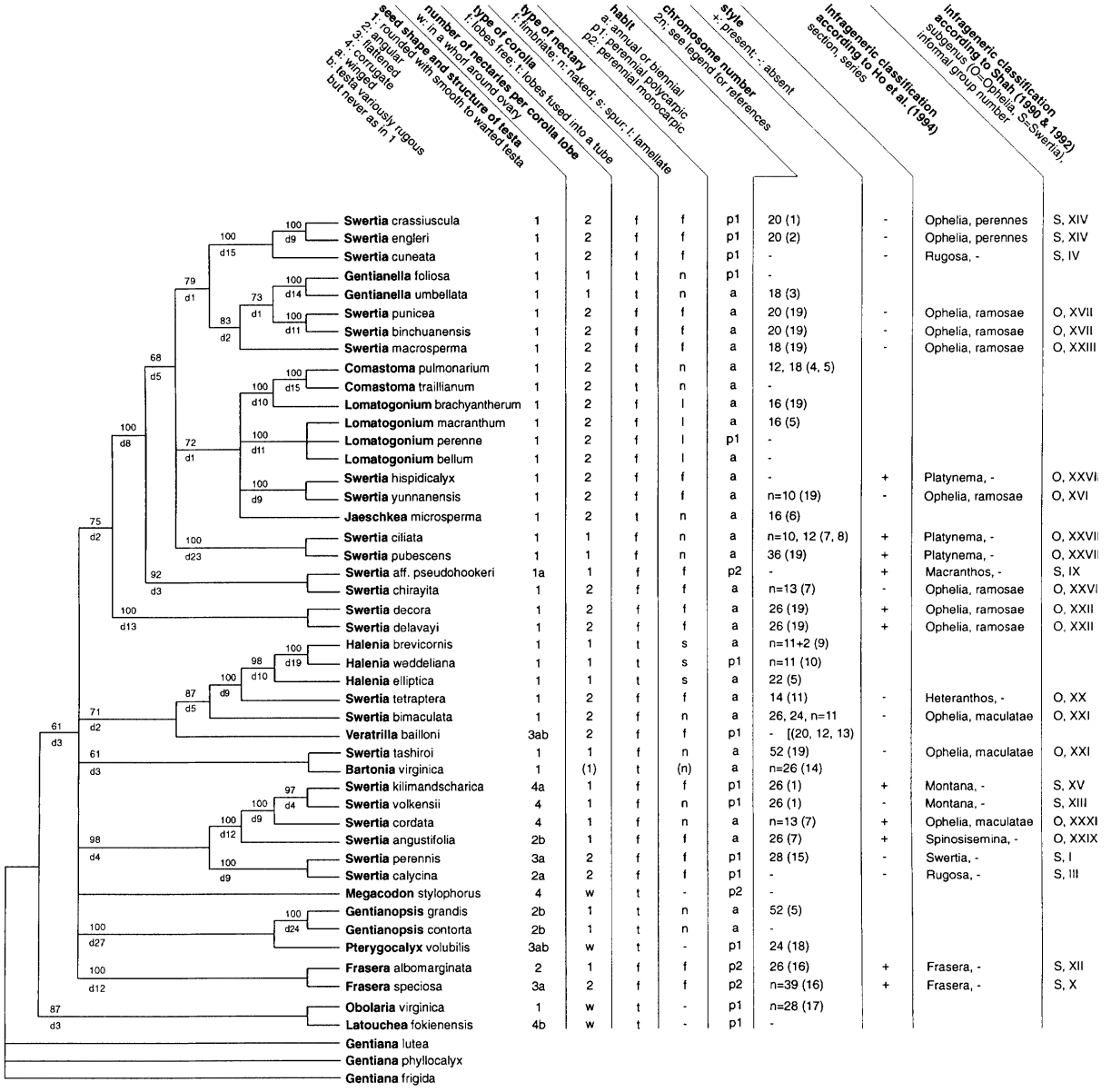

Fig. 2. Strict consensus tree of 48 most parsimonious trees of 1649 steps, CI = 0.60, RI = 0.62 (including autapomorphies) from analysis of combined data. Above branches: jackknife values if greater than 50%. Below branches: decay index. In regard: states for the principal characters discussed in the text. References for chromosome numbers: (1) Hedberg and Hedberg (1977), (2) Nemomissa (1994), (3) Gagnidze et al. (1992), (4) Krogulevitch (1978), (5) Yuan and Küpfer (1993), (6) Yuan et al. (1998), (7) Khoshoo and Tandon (1963), (8) Mehra and Gill (1968), (9) Weaver and Rudenberg (1975), (10) Pringle (1981), (11) Ho et al. (1999), (12) Shigenobu (1983), (13) Roy et al. (1988), (14) Rork (1949), (15) Favarger (1952), (16) Post (1956), (17) Kondo (1970), (18) Yuan and Küpfer (unpublished), (19) present study, (20) Wada (1954)

clades consist of geographically isolated taxa, and/or genera represented by only a few species. *Swertia tashiroi* is an insular endemic in Taiwan and the south-western islands of Japan. Its fleshy habit and chromosome number ($2n = 52$) distinguish it from all the other *Swertia* species included in the basal polytomy. *Megacodon* is a distinct genus comprising only two species restricted to the eastern Himalayas. *Veratrilla* is morphologically very close to *Swertia* but its two species are clearly dioecious, a very rare trait in the *Gentianella*-lineage. *Latouchea* (1 species) is endemic to south-eastern and south-western China. *Bartonia* (4 species) and *Obolaria* (1 species) are North American saprophytes. *Frasera* (14 species) is also restricted to North America and, despite an indubitable resemblance with *Swertia*, seems to have differentiated independently from the remaining *Swertia* species.

As suggested in Yuan and Küpfer's (1995) ITS tree and von Hagen and Kadereit's (2001) *mat*K/ITS tree, *Comastoma* and *Lomatogonium* also group together in our phylogeny, but in a broader clade including *Swertia* pro parte and *Jaeschkea*. *Lomatogonium* was found to be polyphyletic by von Hagen and Kadereit (2001) and our results also suggest its paraphyly in relation to *Comastoma*. *Lomatogonium brachyantherum* has none of the characteristics of *Comastoma* (corolla lobes fused in a tube with fringed scale at its base). The scenario that such an observed clustering of *L. brachyantherum* with *Comastoma* has its root in an erroneous definition of the taxon is ruled out. The *Gentianella* species form a clade corresponding to the *Gentianella* s. str. (uninectariate) clade of von Hagen and Kadereit (2001) with the same *Swertia* species as sister clade. Binectariate *Gentianella* species are absent from our taxon sample but were placed clearly outside of the *Gentianella* s. str. clade and close to *Lomatogonium* species in von Hagen and Kadereit's (2001) study. Note that *Jaeschkea microsperma* is included in the broad *Lomatogonium* – *Comastoma* – *Swertia* clade in our combined phylogeny, whereas *J. oligosperma* was found to be clearly separated from the former two genera in von Hagen and Kadereit's (2001) *mat*K/ITS tree.

Morphology and molecular data. The *Gentianella*-lineage is an assemblage of genera that exhibit intrageneric polymorphism on one hand, and unprecedented morphological ties with each other on the other. The presence of corolline nectaries, regardless of the fine details, is one major feature shared by nearly all genera and has been emphasised as representing a different line of evolution in subtribe Gentianinae (Meszaros 1994). According to Ho and Pringle (1995), nectaries are sometimes absent in *Pterygocalyx*. *Megacodon*, *Latouchea* and *Obolaria* are different by bearing nectaries in a whorl at the base of the ovary like in *Gentiana*. Regarding *Obolaria*, Holm (1897) states that "the corolla bears nectaries", and describes a scale halfway between the base of the filament and the base of the corolla tube. He then adds that "the grooves are very imperfect". However, Lindsey (1940) reports glandular material at the base of the ovary. Wood and Weaver (1982) believe that the latter are more likely the nectaries. Concerning *Bartonia*, neither Wood and Weaver (1982) nor Holm (1907) mention nectaries. A recent collection of *B. Virginica* was examined by the authors. The basal part of the corolla lobes was found to be thickened and of brownish-green colour. It was not possible to assess if this tissue is glandular but it is reminiscent of the nectariferous tissue found in *S. tashiroi* or *S. bimaculata*.

Corolline nectaries are either fimbriate or naked, with or without well-developed marginal scales, or even sunken in spurs as in *Halenia*. The nectary features such as number, as well as their nakedness or fimbriation were used for the segregation of genera. But in regard to the molecular phylogeny, nectaries cannot be considered as reliable synapomorphic characters at the generic level. Their number may be identical between two distantly related clades, and, similarly, naked nectaries characterise distantly related genera like *Bartonia* (?), *Comastoma*, *Gentianella*, *Gentianopsis*, *Pterygocalyx* as well as some species of *Swertia* included in this study, e.g.

S. bimaculata, *S. volkensii* and *S. tashiroi*. Fimbriate nectaries are found in *Frasera*, *Veratrilla* and *Swertia*, where the fimbriae can also occur in association with a more or less developed marginal scale. Spurs however are restricted to *Halenia* sect. *Haleniastrum* (Allen 1933). At the infrageneric level however, von Hagen and Kadereit (2001) have shown that nectary number and fimbriation are representative of phylogenetic relationships in *Gentianella*. Such a pattern is not found for *Swertia* species.

Little has been published on the pollination biology of *Swertia* and allied genera. In their observations, Khoshoo and Tandon (1963) note that even in mixed populations of cross-pollinated *Swertia*, nearly each studied species has its specific pollinator and that the shape and fimbriation of the nectaries seem to play an important role in the pollinator specificity. Under such pollinator-mediated selective pressure, both fimbriate and naked nectaries may have evolved several times and been acquired independently in the different lineages of the *Gentianella*-lineage from plesiomorphic whorled nectaries situated around the ovary as is seen in *Latouchea*, *Megacodon* and *Obolaria*. To be noted, is that in a majority of cases, the nectary itself is devoid of fimbriae in those taxa where it is not readily accessible, i.e. when the corolla lobes are fused (*Bartonia* (?), *Comastoma*, *Gentianella*, *Gentianopsis*, *Jaeschkea*), or when hidden inside a spur as in *Halenia* sect. *Haleniastrum* (Allen 1933). Similarly, a parallel tendency towards conspicuous fimbriation can be seen with the vascularised fimbriae at the base of the corolla lobes in *Gentianella* and the comparable but nonvascularised structure in *Comastoma*. In *Gentianopsis*, fimbriae are absent, but the petals are fringed in many species.

When viewed in the context of the molecular phylogeny, the character state of seed shape and ornamentation doesn't appear as representative of relationships between genera. Many unrelated taxa have smooth rounded seeds, and, similarly, *Veratrilla bailloni*, *Swertia perennis* and *Frasera speciosa* for example all have flat annular winged seeds. Nevertheless, seeds with well-developed ridges, wings or coarsely papillose, and not rounded in shape occur only in basal lineages, i.e. *Latouchea*, *Pterygocalyx*, *Gentianopsis*, *Frasera*, *Megacodon*, *S. perennis – S. kilimandscharica* clade, *Veratrilla*. Concerning the sampled species of African *Swertia*, this character seems to be congruent with the molecular data and confirms the grouping proposed by Nemomissa (1994). For *Swertia* as a whole, it can be noted that seeds in the *S. perennis – S. kilimandscharica* clade are different from those of all other species. They are either corrugate-cristate (*S. volkensii*, *S. calycina*, *S. cordata*), polyhedral with spinose projections (*S. angustifolia*) or flat with an annular wing (*S. perennis*). All other species of *Swertia* have seeds more or less rounded, with a smooth to warted testa (rarely small wings in the *S.* aff. *pseudohookeri* group). Future investigation of a broader taxon sample will allow assessing with more precision the reliability of this character.

Molecular results were also compared with the detailed pollen studies carried out by Nilsson (1964, 1967, 1970), Nilsson and Skvarla (1969) and Jonsson (1973). According to Nilsson, pollen morphological data are difficult to interpret due to similar pollen types in unrelated taxa throughout the *Gentianella*-lineage, and also to transitional sexine patterns when more material is investigated. Only few suggestions regarding inter-generic pollen morphological affinities were made. Nilsson's remark that the pollen structure of *Comastoma* and *Gentianopsis*/*Pterygocalyx* is clearly different from that of *Gentianella* is supported by our tree, as well as the affinity of *Comastoma* with *Lomatogonium*. Likewise, the pollen of *Frasera* is different from that of most other *Swertia* species. On the other hand, the similarity between the pollen of *Halenia* and *Lomatogonium*/*Comastoma* is not congruent with the molecular data. Concerning *Swertia*, Nilsson treated the species according to their geographic distribution and only made comments on a few interesting taxa. The African species are divided in 3 groups. In the *S. crassiuscula* type, the exine is beset with spinules.

The distinction between the other two groups (*S. kilimandscharica* and *S. volkensii* types) is less marked, and there are a number of species with transitional pollen morphology (Jonsson 1973), but both types are devoid of spinules. This is congruent with the molecular tree. Regarding the Asiatic species, such a clear correlation between Nilsson's five pollen types and our tree cannot be found. Spinuliferous exine seems to be a good character in discriminating the two African clades, but in the context of the whole *Gentianella*-lineage, this trait appears to be homoplasious. Beside the African species of the *S. crassiuscula* type, spinules are found in *S. cuneata*, the only Himalayan species belonging to the African *S. crassiuscula* clade, *S. tashiroi* and *Lomatogonium* p.p.

The presence of a style has been used as a distinguishing feature in the segregation of *Frasera* from *Swertia* by Bentham and Hooker (1876) and has been commonly retained by later authors as a diagnostic character of the taxon. At that time, little was known of the global variability of *Swertia*, but it is now evident that styled stigmata are found in many *Swertia* species. Besides, as Pringle (1990) mentions for *Swertia perennis* and *Frasera*, stylar differences are "a matter of relative length rather than being qualitative". When viewed in regard to the molecular tree, it is clear that this trait does not reflect phylogenetic relationships in the genus.

Another morphological marker worth considering is the fusion of corolla lobes to form a tube. Although petals are fused in all the taxa of the *Gentianella*-lineage, the tube is nearly inconspicuous in *Frasera*, *Lomatogonium*, *Swertia* and *Veratrilla*, in contrast to the other genera where it is well developed. This floral trait has significantly contributed to the confusion in the taxonomy of the *Gentianella*-lineage. As shown by the phylogenetic tree, corolla tubes have appeared and disappeared several times in the group. Therefore, phylogenetic inferences and subsequent grouping based on the extent of corolla tube formation may be misleading.

Ho et al. (1994) have suggested a polarity in the evolution of morphological characters for the purpose of phylogeographic hypotheses in *Swertia*. They define ancestral characters as tall, perennial plants with a solitary stem, with few, rather large pentamerous flowers and corrugate-cristate seeds (sect. *Rugosae*). More advanced species would be rather small annuals with many tetramerous flowers borne on branched stems, with smooth to warted, rounded seeds. A comparison with our phylogeny calls for the following remarks. The (perennial versus) annual habit (of the species) seems to be polyphyletic and reflects more ecological requirements than phylogenetic relationships. There are two examples in the tree where perennials and annuals occur in the same clade. *Swertia calycina* and related species are all perennial and mainly of alpine ecology. These species are closely related to annual species of the *S. angustifolia* type that occur at lower elevations. The same pattern is valid for the *S.* aff. *pseudohookeri* (perennial) and *S. chirayita* (annual) groups of species. As discussed above, the character state of rugose or winged seeds does seem to be partly synapomorphic with respect to our phylogeny and proper to the basal *S. calycina* – *S. kilimandscharica* lineage of *Swertia*.

Cytological data and taxonomy. As pointed out by Pringle (1990), earlier taxonomic definitions and phyletic hypotheses based on chromosome counts of a restrained number of species of the *Swertia* complex have not been confirmed by the increasing number of counts of new taxa. Many species in the *Gentianella*-lineage have been recently studied cytologically, giving rise to new considerations (summarised by Yuan 1996 and Pringle 1990). Concerning *Swertia* s. l., Pringle notes that there are two prevailing base numbers, i.e. $x = 13$ and $x = 10$, with a few aneuploid and polyploid species in both groups. This is congruent with our results, where all species from *S. pubescens* upward in the tree have $n = 9$, 10, 18, all others sharing $n = 13$, 14, 26. Only *S. tetraptera* ($n = 7$) (Ho et al. 1999) departs from this picture. More generally, and despite the lack of data for

several genera, x = 13 seems to be common in the basal lineages, although there are a few variations around this number. The paucity of species investigated in the more derived clades (*S. pubescens* upward and particularly *Lomatogonium* and *Comastoma*), coupled with a greater variability of chromosome numbers still keeps us from understanding karyological relationships. Yuan and Küpfer's (1993) suggestion that *Comastoma* (x = 8, 9) shows more affinity with *Lomatogonium* (x = 8) than with *Gentianella* (x = 9) from a karyological and morphological point of view is also supported by our phylogeny. The position of *Jaeschkea* (n = 8) close to *Lomatogonium* and *Comastoma* is also congruent with the karyology, although different chromosome numbers have been reported for other *Jaeschkea* species (n = 9, 11 for *J. oligosperma* (as *J. gentianoides*) (Gohil et al. 1981, Koul and Gohil 1973) and n = 10 for *J. canaliculata* (as *J. latisepala*) (Mehra and Vasudevan 1972, Vasudevan 1975)).

The importance of polyploidy associated to dysploidy and/or hybridisation has been stressed in the explanation of the karyotype evolution of *Gentianopsis* and particularly of *Gentiana* (Yuan and Küpfer 1993, Yuan 1996). Several examples of polyploidy can be found in our phylogeny (e.g. *Bartonia*, *Frasera speciosa*, *Obolaria*, *Swertia tashiroi*, S. *pubescens*). Cases of up dysploidy are found in *S. perennis* and *Obolaria*. Down dysploidy seems to be more frequent, e.g. *Pterygocalyx*, *S. bimaculata*, *Halenia* spp., *S. pubescens*. The case of *S. tetraptera* illustrates the importance of this factor in the karyological evolution of the Swertiinae. Its position in the phylogeny suggests that it is derived from an ancestor with n = 13 and that dysploidy may have affected up to 12 chromosomes during the evolution of this taxon. In regard to the molecular phylogeny, it seems that parallel evolution of karyotypes due to the aforementioned factors may have led to similar chromosome numbers in unrelated lineages, e.g. between *Gentianella* spp. (n = 9, 18) and *S. pubescens* (n = 18), or between *Halenia* spp. (n = 11) and *Gentianopsis ciliata* (n = 22) (Favarger 1949), at different ploidy levels.

Systematics and the definition of *Swertia*. In regard of the molecular phylogeny, most of the genera in the *Gentianella*-lineage seem to be monophyletic and relatively well circumscribed. This is not the case for *Lomatogonium*, that could be paraphyletic towards *Comastoma*, or for *Swertia*. The latter seems to have acted as a stem group, giving rise to different lineages, some of which eventually differentiating enough from the typical swertioid aspect as to be recognised at a generic level, e.g. *Halenia*, *Gentianella*, while other lineages exhibit nearly no variation.

The absence of clear morphological differences between lineages of *Swertia*, combined with parallel evolution of traits commonly referred to in the taxonomy of the genus has contributed much to the profusion of speculative controversies among different authors regarding its generic segregates. In his monograph, Shah (1990, 1992) notes that many earlier synonyms were published by authors unaware of previous descriptions or unfamiliar with the global morphological variability exhibited by species on other continents. He further remarks that erroneous descriptions of type material has also contributed to misleading taxonomic treatments. Although most present day authors agree to consider *Swertia* as a large genus including all previous segregates, some synonyms are still debated and pertinent to the molecular phylogeny. These are *Anagallidium*, *Frasera* and *Ophelia*, and shall be briefly discussed here. Grisebach (1836) separated *S. dichotoma* and *S. tetrapetala* to the genus *Anagallidium*. Later, Ma (1976) also placed *S. tetraptera* in this genus. Even if there are no reliable characters in Grisebach's generic diagnosis, these three species are taxonomically closely related (Shah 1992). Embryological studies in *S. tetraptera* (Xue et al. 1999) reveal more affinities with *Halenia* than with *Swertia*, and the position of *S. tetraptera* in our phylogeny suggests that *Anagallidium* might be a justified taxon, although its proper rank and its relation to *S. bimaculata* may be difficult to determine. The status of *Frasera* has been widely debated,

although no reliable character has ever been brought up to justify its separation from *Swertia* (Pringle 1990). Nilsson's studies indicate that the species of *Frasera* may have a monophyletic origin. Only two species are included in our tree, but preliminary results including all 14 species (not shown) support the monophyly of this lineage without a close link to any other *Swertia* species, contrarily to Toyokuni's (1965) suggestions. At present, this taxon is generally regarded as a section of *Swertia* (Pringle 1990) but its position in our phylogeny at the same level as well differentiated genera might favour its recognition at a generic rank, a position also adopted by Struwe et al. (unpubl. data). The definition and taxonomic rank of *Ophelia* have varied depending on the authors. At present, the character referred to for treating *Ophelia* as a distinct taxon under *Swertia* is the annual habit of the plants (excepted sect. *Ophelia* ser. *perennes* in Ho et al. 1994). Our phylogeny clearly suggests that the annual or perennial habit of the species is not representative of relationships in *Swertia*, neither at a generic level, nor at a sectional level.

A number of local infrageneric classifications have been attempted for *Swertia* (see Nemomissa (1994) for a review), but only three publications offer a treatment of the whole genus (Shah 1990, 1992; Ho et al. 1994). Shah divided *Swertia* into two subgenera (*Swertia* and *Ophelia*) and proposed 35 informal groups according to 1) geographical distribution, and 2) morphological similarities. Ho et al. proposed 11 sections and 16 series based on the hypothetical evolutionary polarity of morphological characters. When compared, these two classifications are contradictory in many ways, that is, species in one of Shah's groups are placed in different series by Ho et al. and vice versa. Figure 2 shows the position of species in the subdivisions of Shah and Ho et al. respectively. Both classifications are not congruent with the molecular data. The subdivisions of *Swertia* according to the perennial versus annual habit of the species in Shah represents the main incongruence. For the rest, with 35 unranked groups for ca. 150 species, Shah was aware not only of the variability within *Swertia* but also of the hypothetical nature of relationships based on morphology alone. The classification of Ho et al. contrasts with our phylogeny in several ways. The sectional rank seems to be overestimated in some cases, especially in the *S. perennis* – *S. kilimandscharica* clade, with 5 sections represented. Another incongruence is to be found in sect. *Ophelia*, which is present in 8 different clades in our tree. Furthermore the sect. *Ophelia* itself is divided in three series, that are also not congruent with the molecular data. Besides, the hypothesis of Ho et al. that African species are monophyletic and derived from *Ophelia* is not supported. According to our results, the African species of *Swertia* represent two distinct lineages (*S. crassiuscula* clade, *S. kilimandscharica* clade). Problems concerning the definition and circumscription of *Ophelia* are also due to the absence of a clearly designated type by either D. Don (1836), or by Grisebach (1836). Garg (1987) proposed *S. ciliata* as lectotype. In the chinese edition of the Flora of China, Ho et al. (1988) give *S. angustifolia* as type of sect. *Ophelia*. Note that *S. angustifolia* perfectly fits the description of sect. *Spinosisemina* published later by Ho et al. (1994).

In order to elaborate a satisfactory classification of *Swertia* and allied genera, and to better understand the phylogeography of this group, it seems necessary to first establish clear relationships between taxa and to identify reliable morphological characters correlated with the paraphyletic *Swertia* clades identified in the phylogeny. Discussing biogeographic issues and character polarisation section by section as attempted by Ho et al. (1994) is not convincing in absence of the aforementioned conditions. A phylogenetic analysis of a much wider taxon sampling (ca. 150 species) is underway in our laboratory and should allow proposing a comprehensive taxonomic treatment reflecting evolutionary relationships

within the *Gentianella*-lineage as well as new phylogeographic hypotheses.

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Addresses of the authors: Philippe Chassot (correspondence), Yong-Ming Yuan, Philippe Küpfer, Institut de Botanique, Laboratoire de Phanérogamie, Université de Neuchâtel, Emile-Argand 11, CH-2007 Neuchâtel 7, Suisse (E-mail: philippe.chassot@bota.unine.ch). Sileshi Nemomissa, The National Herbarium, Biology Department, Addis Ababa University, P.O. Box 3434, Addis Ababa, Ethiopia (E-mail: sileshin@hotmail.com).

II

Chromosomal evolution in the Swertiinae (Gentianaceae – Gentianeae)

with karyological observations on 60 species

P. Chassot, Y.-M. Yuan & P. Küpfer

Abstract. Recent molecular studies in the subtribe Swertiinae (Gentianaceae – Gentianeae) have revealed phylogenetic relationships markedly at contradiction with current generic circumscriptions. To compare these results with karyological data, we counted the chromosome numbers for 60 species and reviewed the data available from literature for 171 species. Chromosome numbers were confirmed for 25 species, and numbers differing from previous reports were found for 10 species. 25 species were examined for the first time, mainly in the genera *Swertia* and *Lomatogonium*. Eight numbers were new for a genus, i. e. the tetraploid level (2n = 52) for *Frasera*, 2n = 10, 12, 16 and 38 for *Gentianella*, 2n = 24 for *Pterygocalyx*, and 2n = 12 and 42 for *Swertia*. Chromosome numbers were also counted for several taxa not related to the Swertiinae (6 species of *Gentiana*, *Tapeinostemon zamoranum* and *Microrphium pubescens*). Polyploidy was found to affect mainly genera that have diversified in North America, such as *Bartonia* (4x), *Frasera* (2x, 4x, 6x), and *Obolaria* (4x), or in South America, such as *Gentianella* (2x, 4x, 6x). Polyploidy is absent from Africa, and rare in Asia. Primary basic chromosome numbers in the Swertiinae range from x = 5 to x = 15, the most frequent being x = 8, 9, 10 and 13, while 2 species only have the secondary basic numbers x = 19 and 21. The variability in basic chromosome numbers is concentrated in *Comastoma*, *Lomatogonium*, *Jaeschkea*, binectariate species of *Gentianella*, and *Swertia*, a genus which shows a level of variation nearly equal to that of the entire subtribe. The basic numbers x = 13 or x = 14 could be ancestral in the subtribe, and lower basic numbers are likely to be derived from x = 13. Species sharing the basic numbers x = 13 or x = 14 were found to be restricted to the basal lineages in the molecular phylogeny of the subtribe, while lower basic chromosome numbers characterise species occupying a more derive position. Frequent dysploidy in the latter derived lineages often resulted in overlapping chromosome numbers in distantly related taxa, whereas occasional severe descending dysploidy may have brought about rather different chromosome numbers in closely related species. Of the various lineages identified in molecular phylogenetic studies, none confines to a unique and distinctive chromosome number. However, the relatively clear-cut cleavage between basal taxa of *Swertia* with x = 13 or 14, and those with a lower basic chromosome number is consistent with molecular phylogenetic results, and may be insightful for taxonomic considerations in this genus.

Key words: cytoevolution, cytology, dysploidy, Gentianaceae, karyology, polyploidy, Swertiinae.

Introduction

The subtribe Swertiinae (Gentianaceae, tribe *Gentianeae*) includes 15 genera representing over 500 species distributed in temperate, alpine or arctic habitats. According to Struwe *et al.* (2002), half of these genera count no more than 4 species, e. g. *Bartonia* Muhl (4 spp.), *Jaeschkea* Kurz (4 spp.), *Latouchea* Franch. (1 sp.), *Lomatogoniopsis* T. N. Ho & S. W. Liu (3 spp.), *Megacodon* (Hemsl.) Harry Sm. (2 spp.), *Obolaria* L. (1 sp.), *Pterygocalyx* (1 sp.), *Veratrilla* Baill. (2 spp.). *Comastoma* Toyokuni, *Frasera* Walter, *Gentianopsis* Ma and *Lomatogonium* A. Braun are of intermediate size with 14-25 species. The wide majority of species of the subtribe are encompassed by *Gentianella* Moench (ca. 250 spp.), *Halenia* Borkh. (ca. 80 spp.) and *Swertia* L. (ca. 138 spp.). However, generic circumscription is vague in the subtribe Swertiinae, and segregates have been abundantly proposed (see Chassot *et al.*, 2001 and Hagen & Kadereit, 2001 for examples). Most of the genera have been delimited with floral morphological characters such as position and structure of the nectaries and their appendages, presence or absence of a corolla tube, stylar and staminal traits, etc. All these characters are likely to undergo strong selection and mask affinities between species (Wagenitz, 1997). Moreover, recent molecular surveys have revealed non-monophyly for the genera *Gentianella*, *Lomatogonium* and *Swertia*, particularly pronounced in the latter, supporting the view that the floral morphological characters currently used for generic circumscription are subject to high levels of homoplasy and thus unreliable (Chassot *et al.*, 2001; Hagen & Kadereit, 2001, 2002).

Chromosome numbers have been widely used in addressing taxonomic and phylogenetic issues (e. g. Moore, 1978; Hong, 1990), but, as other characters, the general significance and implications of chromosome numbers greatly depends on the comprehension and extensive documentation of the largest possible sample of the taxa under study. Previous phylogenetic and taxonomic inferences based on chromosome numbers in this subtribe (e. g. Favarger, 1962; Hitchcock, 1959; Löve, 1953; Löve & Löve, 1976; Toyokuni, 1965b; Wood & Weaver, 1982), however, evidently suffered from the scarcity of chromosome data. Therefore, we have counted chromosome numbers for 60 species in this subtribe, 25 of which are reported here for the first time (Table 1). A thorough review of the chromosomal data available from literature was also carried out (appendix). Including the present results, chromosome numbers are now available for 196 species in the Swertiinae, representing all the genera of the subtribe except for *Latouchea* and *Veratrilla*. The following somatic chromosome numbers have been reported: 2n = 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, (30), 32, 36, 38, 40, 42, 44, 48, 52, 54, 56, 60, 78. However, the basic chromosome numbers x = 8, 9, 10 and 13 are by far the most common. While genera such as *Comastoma*, *Lomatogonium*, *Gentianella* and *Jaeschkea* show substantial variation in

chromosome numbers, the genus *Swertia* shows the largest range of chromosome numbers, nearly equal to that of the entire subtribe. This fact reflects the high paraphyly revealed in this genus by molecular phylogenetic studies. Indeed, evidence suggests that *Swertia*, as currently defined, has acted as a stem group in the Swertiinae, from which several genera have evolved in the course of time (Chassot *et al.*, 2001; Hagen & Kadereit, 2001, 2002).

In order to explore the usefulness of chromosome numbers as taxonomic character in the Swertiinae, as well as the relative importance of cytoevolutionary mechanisms such as dysploidy and polyploidy, that may lead to parallel evolution of karyotypes, chromosome data was evaluated in the context of molecular phylogenetic results.

Materials & methods

The species examined are listed in Table 1. All voucher specimens are deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU). When available, flower buds were fixed in the field in 1:3 glacial acetic acid and ethanol (absolute), otherwise seeds were collected. For the observations on root-tips, seeds were germinated on wet filter papers in petri dishes, and suitable root-tips were pre-treated with a saturated aqueous solution of α-bromonaphthalene for 1 hr and 20 min. prior to fixation. Fixed material was stained with aceto-carmine and squashed on temporary slides. Chromosome observations on fixed flower buds were made preferably from pollen mother cells, or first mitosis of pollen grains, but also from mitoses in young cells of the ovary wall. In few cases chromosomes were counted from second pollen mitosis or various floral parts. 2 species offered clear mitoses in the triploid albumen. Drawings were made with a camera lucida apparatus.

Results

Chromosome numbers have been determined for 83 samples of 60 species representing 8 genera of the Swertiinae. The results are shown in Table 1. The chromosome numbers for 25 species are reported for the first time, and for 10 species different numbers from previous reports were encountered. Eight numbers reported here were found to be new for a genus, i. e. the tetraploid level (2n = 52) for *Frasera*, 2n = 10, 12, 16 and 38 for *Gentianella*, 2n = 24 for *Pterygocalyx*, and 2n = 12 and 42 for *Swertia*. Additionally, 2n = 22 in the genus *Swertia* and 2n = 18 in the genus *Lomatogonium* appeared to be more frequent than suggested by previously published data. Some additional new counts of chromosome numbers of *Gentiana* L., *Microrphium* C. B. Clarke and *Tapeinostemon* Benth. are also presented without further discussion.

Table 1. Chromosome numbers and origin of material of examined species. *: first report for the species; !: different from previous reports (cf. appendix); #: binectariate species of *Gentianella*.

Taxon	Locality	Alt. [m]	Collect. No. NEU	n	2n
***Comastoma* Toyokuni**					
* *C. disepalum* H.W.Li ex T.N.Ho	China: Lijiang, Yunnan	3500	92-219		18
C. pedunculatum (Royle ex G.Don) Holub	Nepal: Langtang, Bagmati	3000	97-9		32
C. traillianum (Forrest) Holub	China:Zhongdian, Yunnan	3210	92-197		18
	China: Zhongdian, Yunnan	3900	99-49	9	18
***Frasera* Walter**					
F. fastigiata A. Heller	USA: Idaho Co., Idaho	1200	00-41		78
! *F. gypsicola* (Barneby) D. M. Post	USA: Nye Co., Nevada	1580	00-31		52
! *F. paniculata* M. E. Jones	USA: Navajo Co., Arizona	1500	00-29	26	52
! *F. puberulenta* Davidson	USA: Inyo Co., California	2900	00-37	26	52
F. tubulosa Coville	USA: Inyo Co., California	2830	00-36	13	
***Gentianella* Moench**					
G. amarella (L.) Harry Sm.	USA: Taos Co., New Mexico	2800	00-26	9	
!# *G. arenaria* (Maxim.) T. N. Ho	China: Aksu, Xinjiang	2800	Glla-95.8.23.2.1	3n=24	
	China: Dari, Qinghai	4080	Glla-94.9.5.3b		16
!# *G. azurea* (Bunge) Holub	China: Baqin, Tibet	4130	92-139		10
G. cerastioides (Kunth) Fabris	Ecuador: Iliniza, Pichincha	4000	00-1	18	
* *G. foliosa* (Kunth) Fabris	Ecuador: Iliniza, Pichincha	4500	00-3	18	
# *G. gentianoides* (Franch.) Harry Sm.	China: Lijiang, Yunnan	2700	99-201		22
* *G. gilioides* (Gilg) Fabris	Ecuador: Cajanuma, Loja	3000	00-16		36
!# *G. moorcroftiana* (Wall.ex Griseb.) Airy Shaw	China: Nyalam, Tibet	4500	92-107		12
	Nepal: Jumla, Karnali	3000	97-66		12
	Nepal: Rara Lake, Karnali	3800	97-70		12
! *G. rapunculoides* (Willd.ex Schultes) J.S.Pringle	Ecuador: Cuicocha, Imbabura	3380	00-10	19	38
***Gentianopsis* Ma**					
* *G. contorta* (Royle) Ma	Nepal: Chauta, Karnali	2300	97-69		26
	China: Deqin, Yunnan	2500	99-101		26
Halenia					
H. brevicornis (Kunth) G.Don	Ecuador: Cuicocha, Imbabura	3380	00-8		22
H. weddelliana Gilg	Ecuador: Cuicocha, Imbabura	3380	00-9		22
	Ecuador: Iliniza, Pichincha	4000	00-5		22
***Lomatogonium* A. Braun**					
! *L. carinthiacum* (Wulfen) Reichb.	China: Aksu, Xinjiang	2820	L-95.8.23.1	8	
* *L. forresti* Fernald	China: Zhongdian, Yunnan	3000	99-130		18
* *L. gamosepalum* (Burkill) Harry Sm. in S. Nilsson	China: Zhongdian, Yunnan	4100	99-135	3n=27	
	China: Luqü, Gansu	3200	L-94.8.30.1.2		18
* *L. graciliflorum* Harry Sm.	Nepal: Rara lake, Karnali	3700	97-73		16
* *L. lijiangense* T.N.Ho	China: Yu Hu, Lijiang	2700	99-205	9	18
* *L. micranthum* Harry Sm.	China: Zhongdian, Yunnan	3300	99-151		16
* *L. oreocharis* Marquand	China: Lijiang, Yunnan	3500	99-219		18
* *L. sikkimense* (Burkill) Harry Sm. in S. Nilsson	Nepal: Langtang, Bagmati	4800	97-13		16
***Pterygocalyx* Maxim.**					
! *P. volubilis*	China: Jiuzaigou, Sichuan	3000	Pt-94.9.13.3		24
***Swertia* L.**					
S. angustifolia Buch.-Ham. ex D. Don	Nepal: Langtang, Bagmati	2400	97-18		26
	Thailand: Chiang Mai	1600	99-229		26
S. bimaculata Hook. f. & Thoms. ex C. B. Clarke	China: Emei Shan, Sichuan	1280	99-225		26
	China: Gongshan, Yunnan	1435	cn2k-24		26
	China: Gongshan, Yunnan	1550	cn2k1-16		26

	Taxon	Locality	Alt. [m]	Collect. No. NEU	n	2n
	S. binchuanensis T. N. He & S. W. Liu	China: Dali, Yunnan	2300	99-197	10	
*	*S. calcicola* Kerr	Thailand: Chiang Mai	2000-	99-241		20
	S. chirayta Karst.	Nepal: Langtang, Bagmati	2200	97-2		26
		Nepal: Tumlingtar, Kosi	2400	97-21		26
	S. ciliata (G. Don) B. L. Burtt	Nepal: Langtang, Bagmati	2200	97-6		20
		Nepal: Langtang, Bagmati	3000	97-8	10	
*	*S. cincta* Burkill	China: Kunming, Yunnan	2200	99-33		36
	S. cordata Wall.	Nepal: Langtang, Bagmati	2800	97-17	13	
*	*S. cuneata* Wall. & G. Don	China: Nyalam, Tibet	4500	92-110		20
	S. delavayi Franch.	China: Lijiang, Yunnan	2200	99-213		26
*	*S. erythrosticta* Maxim.	China: Songpan, Sichuan	2530	S-94.9.15.2.1		26
	S. franchetiana Harry. Sm.	China: Lanzhou, Gansu	2000	94-02		20
		China: Xinin, Qinghai	2400	94-S13		20
	S. japonica Makino	Japan: Aso, Kumamoto pref.		99-20	10	20
*	*S. kouitchensis* Franch.	China: Emei Shan, Sichuan	1280	99-226	10	20
	S. laticalyx J. Shah	China: Lijiang, Yunnan	3100	99-220		36
	S. macrosperma C. B. Clarke	China: Dali, Yunnan	2200	99-181	9	
		China: Zhongdian, Yunnan	3600	99-60		18
		China: Gongshan, Yunnan	2000	cn2k-25		18
		China: Kunming, Yunnan	2200	99-31	9	18
*	*S. membranifolia* Franch.	China: Dali, Yunnan	3300	99-186		26
*	*S. multicaulis* D.Don	Nepal:Langtang, Bagmati	4300	97-12		26
	S. nervosa Wall.	China: Dali, Yunnan	2300	99-198		26
		China: Emei Shan, Sichuan	1280	99-227		26
		China: Kunming, Yunnan	2200	99-30		26
*	*S. patula* Harry Sm.	China: Lijiang, Yunnan	2200	99-211		26
*	*S. af. pinetorum* Kerr	Thailand: Chiang Mai	2120	99-236	10	
	S. punicea Hemsl.	China: Zhongdian, yunnan	2550	99-68	10	
*	*S. randaiensis* Hayata	Taiwan: Kuanwu, Taipei	2450	99-2		18
*	*S. rosulata* (Baker) J. Klackenberg	Madagascar: Tsaratanana, Mahajanga	2400	M023		42
	S. speciosa D. Don	Nepal: Jumla, Karnali	3200	97-68		26
*	*S. striata* Collett & Hemsl.	Thailand: Chiang Mai	2000	99-240		20
	S. tashiroi Makino	Taiwan: Kuanwu, Taipei	2000	99-1		52
*	*S. tenuis* T. N. Ho & S. W. Liu	China: Lijiang, Yunnan		92-210	11	
		China: Lijiang, Yunnan	2000	99-159	11	
		China: Zhongdian, Yunnan	2900	99-85		22
		China: Dali, Yunnan	2200	99-175	11	22
!	*S. tetraptera* Maxim.	China: Dalijia Shan, Gansu	3800	94-12		12
*	*S. tozanensisi* Hayata	Taiwan: Tayuling, Hualien	2600	99-7	13	
	S. yunnanensis Burkill	China: Dali, Yunnan	2500	99-162		22
		China: Zhongdian, Yunnan	3500	99-62	11	
		China: Zhongdian, Yunnan	3200	99-69	11	22
	Other Gentianaceae					
	***Gentiana* L.**					
*	*G. otophora* Franch. ex Hemsl.	China: Gongshan, Yunnan	3570	cn2k-34	14	
	G. davidii Franch.	China: Wuyishan, Fujian	2160	cn2k-12		24
*	*G. sikkimensis* C. B. Clarke	China: Wuyishan, Fujian	3400	cn2k-32		24
*	*G. exquisita* Harry Sm.	China: Gongshan, Yunnan	3500	cn2k-33		36
	G. chinensis Kusnezow	China: Emeishan, Sichuan	2400	93-128		24
!	*G. hyalina* T.-N. Ho	China: Maduo, Qinghai	4250	93-36		12
*	***Microrphium** pubescens* C. B. Clarke	Thailand: PhangNga	20	99-243	18	
*	***Tapeinostemon** zamoranum* Steierm.	Ecuador: Zamora	1500	00-17	11	22

Taking the present results into account, chromosome numbers have now been reported for 196 species in the Swertiinae, representing 13 genera. Chromosome data for the two genera *Latouchea* and *Veratrilla* are still not available. For each genus of the subtribe Swertiinae, all available chromosome number data (see appendix), including the results presented here (Table 1), were summarised in Fig. 1, showing the distribution of species sharing a given chromosome number, as well as the number of reports per species.

***Comastoma*.** *Comastoma pedunculatum* from Nepal is documented here as a tetraploid with 2n = 32. *Comastoma traillianum* and *C. disepalum*, were found to have 2n = 18 as most other species in the genus.

***Frasera*.** In this well documented genus, we found here for the first time the tetraploid level (2n = 52) for *F. gypsicola*, *F. paniculata*, and *F. puberulenta*. *Frasera fastigiata* was confirmed as hexaploid (2n = 78), and *F. tubulosa* as diploid (2n = 26).

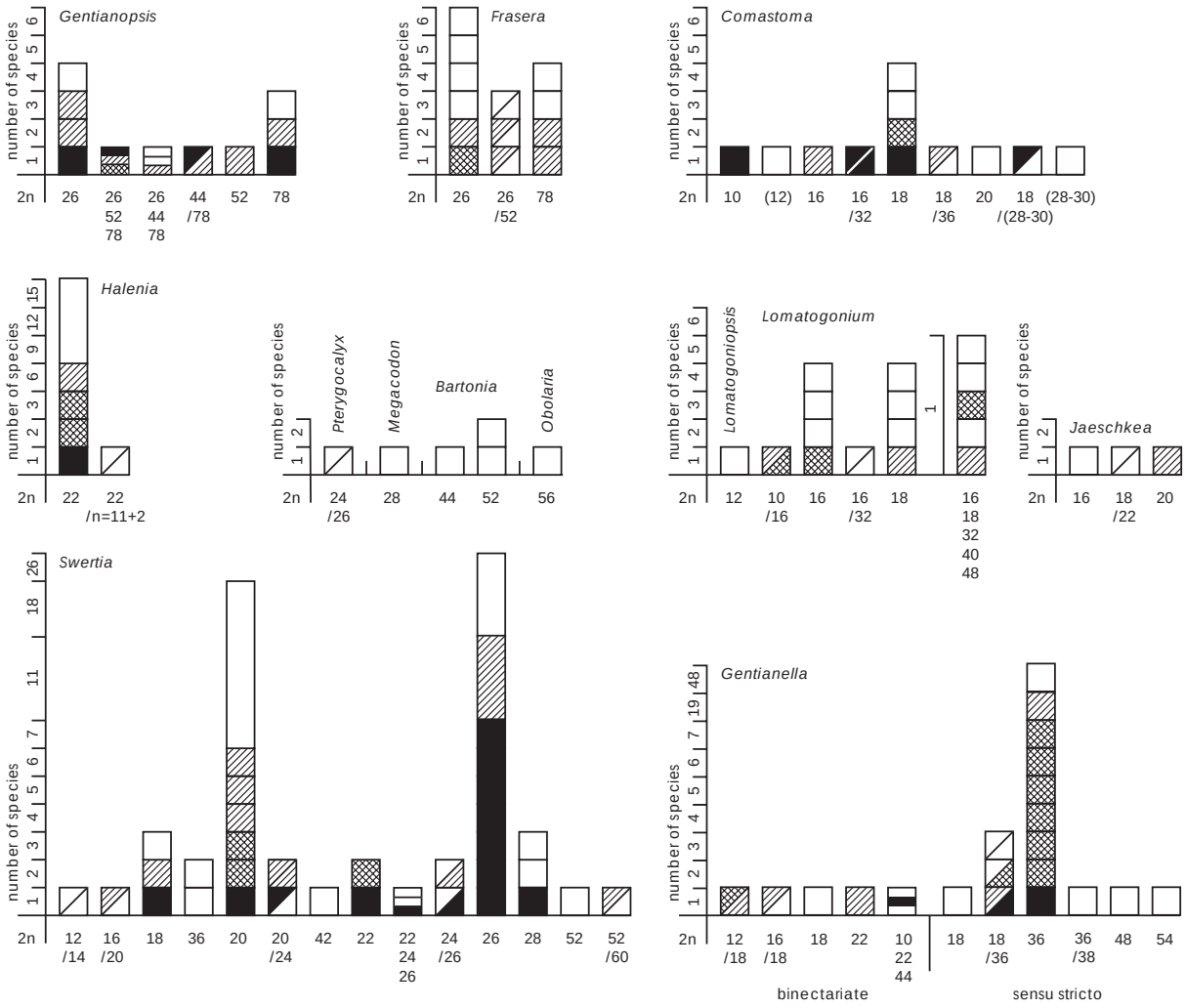

Figure 1. Diploid chromosome numbers for 13 genera of the Swertiinae. Numbers in parenthesis indicate approximate counts. Black boxes: > 3 reports for the species; cross-hatched boxes: 3 reports; simple-hatched boxes: 2 reports; white boxes: 1 report.

***Gentianella*.** Of the taxa studied here, *G. cerastioides*, *G. foliosa* and *G. gilioides* were tetraploid with 2n = 36. *Gentianella rapunculoides* had 2n = 38 but showed 2 small chromosomes indicating possible dysploidy from 2n = 36. *Gentianella amarella* from North America was confirmed as diploid with 2n = 18. The chromosome number obtained here for *G. arenaria* (2n = 16, 3n=24) is different from a previous report of 2n = 18, indicating possible dysploidy in this taxon. Similarly, individuals of *G. moorcroftiana*, collected from two distant localities in Nepal and also from China, were always counted as 2n = 12, whereas 2 previous reports state 2n = 18 for this species. *Gentianella gentianoides* had 2n = 22, a rare number in the genus. Its chromosomes are also among the smallest observed during this study (Fig. 2b). *Gentianella azurea*, otherwise known to have 2n = 22 or 44, was counted here with only 2n = 10 chromosomes.

***Gentianopsis*.** This genus is karyologically well documented (Yuan & Küpfer, 1993), and has an important proportion of polyploid species. For *G. contorta*, individuals from China and Nepal were always found to be strictly diploid with 2n = 26.

***Halenia*.** All previously documented species have 2n = 22 chromosomes, with only one exceptional report of n = 11+2 for *H. brevicornis*. In our observations, only 2n = 22 was found for this species. *Halenia weddelliana* was confirmed to have 2n = 22.

***Lomatogonium*.** The individuals of *L. carinthiacum* studied here were diploid with 2n = 16. Previous records indicate that this species is usually tetraploid with 2n = 32 and occasionally hexaploid with 2n = 48. *Lomatogonium graciliflorum*, *L. micranthum*, and *L. sikkimense* were also counted with 2n = 16. For *L. gamosepalum*, *L. forresti*, *L. lijiangense* and *L. oreocharis*, the chromosome number 2n = 18 was found.

***Pterygocalyx*.** The number 2n = 24 found for *P. volubilis* was different from the previous report of 2n = 26.

***Swertia*.** As indicated in Fig. 1, the predominant diploid chromosome numbers in this genus are 20 and 26. In our present observations, the following species were found with 2n = 20: *S. binchuanensis*, *S. calcicola*, *S. cuneata*, *S. franchetiana*, *S. japonica*, *S. kouitchensis*, *S. af. pinetorum*, *S.*

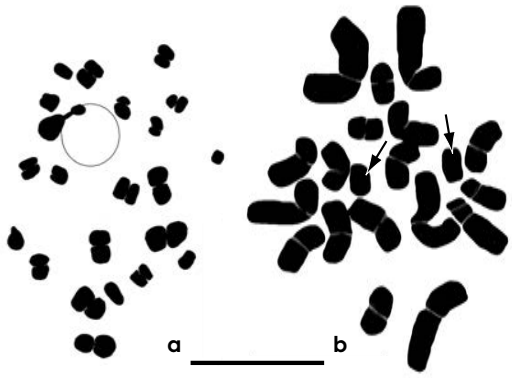

Figure 2. Drawing of a: mitotic metaphase of *Swertia ciliata*, 2n = 20, showing 2 small chromosomes; b: mitotic premetaphase of *Gentianella gentianoides*, 2n = 22, showing the smallest chromosomes observed in the subtribe Swertiinae. Scale = 5µm.

punicea and S. *striata*. This number was also confirmed for *S. ciliata*, but this species showed 2 small chromosomes (Fig. 2a) absent from other species with 2n = 20, suggesting that 2n = 20 in *S. ciliata* may be the result of ascending dysploidy from a lower diploid number. The only *Swertia* species from Madagascar, *S. rosulata*, had 2n = 42 chromosomes. 13 species were found to have 2n = 26 chromosomes: *S. angustifolia*, *S. bimaculata*, *S. chirayita*, *S. cordata*, *S. decora*, *S. delavayi*, *S. erythrosticta*, *S. membranifolia*, *S. multicaulis*, *S. nervosa*, *S. patula*, *S. speciosa* and *S. tozanensis*. *Swertia tashiroi* was found with 2n = 52, which may indicate that it is tetraploid. A less frequent chromosome number, 2n = 18, was constantly found in two morphologically very similar species, *S. macrosperma* and *S. randaiensis*. Additionally, *S. cincta* and *S. laticalyx* appeared to be tetraploid with 2n = 36. The chromosome number found for *S. tenuis* and *S. yunnanensis* was surprising: both species, counted 4 and 3 times respectively, constantly had 2n = 22 chromosomes. This number was otherwise known from a single species in the genus, *S. bimaculata*, that was more frequently reported as 2n = 26.

Discussion

Polyploidy and Geographical Distribution

Polyploidy is not uncommon in the subtribe Swertiinae, although concentrated in *Bartonia*, *Frasera*, *Gentianella*, *Gentianopsis* and *Obolaria* (Fig. 1, Table 2). Its occurrence also seems to be correlated with the geographical distribution of the species affected, and may have little or no taxonomic usefulness. A few genera such as *Bartonia* (2n = 44, 52) and *Obolaria* (2n = 56), with a strictly North American distribution, are entirely tetraploid. These taxa were found to represent rather ancient lineages (Chassot *et al.*, 2001; Hagen & Kadereit, 2002) and may be considered as paleo-tetraploids. Regarding the genus *Gentianopsis*, polyploidisation was proposed as the main course of adaptive radiation from the Himalaya and South-western and Western China, where only diploid and tetraploid species occur, towards the higher latitudes of Europe, the Russian far East and North America, from where only hexaploid cytotypes are known (see Yuan & Küpfer, 1993).

In the species-rich lineage (ca. 250 spp.) of *Gentianella* s. str. (sensu Hagen & Kadereit, 2001), the quasi totality of documented species are tetraploid (2n = 36). *Gentianella acuta*, *G. amarella* and *G. aurea* are the only species know with both di- and tetraploid cytotypes, while *G. umbellata* has only been recorded once with 2n = 18. This lineage is of recent origin in the Swertiinae (Hagen & Kadereit, 2001) and has been the most successful of the entire tribe *Gentianeae* in colonising the young mountain systems formed at the close of the tertiary in

the Southern hemisphere. The impressive number of species in this lineage was explained by the vast area open to colonisation in South America, rather than an increased speciation rate (Hagen & Kadereit, 2001). However, polyploidy may have influenced the tempo of speciation in this lineage, due to an increased allelic diversity (e. g. Bretagnolle *et al.*, 1998), and/or to increased hybridisation by breaking down the postzygotic barriers that keep diploid taxa isolated (e. g. Ramsey & Schemske, 1998).

Frasera is particular in the Swertiinae in having an exclusively North American distribution, but a very close morphological resemblance with *Swertia*. Molecularly, no particular Asian lineage of *Swertia* was found to be tightly related to *Frasera* (Chassot *et al.*, 2001), suggesting that this genus diversified entirely in North America. Two morphological groups can be distinguished in *Frasera*. One consists of robust perennial hexaploids with flat, green-margined leaves, growing in mesic habitats. The other is composed of smaller di- or tetraploid plants with white-margined, conduplicate leaves, adapted to the xeric conditions of the intermountain basin. The occurrence of several diploid taxa in the latter highly specialised group indicates that the initial radiation of *Frasera* in North America took place at the diploid level, and that the hexaploid cytotypes observed in the robust perennials may be of more recent origin.

The remaining genera of the Swertiinae have a mainly Eurasian and African distribution. They count relatively few polyploid species (Table 2), and none reported from Africa. Polyploidy in these taxa does not seem to follow a precise pattern, but notably occurs in some relatively rare island endemics. In the genus *Lomatogonium*, *L. brachyantherum* (Sino-Himalayan; 2n = 16, 32) occurs both as di- and tetraploid. *Lomatogonium carinthiacum* (Eurasian; 2n = 16, 32, 48) has the widest range of documented chromosome numbers in the genus with di-, tetra- and hexaploid cytotypes, and some records suggesting dysploidy and/or aneuploidy (2n = 18, 40). It is also the most widespread species of *Lomatogonium*. In *Comastoma*, di- and tetraploid counts are reliable for the widespread *C. pedunculatum* (Sino-Himalayan; 2n = 16, 32) and *C. beesianum* (Southwest China; 2n = 18, 36), while unconfirmed numbers also indicate possible polyploidy in *C. falcatum* and *C. nanum* (2n = ca 28-30). Among the few binectariate species of *Gentianella*, that were clearly excluded from the mainly tetraploid *Gentianella* s. str. lineage by molecular phylogenetic results (Hagen & Kadereit, 2001, 2002), *Gentianella azurea* (throughout Asia, 2n = 10, 22, 44) is the only polyploid species. In the genus *Swertia*, where polyploidy is rather infrequent in view of the total number of species (ca. 138), only 5 species were reported as polyploid at date. *Swertia cincta* and *S. laticalyx* (Southwest China; 2n = 36), closely related to *S. ciliata* (2n = 20, 24)

and *S. paniculata* (2n = 16, 20) (Shah, 1984), appear to be tetraploid with a basic chromosome number of x = 9 . *Swertia swertopsis* (Japan; 2n = 52) is probably also tetraploid, but with a basic number of x = 13 (Shigenobu, 1983). The chromosome number of *S. rosulata* (Madagascar; 2n = 42) suggests a basic chromosome number of x = 21. However, in view of the close morphological relationship of this species with *S. abyssinica* (Africa; 2n

Genus	Basic number	Ploidy level	No of species	Spp. documented / spp. in genus
Bartonia	11	4x	1	3 / 4 [(1)]
	13	4x	3	
Comastoma	5	2x, (4x, 6x)	1 (3)	11 / 7-25 [(1)]
	(6)	(2x)	(1)	
	8	**2x**, 4x	2	
	9	**2x**, 4x	6	
	10	2x	1	
Frasera	**13**	**2x**, 4x, **6x**	13	13 / 14 [(2)]
Gentianella	8	6x	1	60 / ca 250 [(1)]
s. str.	**9**	2x, **4x**, 6x	54	
	19	2x	1	
Gentianella	5	2x	1	
binectariate	6	2x	1	
	8	2x	1	
	9	2x	3	
	11	2x, 4x	2	
Gentianopsis	11	4x	2	11 / 16-24 [(1)]
	13	**2x**, 4x, **6x**	11	
Halenia	**11**	2x	16	16 / 80 [(1)]
Jaeschkea	8	2x	1	3 / 4 [(1)]
	9	2x	1	
	10	2x	1	
	11	2x	1	
Latouchea	—	—	—	— / 1 [(1)]
Lomatogoniopsis	6	2x	1	1 / 3 [(3)]
Lomatogonium	5	2x	1	11 / 18 [(4)]
	8	**2x**, 4x, 5x	7	
	9	2x	5	
Megacodon	14	2x	1	1 / 2 [(1)]
Obolaria	14	4x	1	1 / 1 [(1)]
Pterygocalyx	12	2x	1	1 / 1 [(1)]
	13	2x	1	
Swertia	6	2x	1	64 / 138 [(5)]
	7	2x	1	
	8	2x	1	
	9	**2x**, 4x	5	
	10	**2x**	21	
	11	2x	3	
	12	2x	5	
	13	**2x**, 4x	31	
	14	2x	3	
	15	4x	1	
	21	2x	1	
Veratrilla	—	—	—	— / 2 [(1)]
				196 / 541-567

Table 2. Basic chromosome numbers and ploidy levels in the subtribe *Swertiinae*. Bold face indicates the most frequent situation. The fourth column indicates the number of species showing the basic chromosome number in regard (polybasic species represented more than once). The last column shows the number of species with documented chromosome numbers out of the total number of species in each genus. [(1)] Struwe *et al.* (2002), [(2)] Shah (1984), [(3)] Ho & Pringle (1995), [(4)] Liu & Ho (1992), [(5)] own estimation.

= 20) (Shah, 1984), x = 21 may be considered as secondary and resulting from ascending dysploidy following tetraploidisation. The highest chromosome number in the genus, 2n = 60, was found in Japanese specimens of *S. tashiroi* by Shigenobu (1982, 1983), who considered this species as hexaploid with a basic chromosome number of x = 10. However, molecular phylogenetic results related this species to other lineages of the Swertiinae with x = 13 (Chassot *et al.*, 2001), suggesting that 2n = 60 may have resulted from ascending dysploidy, and that the Japanese specimens of *S. tashiroi* could be tetraploid with a basic chromosome number of x = 15 rather than x = 10. This interpretation is also consistent with the present result of 2n = 52 from samples collected in Taiwan.

Basic Chromosome Numbers and their Evolution in the Swertiinae

The accumulation of chromosome counts has revealed a high level of heterogeneity of basic chromosome numbers in the Swertiinae, as shown in Table 2. Indeed, except for the genera that were poorly studied, or that comprise only few species (i. e. *Lomatogoniopsis, Megacodon* and *Obolaria*), only two genera in the subtribe, *Frasera* and *Halenia*, show constant basic numbers (x = 13 and x = 11 respectively). All other genera show multiple basic numbers. However, these basic numbers do not occur with the same frequency. One genus usually shows one or two basic numbers more frequent than others. For example, in *Gentianella*, the basic number x = 9 is much more frequent than others, and in *Swertia*, the basic numbers x = 10 and x = 13 are the most common. The evolutionary relationships among these basic numbers are not well understood yet, but, karyological evolution both at generic level (or lineage level for non-monophyletic genera), and within a genus or lineage, can now be examined in the context of phylogenetic affinities revealed by recent molecular studies (fig. 3) (Chassot *et al.*, 2001; Hagen & Kadereit, 2001, 2002).

At generic or lineage level. According to molecular phylogenetic results, the basal lineages in the Swertiinae mostly have the basic number x = 13, and less frequently x = 14 or x = 11. Two monotypic and closely related genera, *Latouchea* from Southeast China and *Obolaria* from North America, were revealed as the basalmost lineage within the subtribe. While the chromosome number of *Latouchea* is yet unknown, *Obolaria* has 2n = 56 chromosomes, which seems to represent a paleo-tetraploid with a basic number of x = 14. The eastern Himalayan endemic *Megacodon* also has the basic number x = 14 (2n = 28), but its affinities with the remaining basal lineages of the Swertiinae is still uncertain, as molecular data failed to resolve these phylogenetic relationships . However, for most of these lineages, the basic number x = 13 is predominant. In *Frasera*, all species documented have x = 13 (2n

= 26, 52, or 78). In *Gentianopsis*, the species mostly have x = 13 (2n = 26, 52, or 78), rarely x = 11 (2n = 44) (see Yuan and Küpfer, 1993). Similarly, in *Bartonia* there are two reports of 2n = 52 and yet one species was recorded with 2n = 44. The monotypic genus *Pterygocalyx* was counted with both 2n = 24 and 2n = 26. In the largely paraphyletic genus *Swertia* (Chassot *et al.*, 2001), the basal lineages consist of species with x = 13 (2n = 26; 2n = 52 and 60 in *S. tashiroi*) or rarely x = 14 (2n = 28), except for *S. tetraptera* (2n = 12, 14). All the species related by Shah (1984) to those representing basal lineages of *Swertia* in Chassot *et al.* (2001), for which chromosome numbers were documented, have x = 13. The genus *Halenia* has the basic chromosome number x = 11 (2n = 22 in all species documented so far). However, this genus was shown to be closely related to a lineage of *Swertia* with x = 13 (2n = 22, 24, 26 in *S. bimaculata*, and 2n = 26 in the morphologically closely related *S. tozanensis*), thus suggesting that x = 11 in *Halenia* is probably derived from x = 13. As it appears, x = 13 is shared by a majority of basal lineages, but whether x = 13 or x = 14 is ancestral in the subtribe cannot be assessed with certainty. Moreover, no unique basic chromosome number was found to confine to a specific lineage.

The derived genera of the subtribe Swertiinae such as *Comastoma*, *Gentianella*, *Jaeschkea*, *Lomatogonium*, *Lomatogoniopsis*, and several lineages of *Swertia*, show a higher variability of chromosome numbers compared to the basal lineages. Multiple basic numbers including x = 5, 6, 8, 9, 10, 11 and 12 were observed, as well as the secondary basic numbers x =19 and x = 21 (*G. rapunculoides* and *S. rosulata* respectively), probably resulting from dysploidy at the tetraploid level. Disregarding numbers confined to a few species only, the prevalent basic chromosome numbers are x = 8, 9 and 10. Noticeably, the aforementioned lineages never show the basic chromosome number x =13, but were revealed as sister to a *Swertia* clade comprising *S. chirayita* with 2n = 26, and *S. barunensis* (as *S. af. pseudohookeri* in Chassot *et al.*, 2001). *Swertia. barunensis* belongs to sect. Macranthos Ho & Liu, for which chromosome data is still entirely missing. However, this section is morphologically closely related to *S. multicaulis* (Shah, 1984), for which 2n = 26 was found in the present study. It seems thus plausible that the lower basic chromosome numbers observed in these derived genera and lineages of the Swertiinae (mainly x = 8, 9 and 10) have evolved from an ancestral basic number of x = 13, possibly repeatedly. However, these lower basic chromosome numbers are largely overlapping across the lineages identified by DNA sequence data, and none uniquely characterises any genus or lineage (cf. Table 2 and Fig. 1). Furthermore, it seems difficult to define a direction of evolution among the basic chromosome numbers x = 8, 9 and 10, hence represented by a cyclic relationship in Fig. 3.

Within genera or lineages. When viewed in the context of molecular phylogenetic results, it appears that parallel gain of identical chromosome numbers may have occurred frequently within different genera or lineages of the Swertiinae. Moreover, closely related species sometimes show markedly different chromosome numbers.

As discussed above, among the basal lineages, where the variability of basic chromosome numbers is relatively low, the most common pattern is the independent evolution of x = 11 from x = 13, as in *Bartonia*, *Gentianopsis* and *Halenia*. In the basal lineages of *Swertia*, discounting occasional intraspecific dysploidy (e. g. *S. bimaculata* with 2n = 22, 24, 26, *S. angustifolia* and *S. alata* with 2n = 24, 26), x = 13 is very stable. Noteworthy exceptions are *Swertia bifolia*, S. *perennis* and *S. wolfgangiana*, that have exclusively 2n = 28. Although closely related, these species were placed in 2 different morphological groups by Shah (1984), thus suggesting that x = 14 may have appeared more than once independently in this *Swertia* lineage comprising numerous Asian plietesial species with x = 13. Clearly at difference from this general picture is the chromosome number of *S. tetraptera* (2n = 12, 14). Indeed, this species was shown to be related to a lineage comprising *S. bimaculata* (x = 11, 12, 13) and *Halenia* (x = 11), suggesting that extensive descending dysploidy may have affected its genome.

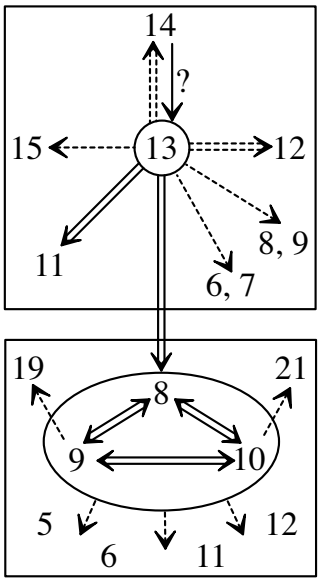

Figure 3. Evolution of basic chromosome numbers in the Swertiinae according to relationships inferred from DNA sequence data. Taxa related to x = 13 and x = 8, 9, 10 in upper and lower boxes respectively. Dashed and double arrows represent rare and multiple occurrences respectively.

The important level of variability of basic chromosome numbers observed in the derived genera and lineages of the Swertiinae (i. e. *Comastoma, Gentianella, Jaeschkea, Lomatogonium, Lomatogoniopsis,* and several lineages of *Swertia*) is not distributed symmetrically. For example, all the karyologically documented species related by Shah (1984) to the *S. punicea* and the *S. cuneata* - *S. crassiuscula* lineages identified in Chassot *et al.* (2001) have 2n = 20, except for *S. tetragona* with a single report of 2n = 18. Similarly, all the documented species of *Gentianella* that belong to the *Gentianella s. str.* lineage have x = 9, except for *G. auriculata,* with a single record of 2n = 48, and the dysploid *G. rapunculoides* with 2n = 36 and 38.

Karyological parallelism and heterogeneity between closely related species is concentrated in *Comastoma*, *Jaeschkea*, *Lomatogonium*, *Lomatogoniopsis*, the binectariate species of *Gentianella* (cf Table 2), and the lineage of *Swertia* including *S. ciliata* (2n = 20, 24), *S. cincta* (2n = 36), *S. paniculata* (2n = 16, 20) and *S. laticalyx* (2n = 36). For example, *Comastoma* and *Lomatogonium*, that form a closely related and derived complex in the subtribe (Chassot *et al.*, 2001; Hagen & Kadereit, 2002), display a puzzling pattern of evolution of chromosome numbers. *Comastoma* is a polybasic genus with x = 5, (6), 8, 9, 10. As it was proposed in Yuan (1993), dysploidy implying large-sized satellites may account for an important part of the variability of chromosome numbers in species of x = 8 and x = 9. However, the karyological relationships between these species and *C. tenellum* (2n = 10) is not understood yet, although their monophyly was confirmed by molecular data (Hagen & Kadereit, 2002). Closely related to *Comastoma* (Chassot *et al.*, 2001; Hagen & Kadereit, 2002), *Lomatogonium* has a similar pattern of basic numbers, with x = 5, 8 and 9. Here again x = 8 and x = 9 are the most common numbers, while only European and American individuals of *L. rotatum* were reported to have 2n = 10. The fact that x = 8 and x = 9 can appear in species belonging to the same section (e. g. *L. gamosepalum* and *L. brachyantherum* in sect. *Sarcorhizoma* Liu & Ho), or even within one species (*L. carinthiacum*), suggests that these numbers may have been derived repeatedly through dysploidy in the genus. The occurrence of the number 2n = 10 in *L. rotatum* is difficult to understand, as molecular phylogenetic results suggested this species to be closely related to other species with x = 8 and x = 9 (Hagen & Kadereit, 2002). Whatever the mechanism that has lead to x = 5, it seems that parallel evolution resulting in a severe reduction of chromosome numbers occurred in both taxa without morphological consequences at the generic level, or even at the species level in the case of *L. rotatum*.

Conversely, important dysploidy may also be accompanied by marked morphological differences, as in *Lomatogoniopsis*. A single report, 2n = 12 in *L. alpina*, is available for this genus, which suggests the distinct basic number x = 6. This species has unique floral characteristics (see Hagen and Kadereit, 2002 for illustrations), and has been variably related to *Comastoma* (Liu, Ho and Chen, 2002) and *Lomatogonium* (Ho & Liu, 1985; Liu & Ho, 1992). However, molecular phylogeny placed *L. aplina* within the core *Lomatogonium* clade, along with species of x = 5, 8 and 9 (Hagen & Kadereit, 2002).

The evolution of chromosome numbers in the binectariate species of *Gentianella* (marked with # in Tables 1 and appendix) is also difficult to understand in the light of relationships suggested by DNA sequence data. Clearly excluded from the *Gentianella s. str.* lineage, these

species, however, were not found to be monophyletic (Hagen & Kadereit, 2001, 2002). Chromosome numbers support their non-monophyly to some extent, being rather dissimilar across species (cf. Fig. 1), except for the identical number of 2n = 22 in *G. azurea* and *G. gentianoides*. However, the karyotype of *G. gentianoides* showed the smallest chromosomes observed in the subtribe (Fig. 2b), suggesting that this number resulted from a severe fragmentation of its genome. A comparison with the karyotype of *G. azurea* in Liu (1994), showing much larger chromosomes, clearly shows that these species are not related cytologically, in agreement with molecular results. Yet, several binectariate species of *Gentianella* appear to be polybasic, and were related by molecular data to other taxa of the Swertiinae showing important differences both in chromosome numbers and morphology. For example, we have found 2n = 12 chromosomes in samples of *G. moorcroftiana* from Nepal and China (2n = 18 was also reported from Western Indian Himalaya), a species molecularly related to *Lomatogonium oreocharis* (2n = 18) and *G. azurea* (2n = 10, 22, 44) (Hagen & Kadereit, 2002), and also to *Jaeschkea microsperma* (2n = 16) (Chassot *et al.*, 2001). Similarly, *G. arenaria* (2n = 16, 18) shows a chromosome number similar to those of the derived lineages of the subtribe, but its position in the phylogeny of the Swertiinae was among basal lineages with x = mostly 13 (Hagen & Kadereit, 2002).

In summary, the interpretation of basic chromosome numbers in the subtribe Swertiinae in the context of phylogenetic relationships (Fig. 3) suggests that 1) at the generic or lineage level, x = 13 or x = 14 could represent the ancestral basic numbers in this subtribe, and that descending dysploidy might have played an important role in the origin of other lower basic numbers in more derived lineages; and 2) closely related taxa may gain rather different basic numbers through severe dysploidy, while parallel evolution implying the gain or loss of 1 or 2 pairs of chromosomes often resulted in identical basic numbers in distantly related taxa or lineages. Therefore, basic chromosome numbers per se cannot be considered as a reliable diagnostic character for any particular genus or lineage in this subtribe. However, the relatively clear-cut distinction between basal taxa of the genus *Swertia* with x = 13 or 14, and those with a lower basic chromosome number is consistent with molecular phylogenetic results, and may be most helpful for future taxonomic considerations in this genus.

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Appendix. Chromosome numbers for 13 genera of the subtribe Swertiinae compiled from literature. #: binectariate species of *Gentianella*.

Taxon	Locality	2n	Reference
***Bartonia* Muhl.**			
B. paniculata (Michx) Muhl.	USA	52	Rork (1949)
B. verna (Michx) Muhl.	USA	n=22	Weaver & Rüdenberg (1975)
B. virginica Britton, Stern & Poggenb.	USA	52	Rork (1949)
***Comastoma* Toyokuni**			
C. arrectum (Franch.) Holub	Yunnan, China	18	Liu & Ho (2002)
C. beesianum (W. W. Smith) Holub	Yunnan, China	18	Liu (1994)
	Yunnan, China	18, 36	Liu (1994)
C. chiuchiense T. N. Ho & J.-Q. Liu	Qinghai, China	20	Liu & Ho (2002)
C. falcatum (Turcz.) Toyokuni	Siberia	ca 28-30	Krogulevich (1978)
	Qinghai, China	18	Liu & Ho (2002)
	Qinghai, China	18	Liu (1994)
	Qinghai, China	18	Liu (1994)
C. nanum (Wulfen) Toyokuni	Italy	ca. 28 or 30	Löve & Löve (1986)
C. pedunculatum (Royle ex G. Don)	Qinghai, China	16	Liu (1994)
Holub	Qinghai, China	n=8	Liu (1994)
	Qinghai, China	32	Liu (1994)
	Qinghai, China	32	Liu (1994)
	Qinghai, China	16	Liu (1994)
	Gansu, China	n=8	Liu (1994)
C. polycladum (Diels & Gilg) T. N. Ho	Gansu, China	16	Yuan & Küpfer (1993)
	Xiahe, Gansu	16	Liu & Ho (2002)
C. pulmonarium (Turcz.) Toyokuni	Gansu, China	18	Yuan & Küpfer (1993)
	Gansu, China	18	Yuan & Küpfer (1993)
	Qinghai, China	18	Liu & Ho (2002)
	Qinghai, China	18	Liu & Ho (2002)
	Qinghai, China	18	Liu & Ho (2002)
	Qinghai, China	n=9	Liu & Ho (2002)
	Qinghai, China	18	Liu & Ho (2002)
	Qinghai, China	18	Liu & Ho (2002)
	Siberia	ca. 12	Krogulevich (1978)
C. tenellum (Rottb.) Toyokuni	Greenland	n=5	Dalgaard (1988)
	Switzerland	10	Favarger (1949)
	Alaska	10	Johnson & Packer (1968)
	Norway	10	Knaben (1950)
	Siberia	ca. 10	Krogulevich (1978)
	Iceland	10	Löve & Löve (1986)
	Iceland	10	Löve (1953)
	Poland	10	Skalinska, Pogan and Jankun (1968)
	NE Siberia	10	Zhukova (1966)
C. traillianum (Forrest) Holub	Yunnan, China	18	Liu & Ho (2002)
***Frasera* Walter — synonymy according to Shah (1984)**			
F. albicaulis Griseb.	USA	26	Post (1956)
as *F. pahutensis* Reveal	Nevada, USA	n=13	Reveal & Styer (1974)
as *F. pahutensis* Reveal	Nevada, USA	n=13	Reveal (1971)
F. albomarginata S. Watson	USA	26	Post (1956)
F. caroliniensis Walter	New York, USA	78	Rork (1946, 1949)
F. fastigiata A. Heller	USA	n=39	Post (1956)
F. gypsicola (Barneby) D. M. Post	USA	26	Post (1956)
F. montana Mulford	USA	26	Post (1956)
F. neglecta H. M. Hall	USA	26	Post (1956)
F. paniculata Torr.	USA	26	Post (1956)
F. parryi Torr.	USA	26	Post (1956)
F. puberulenta Davidson	USA	26	Post (1956)
F. speciosa Griseb.	USA	78	Löve & Kapoor (1967)
	Rocky Mt., USA	78	Löve, Löve and Kapoor (1971)
F. tubulosa Coville	USA	26	Post (1956)
F. umpquaensis M. Peck & Applegate	USA	n=39	Post (1956)
***Gentianella* Moench**			
G. acuta (Michx) Hultén	Siberia	18	Krogulevich (1978)
	Manitoba, Canada	36	Löve & Löve (1982)
G. amabilis (as *Gentiana amabilis* Petrie)	New Zealand	36	Hair, Webb and Beuzenberg (1980)

Taxon	Locality	2n	Reference
G. amarella (L.) Borner	Iceland	36	Löve (1953)
	California	n=9	Post (1983)
	Sweden	36	Löve & Löve (1986)
	Spain	36	Löve & Löve (1986)
	Iceland	36	Löve (1953)
	Iceland	36	Löve & Löve (1986)
G. anglica (Pugsley) E. F. Warb.	England	36	Löve & Löve (1986)
G. anisodonta (Borbas) A. & D. Love	Rossalpe	36	Favarger & Huynh (1965)
	Austria	36	Löve & Löve (1986)
	W Himalaya	n=18	Vasudevan (1975)
G. antarctica (Kirk) T. N. Ho & S. W.	New Zealand	36	Hair *et al.* (1980)
Liu	New Zealand	36	Löve (1983)
G. antipoda (Kirk) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
#*G. arenaria* (Maxim.) T. N. Ho	Qinghai, China	18	Liu (1994)
G. aspera (Hegetsch. & Heer) Dostal	Germany	36	Favarger (1965)
ex Skalicky, Chrtek & J. Gill	Germany	36	Löve & Löve (1986)
G. astonii (Petrie) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
G. auriculata (Pall.) J. M. Gillett	Korjakian Land	48	Sokolovskaya (1968)
G. aurea (L.) Harry Sm.	Greenland	18	Dalgaard (1989)
	Iceland	36	Löve (1983)
	Iceland	36	Löve (1953)
	Iceland	36	Löve & Löve (1986)
G. austriaca (A. & J. Kern.) Holub	Austria	36	Favarger (1952)
	Austria	36	Löve & Löve (1986)
#*G. azurea* (Bunge) Holub	Qinghai, China	22	Liu (1994)
	Qinghai, China	44	Liu (1994)
	Dari, Qinghai	22	Liu (1994)
	Dingqin, Tibet	22	Liu (1994)
	Dari, Qinghai	22	Liu, Ho and Chen (2002a)
G. bellidifolia (Hook f.) Holub	New Zealand	36	Hair *et al.* (1980)
	New Zealand	36	Löve (1983)
G. biebersteinii (Bunge) Holub	Caucasus	36	Gagnidze, Küpfer and Yuan (1992)
G. bulgarica (Velen.) Holub	Bosnia	36	Löve & Löve (1986)
G. campestris (L.) Harry Sm.	Switzerland	n=18	Favarger (1949)
	Sweden	36	Löve & Löve (1986)
	Spain	36	Löve & Löve (1975b)
	Sweden	36	Löve & Löve (1986)
	Iceland	36	Löve (1953)
	Iceland	36	Löve & Löve (1986)
G. caucasea (Lodd. ex Sims) Holub	Caucasus	36	Gagnidze *et al.* (1992)
G. cerastioides (Kunth) Fabris	Ecuador	n=18	Pringle (1981)
G. chathamica (Cheeseman) T. N. Ho	New Zealand	36	Hair *et al.* (1980)
& S. W. Liu			
G. columnae (Ten.) Holub	Italy	36	Löve & Löve (1986)
G. corymbifera (Kirk) Holub	New Zealand	36	Hair *et al.* (1980)
G. corymbosa (Kunth) Weaver &	Colombia	n=18	Weaver & Rüdenberg (1975)
Rüdenberg			
G. crassulifolia (Griseb.) Fabris	Colombia	n=18	Weaver & Rüdenberg (1975)
G. crispata (Vis.) Holub	Italy	36	Löve & Löve (1986)
G. dacrydioides (Gilg) Weaver &	Colombia	n=18	Weaver & Rüdenberg (1975)
Rudenberg			
G. divisa	New Zealand	36	Hair *et al.* (1980)
(as *Gentiana divisa* Cheeseman)			
G. engadinensis (Wettst.) Holub	Switzerland	n=18	Favarger (1965)
	Switzerland	36	Löve & Löve (1986)
	Pirin Mt.	36	Nikolov (1991)
#*G. gentianoides* (Franch.) Harry Sm.	Lijiang, Yunnan	22	Liu *et al.* (2002a)
G. germanica (Willd.) E. F. Warb.	Germany	36	Löve & Löve (1986)
	Boulonnais	36	Leveque & Gorenflot (1969)
	W Himalaya	n=18	Vasudevan (1975)
G. gracilifolia (Cheeseman) T. N. Ho &	New Zealand	36	Hair *et al.* (1980)
S. W. Liu			
G. grisebachii (Hook. f.) T. N. Ho	New Zealand	36	Hair *et al.* (1980)
	New Zealand	36	Löve (1983)
G. hypericifolia (Murb.) Tutin ex	Spain	n=18	Favarger & Küpfer (1968)
Pritchard	Spain	n=18	Küpfer & Favarger (1967)
	Spain	36	Löve & Löve (1975b)
G. insubrica (Kunz) Holub	Tessin	n=18	Favarger (1952)

Taxon	Locality	2n	Reference
G. lineata (Kirk) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
	New Zealand	36	Löve (1983)
G. lutenscens (Velen.) Holub	Slovenia	36	Löve & Löve (1986)
G. magellanica (Gaudich.) Fabris	Argentina	n=18	Moore (1981)
	Argentina	36	Löve (1983)
G. montana (G. Forst.) Holub	New Zealand	36	Hair *et al.* (1980)
	New Zealand	36	Löve (1983)
	New Zealand	36	Hair *et al.* (1980)
G. matthewsii (Petrie) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
#*G. moorcroftiana* (Wall. ex Griseb.) Airy Shaw	W Himalaya, India	n=9	Mehra & Vasudevan (1972)
	W Himalaya	n=9	Vasudevan (1975)
G. nevadensis (Gilg) Weaver & Rudenberg	Venezuela	n=18	Weaver & Rüdenberg (1975)
G. patula (Cheeseman) Holub	New Zealand	36	Hair *et al.* (1980)
G. pavonii (Griseb. ex DC.) Fabris		n=18	Pringle (1981)
G. pilosa (Wettst.) Holub	Slovenia	36	Löve & Löve (1986)
G. praecox (A. & J. Kern.) Dostál ex E. Mayer	Mt. Tatra	36	Skalinska (1951)
G. propinqua (Richardson) J. M. Gillett	Manitoba, Canada	36	Löve & Löve (1982)
	Chukotka	36	Zhukova (1982)
#*G. pygmaea* (Regel & Schmalh.) Harry Sm. in S. Nilsson	Qinghai, China	18	Liu (1994)
G. quinquefolia Small		36	Rork (1946, 1949)
G. ramosa (Hegetschw.) Holub	Switzerland	36	Löve & Löve (1986)
	Switzerland	n=18	Müller (1974)
	W Himalaya	36	Vasudevan (1975)
G. rapunculoides (Willd. ex Schultes) J. S. Pringle	Ecuador	n=18	Pringle (1981)
G. rhaetica (A. & J. Kern.) A. & D. Löve	Grossglockner	36	Favarger (1965)
G. saxosa (G. Forst.) Holub		36	Favarger (1952)
	New Zealand	36	Löve (1983)
G. serotina (Cockayne) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
G. tenuifolia (Petrie) T. N. Ho & S. W. Liu	New Zealand	36	Löve (1983)
	New Zealand	36	Favarger (1952)
G. uliginosa (Willd.) Harry Sm.	Denmark	54	Löve & Löve (1986)
G. umbellata (M. Bieb.) Holub	Caucasus	18	Gagnidze *et al.* (1992)
G. vernicosa (Cheeseman) T. N. Ho & S. W. Liu	New Zealand	36	Hair *et al.* (1980)
	New Zealand	36	Löve (1983)
***Gentianopsis* Ma**			
G. barbata (Froel.) Ma	Yunnan, China	n=13	Yuan & Küpfer (1993)
	Yunnan, China	26	Yuan & Küpfer (1993)
	Gansu, China	52	Yuan & Küpfer (1993)
	Siberia	ca.50	Agapova (1990)
	Yakuta	78	Zhukova, Korobrov and Tikhonova (1977)
	Mt. Chukotkiy	78	Agapova (1990)
	south Ural	78	Löve & Löve (1986)
	Qinghai, China	26	Liu (1994)
	Qinghai, China	26	Liu (1994)
	Yunnan, China	26	Liu (1994)
	Tunkinsky	ca.70	Krogulevich (1976)
	Qinghai, China	26	Liu (1994)
	Qinighai, China	26	Liu (1994)
G. ciliata (L.) Ma	Jura Range	n=22	Favarger (1949)
	Morocco	44	Quézel (1957)
	Mt. Tatra	44	Skalinska, Czapik and Piotrowicz (1959)
	Central Pyrénées	44	Löve & Löve (1975a)
	Central Pyrénées	78	Löve & Löve (1986)
	Eastern Ukraine	78	Löve & Löve (1986)
G. crinita (Froel.) Ma	New York, USA	78	Rork (1949)
	New York, USA	78	Rork (1949)
	Manitoba, Canada	78	Löve & Löve (1982)
	Manitoba, Canada	78	Löve & Löve (1982)
G. detonsa (Rottb.) Ma	Iceland	44	Löve (1953)

Taxon	Locality	2n	Reference
	Iceland	78	Löve (1983)
	Iceland	78	Löve & Löve (1986)
	Kashmir	n=13	Vasudevan (1975)
G. grandis Ma	Yunnan, China	n=26	Yuan & Küpfer (1993)
	Yunnan, China	52	Yuan & Küpfer (1993)
G. holopetala (A. Gray) H. H. Iltis	California, USA	n=39	Post (1983)
G. lutea Ma	Yunnan, China	n=13	Yuan & Küpfer (1993)
	Yunnan, China	26	Yuan & Küpfer (1993)
G. paludosa (Hook. f.) Ma	Gansu, China	26	Liu *et al.* (2002a)
	Qinghai, China	n=13	Liu *et al.* (2002a)
	Qinghai, China	n=13	Liu (1994)
	Qinghai, China	26	Liu (1994)
	Gansu, China	26	Yuan & Küpfer (1993)
	Gansu, China	26	Yuan & Küpfer (1993)
G. procera (T. Holm) Ma	USA	ca.80	Denniston (1913)
	Wisconsin, USA	n=39	Rork (1949)
	Manitoba, Canada	78	Löve & Löve (1982)
G. vvedenskyi (Grossh.) Pisjauk.		26	Agapova (1990)
***Halenia* Borkh.**			
H. brevicornis (Kunth) G. Don	Venezuela	n=11+2	Weaver & Rüdenberg (1975)
H. corniculata Druce	Baikal	22	Belaeeva & Siplivinsky (1975)
	Siberia	22	Krogulevich (1976, 1978)
	Russia	22	Löve & Löve (1986)
	Mongolia	22	Murin, Haberova and Zamsran (1984)
	Japan	22	Wada (1966)
H. crassiuscula B. L. Rob. & Seaton	Mexico	22	Beaman, DeJong and Stoutamire (1962)
H. cuatrecasasii C. K. Allen ex Cuatrec.	Colombia	n=11	Weaver & Rüdenberg (1975)
H. decumbens Benth.		n=11	Niehaus & Wong (1971)
H. deflexa Griseb.	Manitoba, Canada	22	Löve & Löve (1982)
		n=11	Mulligan (1967)
H. elliptica D. Don	Gansu, China	22	Yuan & Küpfer (1993)
	Gansu, China	22	Yuan & Küpfer (1993)
		22	Favarger (1952)
H. inaequalis Wedd.	Venezuela	n=11	Weaver & Rüdenberg (1975)
H. multiflora Benth.		n=11	Niehaus & Wong (1971)
H. perrottetii Griseb.	India	22	Mallikarjuna (1985)
	India	22	Mallikarjuna, Sheriff and Krishnappa (1987)
H. phyllophora C. K. Allen	Colombia	n=11	Weaver & Rüdenberg (1975)
H. rhyacophila C. K. Allen	Costa Rica	n=11	Weaver & Rüdenberg (1975)
H. shannonii Briq.	Guatemala	22	Beaman *et al.* (1962)
H. umbellata Gilg		22	Favarger & Huynh (1965)
	Peru	22	Huynh (1965)
H. viridis Gilg	Venezuela	n=11	Weaver & Rüdenberg (1975)
H. weddelliana Gilg		n=11	Pringle (1981)
***Jaeschkea* Kurz — synonymy according to Garg (1987)**			
J. oligosperma Knobl.	Himalaya	n=11	Gohil, Raina and Ashruf (1981)
(as *J. gentianoides* Kurz)	Kashmir	18	Koul & Gohil (1973)
J. canaliculata (Royle ex D. Don) Kobl.	W Himalaya	n=10	Mehra & Vasudevan (1972)
(as *J. latisepala* C. B. Clarke)	W Himalaya	n=10	Vasudevan (1975)
J. microsperma C. B. Clarke	Tibet	16	Yuan, Küpfer and Zeltner (1998)
***Lomatogoniopsis* T. N. Ho & S. W. Liu**			
L. alpina T. N. Ho & S. W. Liu	Qinghai, China	12	Liu, Ho and Chen (2002b)
***Lomatogonium* A. Braun**			
L. brachyantherum (C. B. Clarke) Fernald	Qinghai, China	16	Liu (1994)
	Qinghai, China	32	Liu (1994)
L. carinthiacum (Wulfen) Rchb.	Austria	40	Fürnkranz (1965)
	Caucasus	48	Gagnidze *et al.* (1992)
	Siberia	32	Krogulevich (1978)
	Romania	32	Löve & Löve (1986)
		n=24	Vasudevan (1975)
	Qinghai	2n=18	Liu *et al.* (2002a)
	Qinghai, China	32	Liu (1994)
L. macranthum (Diels & Gilg) Fernald	Gansu, China	16	Yuan & Küpfer (1993)

Taxon	Locality	2n	Reference
	Qinghai, China	n=8	Liu (1994)
		16	
	Qinghai, China	16	Liu (1994)
L. rotatum (L.) Fries ex Nyman	Gansu, China	16	Yuan & Küpfer (1993)
	Iceland	10	Löve (1953)
	Manitoba, Canada	10	Löve & Löve (1982)
	Iceland	16	Löve & Löve (1986)
	Qinghai, China	16	Liu *et al.* (2002a)
***Megacodon* (Hemsl.) Harry Sm.**			
M. stylophorus (C. B. Clarke) Harry Sm.	Deqin, Yunnan	28	Liu *et al.* (2002b)
Obolaria L.			
O. virginica L.	USA	56	Kondo (1970)
***Pterygocalyx* Maxim.**			
P. volubilis Maxim.	China	26	Ho & Liu (2001)
***Swertia* L. — synonymy according to Shah (1984)**			
S. abyssinica Hochst.	Ethiopia	20	Nemomissa (1994)
S. alata Royle ex D. Don	Himalaya	26	Khoshoo & Tandon (1963)
	W Himalaya, India	n=12	Mehra & Gill (1968)
	W Himalaya	n=13	Vasudevan (1975)
S. angustifolia Buch.-Ham. ex D. Don	Himalaya	26	Khoshoo & Tandon (1963)
	India	26	Mallikarjuna (1985)
	W Himalaya, India	n=12	Mehra & Gill (1968)
	W Himalaya	n=13	Vasudevan (1975)
	India	26	Mallikarjuna *et al.* (1987)
S. beddomei C. B. Clarke	India	26	Mallikarjuna (1985)
S. bifolia Batalin	China, Qinghai	28	Ho, Wang and Xue (1999)
S. bimaculata (Siebold & Zucc.) Clarke	Himalaya	n=11	Roy, Ghosh and Chatterjee (1988)
	Japan	26	Shigenobu (1982b, 1983)
	Japan	24	Wada (1954)
	Japan	26	Wada (1955, 1966)
S. binchuanensis T. N. He & S. W. Liu	China, Yunnan	n=10	Chassot *et al.* (2001)
S. brownii J. Shah (as *S. calycina* N. E. Br.)	Central Africa	20	Auquier & Renard (1975)
S. chirayita Karst.	Himalaya	26	Khoshoo & Tandon (1963)
	W Himalaya	n=13	Vasudevan (1975)
as *S. tongluensis* Burkill	India	26	Sharma & Sarkar (1970)
S. ciliata Royle ex D. Don	Himalaya	20	Khoshoo & Tandon (1963)
(as *S. purpurascens* Wall.)	W Himalaya, India	n=12	Mehra & Gill (1968)
	W Himalaya	n=10	Vasudevan (1975)
S. cordata Wall.	Himalaya	26	Khoshoo & Tandon (1963)
	W Himalaya, India	n=13	Mehra & Gill (1968)
	W Himalaya	n=13	Vasudevan (1975)
S. corymbosa (Griseb.) C. B. Clarke	India	26	Mallikarjuna (1985)
	India	n=13	Mallikarjuna *et al.* (1987)
	India	26	Mallikarjuna (1985)
	India	26	Mallikarjuna *et al.* (1987)
S. crassiuscula Gilg	Africa	20	Hedberg & Hedberg (1977)
	Africa	20	Hedberg (1957)
	E Africa	20	Thulin (1970)
	E Africa	20	Hedberg & Hedberg (1977)
S. decora Franch.	China, Yunnan	26	Chassot *et al.* (2001)
S. delavayi Franch.	China, Yunnan	26	Chassot *et al.* (2001)
S. densifolia (Griseb.) Kashyapa	India	n=13	Mallikarjuna (1985)
S. diluta (Turcz.) Benth. & Hook. f.	China, Gansu	20	Yuan & Küpfer (1993)
	Japan	20	Wada (1966)
	Japan	24	Wada (1954)
(as *Ophelia chinensis* Bunge ex Griseb.)	Russian far East	24	Volkova & Boyko (1989)
S. engleri Gilg	Ethiopia	n=10	Nemomissa (1994)
S. fimbriata (Hochst.) Cufod.	Ethiopia	2n=26	Nemomissa (1994)
S. franchetiana Harry Sm.	China, Xizang	20	Ho *et al.* (1999)
S. iberica Fisch. ex Boiss.	Georgia	26	Davlianidze (1985)
	Caucasus	26	Gagnidze, Gviniaschvil and Tschuradse (1986)
S. japonica Makino	Japan	20, 21	Shigenobu (1983)

Taxon	Locality	2n	Reference
	Japan	20	Wada (1960, 1966)
S. kilimandscharica Engl.	Africa	26	Hedberg (1957)
	Africa	26	Hedberg & Hedberg (1977)
	E Africa	26	Thulin (1970)
	Ethiopia	26	Nemomissa (1994)
S. laticalyx J. Shah	China	36	Chassot *et al.* (2001)
(as *S. pubescens* Franch.)			
S. lugardae Bullock	Ethiopia	n=10	Nemomissa (1994)
S. lurida Royle ex D. Don	W Himalaya, India	n=13	Mehra & Vasudevan (1972)
	W Himalaya	n=13	Vasudevan (1975)
S. macrosepala Gilg	Africa	26	Hedberg (1957)
	Africa	26	Hedberg & Hedberg (1977)
S. macrosperma C. B. Clarke	China	18	Chassot *et al.* (2001)
S. marginata Schrenk	Siberia	26	Krogulevich (1978)
(as *S. komarovii* Pissjaukova)			
S. minor (Griseb.) Knobl.	India	20	Mallikarjuna (1985)
	India	20	Mallikarjuna *et al.* (1987)
S. nervosa Wall.	India	26	Sharma & Sarkar (1970)
S. paniculata Wall.	Himalaya	16	Khoshoo & Tandon (1963)
	W Himalaya	n=8	Vasudevan (1975)
(as *S. dilatata* C. B. Clarke)	Himalaya	20	Khoshoo & Tandon (1963)
S. pauciflora J. Sha*h*	India	26	Mallikarjuna (1985)
(as *S. lawii* Burkill)	India	26	Mallikarjuna *et al.* (1987)
S. perennis L.	Switzerland	28	Favarger (1952)
	Alaska	28	Dawe & Murray (1979)
	Spain	28	Löve & Löve (1986)
	Colorado	n=14	Post (1983)
	Byelorussia	28	Semerenko (1985)
(as *S. cuspidata* (Maxim.) Kitagawa)	Japan	28	Shigenobu (1983)
(as *S. stenopetala* (Regel & Tiling) Pissjaukova)	Siberia	n=14	Zhukova, Petrovsky and Plieva (1973)
S. petiolata Royle	Kashmir	n=13	Jee, Dhar and Kachroo (1989)
	Himalaya	26	Khoshoo & Tandon (1963)
	Kashmir	26	Koul & Gohil (1973)
	Himalaya	n=13	Vasudevan (1975)
S. pseudochinensis Hara	Japan	20	Shigenobu (1983)
S. punicea Hemsl.	China	n=10	Chassot *et al.* (2001)
S. speciosa Wall.	Himalaya	26	Khoshoo & Tandon (1963)
	W Himalaya, India	n=13	Mehra & Gill (1968)
	W Himalaya	n=13	Vasudevan (1975)
S. subnivalis T. C. E. Fr.	Africa	26	Hedberg & Hedberg (1977)
S. swertopsis Makino	Japan	52	Shigenobu (1982b)
S. tashiroi Makino	Japan	60	Shigenobu (1982a)
	Taiwan	52	Chassot *et al.* (2001)
S. tetragona Edgew.	Himalaya	18	Khoshoo & Tandon (1963)
	W Himalaya	n=9	Vasudevan (1975)
S. tetraptera Maxim.	China, Qinghai	14	Ho *et al.* (1999)
S. tetrandra Hochst.	Ethiopia	n=10	Nemomissa (1994)
S. thomsoni C. B. Clarke	Himalaya	26	Khoshoo & Tandon (1963)
	W Himalaya	n=13	Vasudevan (1975)
S. tosaensis Makino	Japan	20	Wada (1955, 1966)
(as *S. hickinii* Burkill)	China, Anhui	20	Ho *et al.* (1999)
S. umbellata (Hook.) J. Shah	India	26	Mallikarjuna (1985)
(as *S. trichotoma* C. B. Clarke)	India	26	Mallikarjuna *et al.* (1987)
S. uniflora Mildbr.	Africa	26	Hedberg & Hedberg (1977)
S. volkensii Gilg	Africa	26	Hedberg & Hedberg (1977)
S. wolfgangiana Gruning	China, Qinghai	28	Ho *et al.* (1999)
S. yunnanensis Burkill	China, Yunnan	22	Chassot *et al.* (2001) (as 2n = 20 , erratum)

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III

Pollen Morphology of Subtribe Swertiinae (Gentianaceae – Gentianeae): Phylogenetic Considerations

P. Chassot & K. B. von Hagen [1]

[1] Institut für Geobotanik und Botanischer Garten, Martin-Luther-Universität Halle-Wittenberg

Abstract. The significance of exine pattern variation in subtribe Swertiinae (Gentianaceae-Gentianeae) was re-evaluated in comparison with recent molecular phylogenetic surveys of the Swertiinae that revealed unexpected relationships from a morphological point of view, and polyphyly in the genera *Gentianella*, *Jaeschkea*, *Lomatogonium* and *Swertia*. Pollen grains of 65 species representing all but 2 lineages (*Megacodon* and *Veratrilla*) of the Swertiinae identified by molecular results, with a particular focus on species of the genus *Swertia*, were examined by scanning electron microscopy as a complement of Nilsson's (1967) survey of tribe Gentianeae using light microscopy. The main exine patterns found in the Swertiinae are striate, striato-reticulate, rugulate, reticulate, punctate, perforate, verrucate, spinose, shortly ridged or scabrate to smooth. Many distantly related genera such as *Frasera*, *Halenia*, *Gentianella*, and lineages of *Swertia* share a common striato-reticulate to reticulate exine pattern, whereas some closely related taxa such as *Comastoma* and *Lomatogonium* p. p. or *Latouchea* and *Obolaria* have very different pollen types. In general, relationships between genera and lineages of a genus revealed by molecular analyses were not well supported by pollen morphology, except for *Pterygocalyx* and some species of *Gentianopsis*. However, in combination with other morphological and karyological characters, pollen morphology was particularly useful in characterising several lineages of the polyphyletic genus *Swertia*. According to exine characters, and in agreement with other results, the Asian *S. cuneata* is related to an African lineage of *Swertia*, *S. yunnanensis* is related to species of *Lomatogonium*, *S. swertopsis* is distinct from all other species, *S. tashiroi* and *S. rosularis* are not related to *S. bimaculata* and *S. tenuis* respectively, *S. macrosperma* is distinct from most other Asian annual or biennial species, as well as are *S. chirayita* and *S. membranifolia*. Among species not sampled in molecular studies, *S. minor* is palynologically related to *S. cuneata* and African species, and *S. piloglandulosa* to *S. macrosperma*.

Keywords: Swertiinae, pollen, taxonomy.

Introduction

According to the most recent classification of Gentianaceae (Struwe *et al.*, 2002), subtribe Swertiinae of the Gentianeae includes 14 genera (15 with *Lomatogoniopsis* T.-N. Ho & S.-W Liu) representing over 500 species distributed mainly in temperate, alpine or arctic habitats. Of these, *Gentianella* Moench (ca. 250 spp.) and *Swertia* L. (ca. 138 spp.) are the most speciose, and also include species representing an important level of morphological heterogeneity, as attest the numerous segregates proposed for these genera (for examples see Chassot *et al.*, 2001, and Hagen & Kadereit, 2001). Furthermore, recent molecular phylogenetic surveys (Chassot *et al.*, 2001 and in ms.; Hagen & Kadereit, 2001; Hagen & Kadereit, 2002) have revealed non-monophyly for *Gentianella*, *Lomatogonium* and *Swertia*, particularly pronounced in the latter, supporting the view that floral gross morphological characters such as nectaries and their appendages, presence or absence of a corolla tube, stylar and staminal traits, etc., currently used in the taxonomy of Swertiinae, are subject to high levels of homoplasy and thus unreliable for the definition of the lineages identified by the analysis of DNA sequence data. Particularly problematic is the characterisation of the various lineages of the genus *Swertia*. Although admittedly a highly polymorphic genus from a gross morphological point of view (Shah, 1984), synapomorphies confined to the lineages identified by molecular results are scarce. Chromosome numbers in the Swertiinae support molecular phylogenetic results, but only as regards the distinction between a basal grade composed of *Bartonia* Muhl., *Frasera* Walter, *Gentianopsis* Ma, *Halenia* Borkh., *Latouchea* Franch., *Megacodon* (Hemsl.) Harry Sm., *Obolaria* L., *Veratrilla* Baill. and lineages of *Swertia*, and a more derived clade including *Comastoma* Toyokuni, *Gentianella*, *Jaeschkea* Kurz, *Lomatogoniopsis* T. N. Ho & S. W. Liu, *Lomatogonium* A. Braun and again lineages of *Swertia* (Chassot *et al.*, in ms.). Within each of these broad groups, chromosome numbers are identical or largely overlapping between lineages, precluding their use in reliably characterising these lineages.

Pollen morphology in the Swertiinae is rather well documented, and has already contributed to clarifying taxonomic and phylogenetic issues in the Swertiinae. Liu & Ho (1992) complemented Nilsson's (1964) investigations in *Lomatogonium* and used some pollen features in their sectional classification of the genus. In a broad palynological survey of Gentianeae based on light microscope observations, Nilsson (1967), presented evidence of the pollen morphological distinctness of debated taxa such as *Comastoma*, *Gentianopsis* and *Megacodon*, that were recently shown to be entirely justified by molecular phylogenetic studies (Yuan & Küpfer, 1995; Struwe *et al.*, 1998, 2002; Chassot *et al.*, 2001; Hagen &

Kadereit, 2002). The inclusion of the mycotrophic genera *Bartonia* and *Obolaria* in the Swertiinae on grounds of DNA sequence data (Struwe *et al.*, 2002) also finds some palynological support (Nilsson *et al.*, 2002). As regards the genus *Swertia*, Nilsson (1967) noted that its species were pollen morphologically heterogeneous, but considered that a better taxonomic understanding of the genus was necessary before drawing further conclusions. On a regional scale, two main pollen morphological groups based on the presence or absence of spinules were recognised in African species of *Swertia* (Hedberg, 1957; Jonsson, 1973; Nemomissa, 1994), that agreed well with other morphological and karyological characters. References to additional investigations on the pollen morphology of the genera of the Swertiinae may be found in Nilsson (2002).

Following the recent detailed molecular phylogenetic surveys of subtribe Swertiinae (Chassot *et al.*, 2001 and in ms.; Hagen & Kadereit, 2001; Hagen & Kadereit, 2002), and the extensive morphological and taxonomic reviews of the genus *Swertia* (Shah, 1984; Ho *et al.*, 1988; Ho *et al.*, 1994; Nemomissa, 1994), it appeared timely to complement the pollen data available for this group, and to re-interpret pollen morphological variation in this new context. Pollen grains of 65 species representing all genera of the Swertiinae except *Megacodon* (Hemsl.) Harry Sm. and *Veratrilla* Baill. ex Franch. were thus observed with scanning electron microscopy. This work was initiated in collaboration with Siwert Nilsson, whose immense knowledge of Gentianaceae pollen was invaluable, but unfortunately had to be fulfilled by the authors alone.

Material and methods

The taxon sample includes all but 2 genera of the Swertiinae (sensu Struwe *et al.*, 2002), with special emphasis on the taxa occupying a surprising position in molecular phylogenies, and on the species of *Swertia*. For the latter, representative species of all but 9 of the 32 morphological groups defined by Shah (1984) were investigated. Five of the groups not sampled represent African species that have been studied extensively (Jonsson, 1973), whereas the remaining 4 groups are monospecific and thus not likely to have an important incidence on the pollen variation in this genus.

The pictures of *Latouchea* and most species of *Frasera* were taken by S. Nilsson at the palynological laboratory, Swedish Museum of Natural History, and are reproduced here with permission. All other species were analysed at the Interdisziplinäres Zentrum für Materialwissenschaften at the University of Halle on a Philips ESEM XL 30 FEG microscope. Because the focus of our investigations was mainly the exine structure, pollen

grains were mounted on adhesive tape and observed without further preparation. The shape reported in Table 1 should thus be considered with some caution, as pollen grains were not always homogeneously expanded. Specimens examined and voucher information are given in Table 2.

Results and discussion

Shape, size and exine ornamentation of the pollen grains investigated are given in Table 1 and illustrated in Figs 1-171. Voucher specimens are listed in Table 2. The results are presented and discussed according to the phylogenetic relationships proposed in Chassot *et al.* (2001 and in ms.) unless otherwise mentioned. For the genus *Swertia*, Pollen morphology is additionally compared with the classifications proposed by Shah (1984), and Ho *et al.* (1988; 1994) based on gross morphology.

***Obolaria, Bartonia* and *Latouchea* (Figs. 1-7).** Although closely related from a molecular phylogenetic point of view, the monotypic genera *Latouchea* and *Obolaria* have a very different exine ornamentation. Pollen grains of *Latouchea fokienensis* have a sparsely punctate tectum densely covered by spinules (Fig. 7), whereas in *Obolaria virginica*, the exine is reticulate to finely reticulate, with rounded to angular lumina (Figs. 1-3). Except for the relatively small size of pollen grains (17.1-22.5 x 12.6-17.8 µm), pollen morphology does not bring additional support to the sister relationship of these taxa. Moreover, the pollen of *Latouchea* is unique in the subtribe Swertiinae. On the other hand, *Obolaria* was considered to be related to *Bartonia* on grounds of morphological similarities such as tetramerous flowers, mycotrophic habit and imbricate aestivation, a rare trait in the Swertiinae (Hagen & Kadereit, 2002). In addition, the pollen of *Bartonia virginica* (Figs. 4-6) and *Obolaria virginica* (Figs 1-3) have a similar reticulate exine pattern. Pollen similarities between these two genera were already pointed out by Nilsson & Skvarla (1969), who also noted comparable pollen in other unrelated saprophytic genera such as *Cotylanthera* Blume and *Voyriella* (Miq.) Miq. Yet, molecular results did not support a close affinity between species of *Bartonia* and *Obolaria*.

***Pterygocalyx* and *Gentianopsis* (Figs. 8-13).** As pointed out by Nilsson (1967), both genera have palynological affinities, in agreement with molecular results. However, the exine structure of *Gentianopsis blepharophora* sampled here (Figs. 7-9) resembles more that of *Pterygocalyx volubilis* (Figs. 3-6) than that of, for example, *G. crinita*, where the muri are ring-shaped and not fused with those surrounding the adjacent lumina (see Nilsson, 2002 for illustrations). According to molecular results (Hagen & Kadereit, 2002), *Pterygocalyx* is also

more closely related to *G. ciliata* and *G. blepharophora* that to other species of *Gentianopsis*. This is also consistent with the exotestal seed structure of these species. The seeds of *P. volubilis, G. blepharophora* and *G. ciliata* are flattened with an ellipsoid wing and a smooth testa, whereas other species of *Gentianopsis* usually have ovate, wingless seeds with a testa covered by spinulous processes. Species of *Gentianopsis* with this seed type were overlooked by Chen & Ho (1998), who used this character as supporting evidence for recognising *Pterygocalyx* as a distinct genus. In addition, contrary to the description of *Pterygocalyx* in Ho & Pringle (1995), *P. volubilis* also has epipetalous nectaries similar to those of *Gentianopsis* (Chen & Ho, 1998; pers. obs.). Evidence from sequence data, gross morphology and palynology thus suggests that the differences between *Pterygocalyx* and *Gentianopsis* may in fact only be rather faint.

***Frasera* (Figs. 14-28).** The status of *Frasera* is probably the most controversial of all the taxa segregated from *Swertia* (see Shah, 1984 for details; Chassot *et al.*, 2001). Nilsson (2002) suggested that pollen data may be useful in assessing the appropriate taxonomic rank of *Frasera*. The species of this genus are pollen morphologically rather homogeneous. Pollen grains are sub-prolate to prolate, 3-colporate, and 25-42.7 x 16.6-36.7 µm. The exine is densely striate-perforate, occasionally somewhat rugulate. Only *F. umpquaensis* deviates from this pattern with an exine coarsely reticulate, and thickened and smooth at the colpus margin (Figs. 17-19). From a molecular phylogenetic standpoint, *Frasera* forms a distinct and independent lineage, but its affinities with other members of the Swertiinae are not clear. The genus was suggested to be related to *Gentianopsis* and *Pterygocalyx* (Struwe *et al.*, 2002), but this is not supported by pollen data. Furthermore, the striate-perforate pattern observed in most species of *Frasera*, although rather densely striate, also occurs in numerous species of *Swertia* and does not seem to bring additional support for the recognition of this taxon at generic level.

***Halenia* (Figs. 29-34).** Nilsson (1967) recognised 3 pollen types in the genus. *Halenia elliptica* sampled here belongs to the *H. foliosa* type. In this species, the exine is striate-perforate, but the lirae are short and arranged so as to form a broadly reticulate secondary pattern (Figs. 29-31). *Halenia weddelliana* belongs to the *H. schiedeana* type, with a regular reticulate exine (Figs. 32-34). The genus has close phylogenetic affinities with *Swertia dichotoma* (Figs. 121-123) and *S. tetraptera* (Figs. 124-126), and also with *S. bimaculata* (Figs. 118-120). These species of *Swertia* show some palynological similarities with *H. elliptica* with respect to the rather broad and rounded lirae, and sparsely perforate tectum.

This pattern is however not confined to these species, and may thus not be considered as supporting evidence for the relationships suggested by sequence data.

***Gentianella* and *Jaeschkea* (Figs. 35-55).** With exception of only a few species, the genus *Gentianella* was shown to be monophyletic (Hagen & Kadereit, 2001). In the present study, we have focused on those species that were excluded from the *Gentianella* s. str. clade by the analysis of sequence data. These species (*G. arenaria* [Figs. 41-43], *G. gentianoides* [Figs. 44-46], *G. moorcroftiana* [Figs. 47-49] and *G. azurea* [Figs. 50-52] in our sample) have 2 widely spaced nectaries per corolla lobe, in contrast to the species of *Gentianella* s. str., where the nectary is solitary or more or less deeply bilobed (*G. rapunculoides* [Figs. 35-37] and *G. turkestanorum* [Figs. 38-40] in our sample). However, evidence from sequence data suggests that the binectariate species of *Gentianella* do not form a monophyletic lineage (Hagen & Kadereit, 2002; Chassot *et al.*, in ms.). This has palynological support, as these species all have a different exine ornamentation. However, except for *G. arenaria* which has a very particular striation pattern, the variation of exine ornamentation in these species does not exceed the variability reported for species of *Gentianella* s. str. in Nilsson (1967, 2002).

Two species of *Jaeschkea*, *J. canaliculata* and *J. oligosperma* have similar pollen grains (see Nilsson, 1967, 2002 for illustrations) and are molecular phylogenetically closely related (Hagen & Kadereit, 2002). This lineage of *Jaeschkea* is related to species of *Gentianella* s. str. and *Swertia* (*S. punicea* and akin species, *S. macrosperma*). The striate-perforate exine with sharply keeled and more or less straight lirae of these species of *Jaeschkea* is similar to that observed in species of *Gentianella* s. str. (e. g. *G. turkestanorum* [Fig. 38-40]) and species of *Swertia* related to *S. punicea* such as *S. binchuanensis* (Figs. 154-156), *S. davidi* (Figs. 157-159) and *S. striata* (Figs. 160-162), but very different from the one observed in *S. macrosperma* (Figs. 163-165). *Jaeschkea microsperma* (Figs. 53-55), however, was related to *Gentianella moorcroftiana* and not to other species of *Jaeschkea*, from which it also differs from a palynological point of view. In this species, the lirae are densely spaced, not keeled, and interconnected by minute muri demarcating very small lumina. Exine ornamentation supports the separate position of *J. microsperma* with respect to other species of *Jaeschkea*, but not its relationship with *G. moorcroftiana*, which has striate-reticulate pollen grains (Figs. 47-49).

***Comastoma, Lomatogonium* and *Lomatogoniopsis* (Figs. 56-78).** As revealed by molecular data, these three genera are closely related. The species of *Comastoma* (e. g. *C. tenellum*, Figs. 56-57) form a morphologically and palynologically homogeneous group (Nilsson, 1967), also found to be monophyletic according to sequence data. In *Lomatogonium*

however, the morphology is more variable (as regards the shape of stigmas and ovary, number and structure of nectaries, habit), and molecular results suggest relationships that are at contradiction with the sectional classification based on gross morphology proposed by Liu & Ho (1992), in addition to paraphyly with respect to *Comastoma*, and a probably polyphyletic origin for certain species (e. g. *L. oreocharis* [Hagen & Kadereit, 2002]). Palynologically, all the species of the genus are now documented, either using light microscopy (Nilsson, 1964; Nilsson, 1967) or electron microscopy (Liu & Ho, 1992, present results). Nilsson (1967) recognised 2 main pollen types, i. e. the *L. gamosepalum* type with a spinose-punctate exine (Figs. 73-75), and the *L. rotatum* type with a striate-perforate to striate-reticulate exine (e. g. *L. carinthiacum*, Figs. 64-66). It is noteworthy that pollen morphology partly disagrees with both molecular phylogenetic results and the sectional classification of Liu & Ho (1992).

For example, some species not included in sect. *Lomatogonium* were found to be related to species of this section by molecular analyses. Of these species, *Lomatogonium micranthum* (Figs. 67-69) of sect. *Pleurogynella* lacks most of the morphological traits of sect. *Lomatogonium*, but has a pollen type similar to that of the species of the section. Conversely, *Lomatogonium longifolium* (Figs. 61-63) and *L. perenne* (see Liu & Ho, 1992 for illustrations) of sect. *Sarcorhizoma* have all the characteristics of sect. *Lomatogonium* except for their perennial habit. Their pollen seems to be intermediate between the spinose-punctate type of other species of sect *Sarcorhizoma* (e. g. *L. oreocharis*, Figs. 58-60) and the shortly ridged-perforate type of *L. forrestii* (see Liu & Ho, 1992 for illustrations) of sect. *Lomatogonium*.

Among the species related to *Comastoma* by molecular data, some, as *Lomatogonium graciliflorum* (Figs. 70-72) (sect. *Pleurogynella*) and *L. sikkimense* (see Liu & Ho, 1992 for illustrations) (sect. *Lomatogonium*), have pollen grains reminiscent of those of *Comastoma*, i. e. striate-reticulate to reticulate and lacking the sexine protrusion over the ora, a characteristic feature of *Lomatogonium* (Nilsson, 1967). Others, as *L. gamosepalum* and *L. brachyantherum*, have pollen grains markedly different from those of *Comastoma*, i. e. spinose-punctate, but nearly identical to those of some species of sect. *Sarcorhizoma* (e. g. *L. oreocharis*). As Nilsson (1967) pointed out, the pollen of *Lomatogoniopsis alpina* (Figs. 76-78) (as *L. bicoronatum* Harry Sm. ined.) has features of both *Lomatogonium* and *Comastoma*. The plant is morphologically also intermediate, but sequence data, however, closely related this species to the core *Lomatogonium* clade.

In conjunction with the incongruence between morphological and molecular data revealed for some species, the occurrence of a similar pollen type in distantly related lineages (e. g. *L. gamosepalum* and *L. oreocharis*), or, inversely, the occurrence of distinctly different pollen types in closely related lineages (e. g. *Comastoma* spp. and *L. gamosepalum*) could point to a hybrid origin of some taxa.

***Swertia* (Figs. 79-173).** The various lineages of *Swertia* identified by the analysis of DNA sequence data are largely in agreement with the grouping based on gross morphology established by Shah (1984), except for the subdivision of *Swertia* into 2 subgenera based on the annual (or biennial) and perennial habit of the species. Combining our palynological results with those of Nilsson (1967) and Jonsson (1973), additional support was found for several lineages of *Swertia* revealed by molecular data, as well as for some relationships that disagreed with the morphological groups of Shah (1984). Possible affinities of a few morphologically particular species that were not included in the analysis of sequence data could also be proposed.

Swertia swertopsis was placed by Shah (1984) in a monospecific group, and formed an independent lineage in molecular analyses. The exine ornamentation of this species is also unique in the genus. The tectum is punctate to slightly perforate, and covered with minute spinules or short, sharp ridge-like processes (Figs. 115-117).

Swertia tashiroi was related by Shah (1984) and Ho *et al.* (Ho *et al.*, 1988) to, i. a., *S. bimaculata*, from which it differs mainly by its single nectaries per corolla lobe and echinate seedcoat (Wang & Lu, 1998). According to molecular results, this species represents an independent lineage not related to *S. bimaculata*. Pollen morphology supports molecular results, as *Swertia tashiroi* has spinose pollen grains (Figs. 112-114), whereas the pollen of *S. bimaculata* is striate (Figs. 118-120).

Spinose pollen also occurs in some African species of *Swertia*, documented by Hedberg (1957), Nilsson (1967), and more extensively by Jonsson (1973) (but see Nemomissa [1994] for misidentified material in the latter). In addition to their pollen morphology, the African species of *Swertia* with spinose pollen are readily separable from other African species by their smooth seedcoat and chromosome number of 2n = 20 (Nemomissa, 1994). One Asian species, *S. cuneata*, also has spinose pollen (Figs. 169-171), 2n = 20 chromosomes (Chassot *et al.*, in ms.), and conforms to the characteristics of African species sharing this type of pollen. Shah (1984) placed *S. cuneata* in a monospecific group, mainly due to its Asian distribution, but Ho *et al.* (1988) included it in sect. *Swertia* (*S. perennis* and related Asian

perennial species; all species documented have striate-perforate, striate-reticulate or reticulate pollen grains [Figs. 88-99]). From a phylogenetic point of view, *S. cuneata* and the African species with spinose pollen form a monophyletic lineage only distantly related to other African species of *Swertia*, in agreement with pollen morphology. *Swertia minor* from western India could possibly also belong to this group, as it has spinose pollen (Figs. 172-173) (but note that Rao & Nagaraj [1982] reported a striate-reticulate pattern), a smooth seedcoat, and a corresponding chromosome number of 2n = 20 (Mallikarjuna, 1985).

The pollen of *Swertia macrosperma* (Figs. 163-165) and *S. piloglandulosa* (Figs. 166-168) has a verrucate to spinose-punctate exine, extremely short colpi, and pollen grains that seem to form polyads. Pollen morphology supports molecular results, as *Swertia macrosperma* formed an independent lineage in a polytomy comprising species of *Gentianella* s. str. (e. g. *G. turkestanorum* [Figs. 38-40], *G. rapunculoides* [Figs. 35-37]), *Swertia* (e. g. *S. binchuanensis* [Figs. 154-156], *S. davidi* [Figs. 157-159] and *S. striata* [Figs. 160-162]) and *Jaeschkea* (e. g. *J. canaliculata* (see Nilsson, 2002 for illustrations), for which this type of pollen was never reported. It also supports the group defined by Shah (1984) for *S. macrosperma* and a few morphologically akin species. No sequence data was available for *S. piloglandulosa*, which Shah (1984) placed in a monospecific group mainly on account of its distribution and perennial habit, but palynology strongly suggests a close relationship of this species with the *S. macrosperma* lineage.

Of special interest are *Swertia hispidicalyx*, *S tenuis* and *S. yunnanensis*. In molecular phylogenetic analyses, they formed a strongly supported clade, along with species of *Gentianella* (*G. azurea*) and *Lomatogonium* (*L. oreocharis* and *L. zhongdianense*). However, these species are morphologically (as regards number and structure of nectaries, shape of ovary, style and stigmas), and palynologically very different. Pollen morphology does not support the inclusion of such heterogeneous taxa in a same clade, but may bring some insight as regards their affinities within the Swertiinae. *Swertia hispidicalyx* (Figs. 83-85) is morphologically (Shah, 1984) related to a group of species including, i. a., *S. ciliata* (Figs. 145-147), *S. laticalyx* (Figs. 148-150) and *S. teres* (Figs. 151-153). This is also supported by pollen morphology [Nilsson, 1967 #160; present results] (pollen grains striate-perforate to striate-reticulate with sharp lirae and branched bacula). *Swertia tenuis* is unique in the genus from a gross morphological point of view (Shah, 1984), but pollen morphology (Figs. 82-84) relates this species to *Lomatogonium* (as regards shape, aperture and colpi), and in particular to *L. forrestii* (exine covered with very short, sharp ridges; see Liu & Ho [1992] for illustrations of *L. forrestii*). Moreover, Shah considered *S. rosularis* (Figs. 133-135) as a

synonym of *S. tenuis*, but, in agreement with molecular results, pollen morphology clearly refutes this synonymy. Similarly, *S. yunnanensis* has unique morphological characteristics in the genus (Shah, 1984), but has pollen grains of the *L. gamosepalum* type (Figs. 79-81). Thus, except for *S. hispidicalyx*, pollen morphology tends to support a relationship of these species with *Lomatogonium*, as suggested by molecular data, and, in combination with gross morphology, could also be interpreted as an indication of a hybrid origin of these taxa.

A few species were found to have almost smooth (e. g. *Swertia multicaulis*, Figs. 142-144) or rugulate-punctate (e. g. *S. hookeri*, Figs. 139-141) pollen grains. These species belong to a group of both molecularly and morphologically closely related taxa. A similar smooth type of pollen was reported by Nilsson (1967) for *S. handeliana*. This species shares some characteristics with *S. multicaulis* (tetramerous flowers with a single nectary per corolla lobe, smooth seedcoat), but was placed in a monospecific group by Shah (1984). As it was not included in molecular analyses, its affinities with other species of *Swertia* are still vague, but pollen morphology may support a relationship with *S. multicaulis*.

Nilsson (1967) pointed out palynological affinities between *Swertia chirayita* and *S. lurida*, two species that belong to a same morphological group, along with *S. membranifolia* sampled here (Shah, 1984). The pollen grains of *S. chirayita* (not shown) and *S. membranifolia* (Figs. 136-138) are among the smallest in the genus. The exine is striate-punctate, but the lirae are wide and low, as in no other species included in our sample. This group of species were molecularly related to *S. multicaulis* and *S. barunensis* (a species similar to *S. hookeri*). Although pollen morphology differs between *S. membranifolia*, *S. hookeri* and *S. multicaulis* as regards size, shape and exine ornamentation, the comparatively broad muri/lirae appear to confine to this group of species.

The remaining species of *Swertia* not discussed above, for which palynological data is presented here or available from other studies, have pollen grains either striate-punctate, striate-perforate, striate-reticulate or reticulate, among which our studies did not allow to distinguish clear-cut pollen types. Among these species, however, *S. decora* (Figs. 130-132) and *S. patula* (Figs. 127-129) were found to have slightly different pollen grains with respect to the thickened and shortly striate colpus margins. These 2 species were also closely related according to molecular results.

In sum, and despite the still preliminary aspect of pollen morphological studies in the Swertiinae and in the genus *Swertia*, palynological features were found to be in good overall agreement with molecular results. Although characteristics of the exine sculpture did not allow to characterise several genera or lineages (e. g. *Frasera*, *Halenia*, some lineages of

Swertia) due to insufficient variation, or did not bring additional support for some molecular relationships for the same reason (e. g. the affinities of *Halenia* with *S. dichotoma* and *S. tetraptera*, and of *Gentianella* s. str. with species of the *S. punicea* lineage), or altogether contradicted some molecular results (e. g. the sister relationship of *Latouchea and Obolaria* , relationships between some species of *Lomatogonium*), pollen morphology was found to be particularly useful in delimiting many lineages of the polyphyletic genus *Swertia* (e. g. the lineages represented by *S. membranifolia* and *S. hookeri*, *S. cuneata*, *S. macrosperma*, *S. rosularis*, *S. swertopsis*, *S. tashiroi*). This suggests that more detailed studies, in particular of cross sections using transmission electron microscopy, may uncover additional and valuable information.

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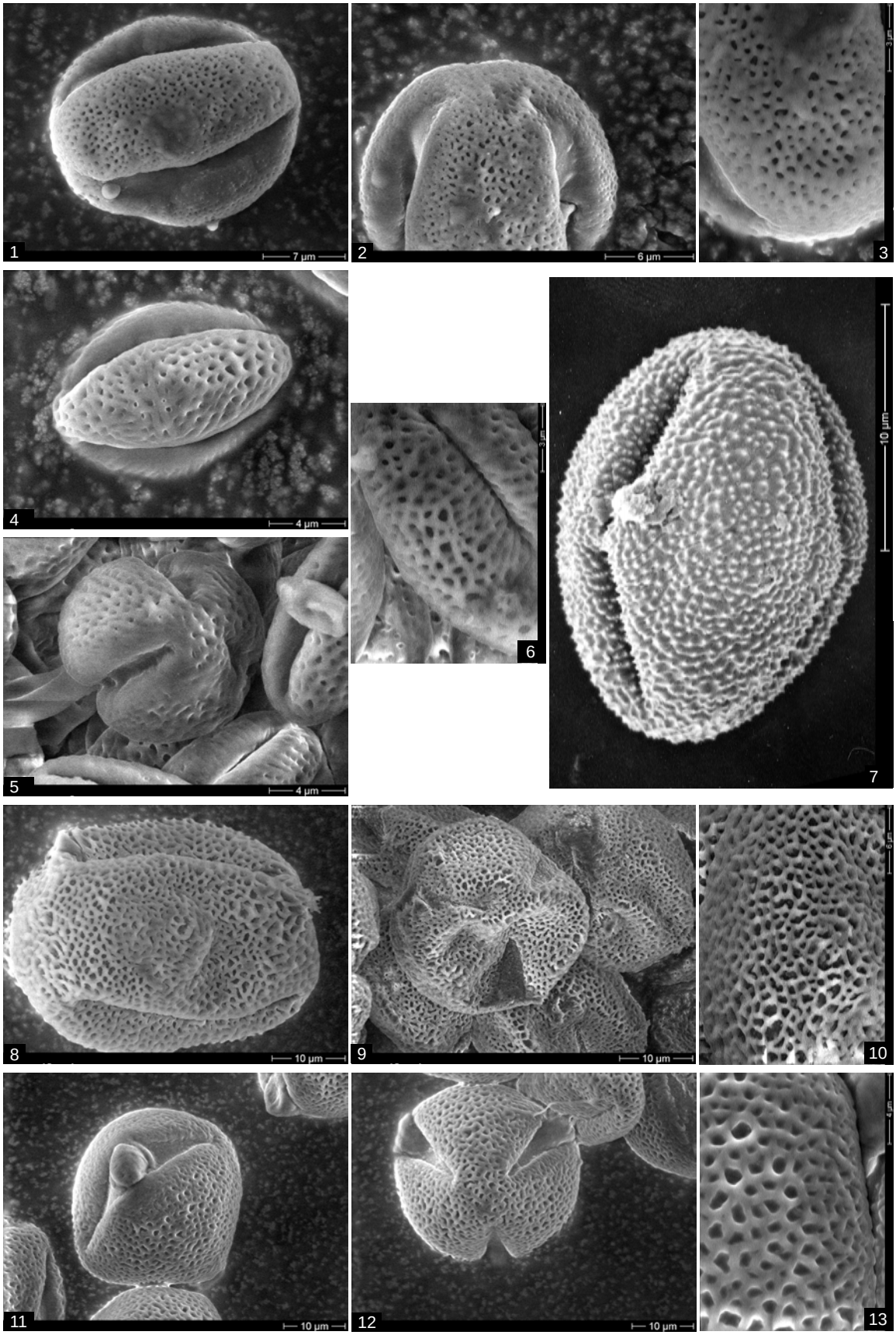
Table 1. Pollen morphology of some species of *Swertia* and other genera of the Swertiinae.

Species	Shape	P. axis [µm]	E. axis [µm]	sexine ornamentation	width of lirae, muri, rugulae, spines or verrucae [µm]	notes	Fig.
Obolaria virginica	subprolate	22.5	17.8	reticulate	0.4-0.5		1-3
Bartonia virginica	perprolate	13.3	6.3	reticulate	0.2-0.4	muri thicker than lumina	4-6
Latouchea fokienensis	prolate	17.1	12.6	spinose-punctate	0.2-0.6	spines dense	7
Pterygocalyx volubilis	subprolate	44.2	34.6	reticulate	0.3-0.6	lumina angular	8-10
Gentianopsis blepharophora	spheroidal	32.5	31.8	reticulate	0.4-0.6		11-13
Frasera speciosa	prolate	41.1	25.7	striate-perforate	0.1-0.3	lirae dense	14-16
Frasera umpquaensis	prolate	28.8	19.2	reticulate	0.7-1.4		17-19
Frasera caroliniensis	subprolate	29.6	26.1	striate-perforate	0.2-0.4		20
Frasera coloradoensis	subprolate	28.3	21.7	striate-perforate	0.2-0.4		21
Frasera gypsicola	subprolate	26.4	20.4	striate-perforate	0.1-0.4	perforations very small	22
Frasera montana	prolate	24.4	16.6	striate (rugulate)-perforate	0.1-0.4		23
Frasera neglecta	prolate	26.9	19.2	striate-perforate	0.2-0.4		24
Frasera paniculata	subprolate	25	20.8	striate-perforate	0.2-0.3		28
Frasera parryi	subprolate	28.6	24.1	striate-perforate	0.2-0.4		25
Frasera puberulenta	prolate	27.1	20.8	striate-perforate	0.1-0.3		26
Frasera tubulosa	subprolate	42.7	36.7	striate (rugulate)-perforate	0.1-1		27
Halenia elliptica	subprolate	30	25.9	irregularly striate-reticulate	0.3-0.6		29-31
Halenia weddelliana	suboblate	33	37.7	reticulate	0.4-1		32-34
Gentianella rapunculoides	suboblate	34.1	40.7	striate-perforate	0.3-0.5	lirae dense, small	35-37
Gentianella turkestanorum	prolate	56.3	30	striate-reticulate	0.3-0.4		38-40
Gentianella arenaria	prolate	33.3	24.8	striate-perforate	0.2-0.3	striae “cross-hatched”	41-43
Gentianella gentianoides	spheroidal	26.3	26.7	striate-imperforate	0.2-0.3	striae dense, striate-reticulate at pole	44-46
Gentianella moorcroftiana	subprolate	53	48.9	striate-reticulate	0.5-0.9	lirae very low	47-49
Gentianella azurea	prolate	46.2	34.5	striate-reticulate	0.3-0.4		50-52
Jaeschkea microsperma	spheroidal	35.3	37.6	striate-perforate	0.4-0.7		53-55
Comastoma tenellum	subprolate	37.9	28.5	reticulate	0.3-0.6	not all lumina perforate	56-57
Lomatogonium oreocharis	spheroidal	43.2	47.4	spinose-punctate	0.7-1.4	spines sparse	58-60
Lomatogonium longifolium	spheroidal	23.7	24.2	tuberculate (spinose)-punctate to perforate	0.4-0.6	tubercles usually dull and elongated, some spiniforme	61-63
Lomatogonium carinthiacum	subprolate	31.4	27.4	wrinkly striate perforate	0.4-0.6		64-66
Lomatogonium micranthum	spheroidal	26.7	27	striate-perforate	0.2-0.5		67-69
Lomatogonium graciliflorum	prolate	38.9	29.1	reticulate	0.5-10.3	reticulation dense	70-72
Lomatogonium gamosepalum	suboblate	38.2	44.1	spinose-punctate	0.7-1.4	spines sparse	73-75
Lomatogoniopsis alpina	subprolate	37.4	31.1	(striate)-reticulate	0.4-0.8	reticulation dense	76-78
Swertia yunnanensis	spheroidal	39.8	45	spinose-punctate	0.4-1.5	spines sparse	79-81

Species	Shape	P. axis [µm]	E. axis [µm]	sexine ornamentation	width of lirae, muri, rugulae, spines or verrucae [µm]	notes	Fig.
Swertia tenuis	suboblate	30.4	35.8	spinose to shortly cristate-punctate	0.3-0.6	spines often short ridge-shaped	82-84
Swertia hispidicalyx	spheroidal	35.7	35.7	striate-perforate	0.3-0.4		85-87
Swertia kingii	spheroidal	36.7	39.2	striate-perforate	0.4-0.7		88-90
Swertia calycina	spheroidal	25.9	29.5	striate-reticulate to reticulate	0.3-0.5		91-93
Swertia erythrosticta	spheroidal	32.1	33	striate-reticulate	0.3-0.9		94-96
Swertia perennis	prolate	41	22.1	striate-perforate	0.2-0.4		97-99
Swertia cordata	prolate	32.5	18.4	striate-perforate	0.2-0.3		100-102
Swertia angustifolia	subprolate	26.7	22.5	striate-reticulate	0.3-0.5		103-105
Swertia beddomei	prolate	26.6	17.7	striate-perforate	0.3-0.4		106-108
Swertia nervosa	subprolate	24	19.2	reticulate	0.2-0.7		109-111
Swertia tashiroi	prolate	39.4	28.8	spinose (tuberculate)-punctate	0.5-1.2	spines dull, sometimes ridge-like	112-114
Swertia swertopsis	prolate	32.4	24.1	spinose-punctate	0.5-1	spines often triquetrous or short, sharp ridge-shaped	115-117
Swertia bimaculata	spheroidal	30.9	29.6	striate-perforate	0.3-0.6	secondary broad reticulation	118-120
Swertia dichotoma	prolate	36.6	27.1	striate-punctate to perforate	0.3-0.7		121-123
Swertia tetraptera	prolate	36.8	20.2	striate-punctate to perforate	0.2-0.4		124-126
Swertia patula	subprolate	33.2	29.1	striate-reticulate	0.5-0.6	colpus margin thickened and densely striate	127-129
Swertia decora	spheroidal	30.6	29	striate-reticulate	0.4-0.7	colpus margin thickened and striate	130-132
Swertia rosularis	perprolate	29.9	12.9	striate-punctate to perforate	0.2-0.4	lirae dense	133-135
Swertia membranifolia	spheroidal	20.4	20.6	striate-perforate	0.3-0.4	lirae low and broad	136-138
Swertia hookeri	spheroidal	31	31.2	rugulate-punctate	0.6-1.4		139-141
Swertia multicaulis	subprolate	26.4	21.1	rugulate-punctate	—	nearly smooth	142-144
Swertia ciliata	subprolate	34.9	27.2	striate-perforate	0.3-0.5		145-147
Swertia laticalyx	spheroidal	32.9	35.5	striate-punctate	0.5-0.6		148-150
Swertia teres	spheroidal	31.1	32.4	striate-punctate	0.5-0.7		151-153
Swertia binchuanensis	prolate	37.5	25.4	striate-reticulate	0.5-0.6		154-156
Swertia davidi	spheroidal	26.7	26.9	striate-imperforate	0.3-0.4		157-159
Swertia striata	subprolate	32.6	26.5	striate-reticulate	0.4-0.7		160-162
Swertia macrosperma	spheroidal	33.2	33.8	verrucate-punctate	0.4-1.4	pollen grains in polyads	163-165
Swertia piloglandulosa	spheroidal	30.3	30.9	verrucate-punctate	0.4-1.4	pollen grains in polyads	166-168
Swertia cuneata	spheroidal	34.6	32.8	spinose-punctate	0.5-1.4		169-171
Swertia minor	subprolate	28.5	21.9	spinose-punctate	0.7-1.4		172-173

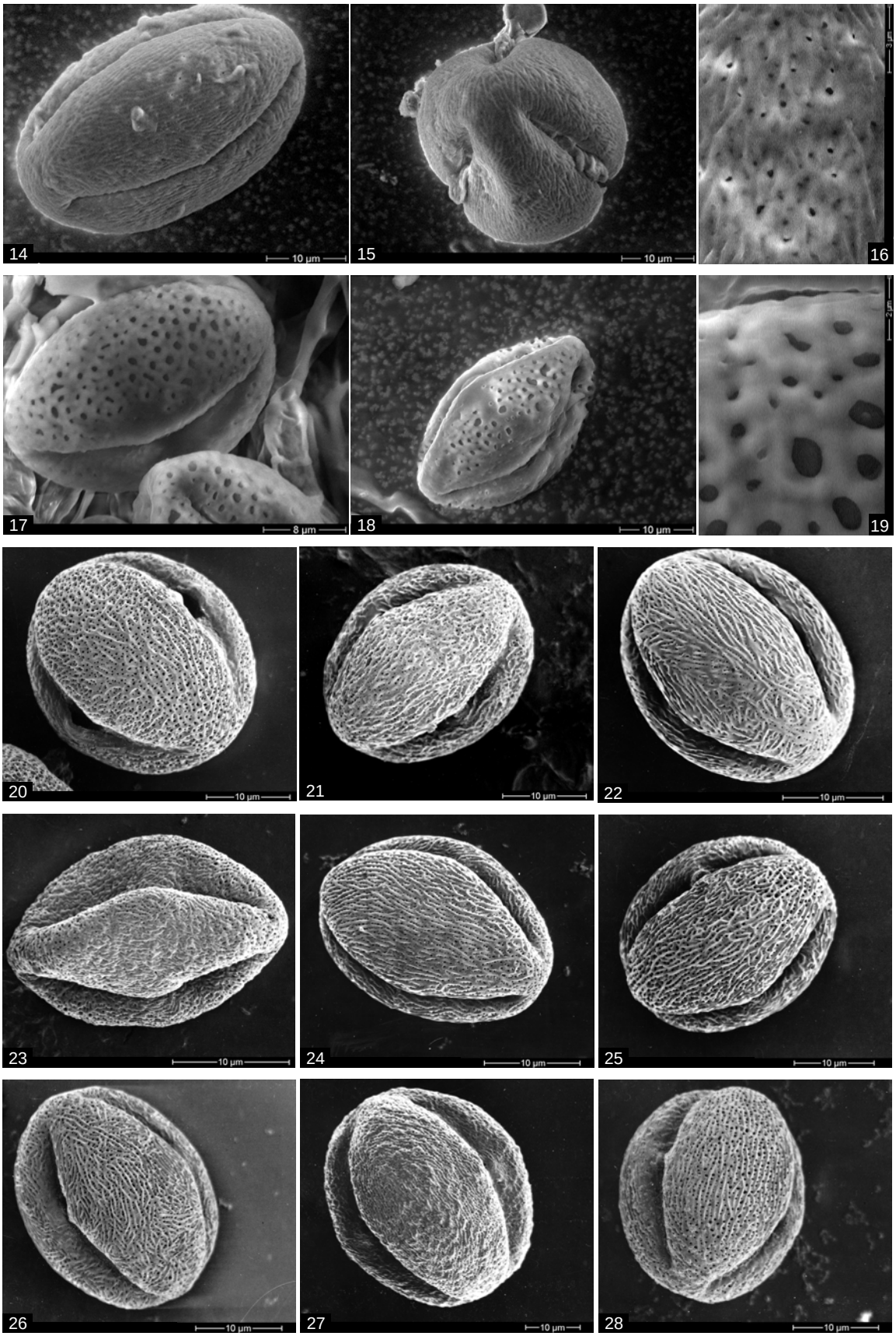
Table 2. Voucher information for the species investigated.

Bartonia virginica Britton, Sterns & Poggenb. (NEU) M. T. Strong 2394
Comastoma tenellum (Rottb.) Toyokuni (NEU) Favarger 270809
Frasera caroliniensis Walter. (NEU) PRINGLE-2727
Frasera coloradensis (Rogers) Post (NEU) Chassot 00-28
Frasera gypsicola (Barneby) Post (NEU) Chassot 00-31
Frasera montana Mulf. (NEU) Chassot 00-39
Frasera neglecta Hall (NEU) Chassot 00-22
Frasera paniculata Torr. (NEU) Chassot 00-29
Frasera parryi Torr. (NEU) Chassot 00-34
Frasera puberulenta Davidson (NEU) Chassot 00-37
Frasera speciosa Griseb. (NEU) Chassot 00-25
Frasera tubulosa Coville (NEU) Chassot 00-36
Frasera umpquaensis M. Peck & Applegate (NEU) Chassot 00-42
Gentianella arenaria (Maxim.) T.-N. Ho (NEU) Yuan & Küpfer 95-95
Gentianella azurea (Bunge) Holub (NEU) Yuan & Küpfer 92-124
Gentianella gentianoides (Franch.) Harry Sm. (NEU) Chassot & Yuan 99-201
Gentianella moorcroftiana (Wall ex Griseb.) Airy Shaw (NEU) Chassot 97-66
Gentianella rapunculoides (Willd ex Schultes) J. S. Pringle (NEU) Chassot 00-10
Gentianella turkestanorum (Gandoger) Holub (NEU) Yuan & Küpfer glla-95.8.23.2.2
Gentianopsis blepharophora (Bordz.) A. I Galushko (NEU) Yuan & Küpfer Gs-1
Halenia elliptica D. Don (NEU) Yuan cn2k2-147
Halenia weddelliana Gilg (NEU) Chassot 00-5
Jaeschkea microsperma C. B. Clarke (NEU) Yuan & Küpfer 93-105
Latouchea fokienensis Franch. (NEU) Yuan cn2k1-4
Lomatogoniopsis alpina T.-N. Ho & S.-W. Liu (NEU) Yuan & Küpfer 92-141
Lomatogonium carinthiacum (Wulf.) Reichb. (NEU) Yuan & Küpfer L-91.8.22
Lomatogonium gamosepalum (Burkill) Harry Sm. (NEU) Yuan & Küpfer L-92.9.15b
Lomatogonium graciliflorum Harry Sm. (NEU) Chassot 97-73
Lomatogonium longifolium Harry Sm. (NEU) Chassot & Yuan 99-78
Lomatogonium micranthum Harry Sm. (NEU) Chassot & Yuan 99-127
Lomatogonium oreocharis C. Marquand (NEU) Yuan & Küpfer L-92.10.3
Obolaria virginica L. (NEU) 270852 s. l.
Pterygocalyx volubilis Maxim. (NEU) Chassot & Yuan 99-100
Swertia angustifolia Buch.-Ham. (NEU) Chassot & Yuan 99-233
Swertia beddomei C. B. Clarke (NEU) Piguet 98-3
Swertia bimaculata (Sieb. & Zucc.) C. B. Clarke (NEU) Chassot 97-22
Swertia binchuanensis T.-N. Ho & S.-W. Liu (NEU) Chassot & Yuan 99-160
Swertia calycina Franch. (NEU) Yuan & Küpfer S-92.10.3d
Swertia ciliata (G. Don) B. L. Burtt (NEU) Chassot 97-8
Swertia cordata (G. Don) C. B. Clarke (NEU) Chassot 97-65
Swertia cuneata D. Don (NEU) Yuan & Küpfer 92-S2
Swertia davidi Franch. (K) Henry 4444
Swertia decora Franch. (NEU) Chassot & Yuan 99-176
Swertia dichotoma L. (NEU) Yuan & Küpfer 95-19
Swertia erythrosticta Maxim. (NEU) Yuan & Küpfer S-94.9.15.2.1
Swertia hispidicalyx Burkill (NEU) Yuan & Küpfer 92-72
Swertia hookeri C. B. Clarke (BM) Stainton 641
Swertia kingii Hook. (NEU) Yuan & Küpfer 92-S3
Swertia laticalyx J. Shah (NEU) Yuan & Küpfer S-92.10.3b
Swertia macrosperma (C. B. Clarke) C. B. Clarke (NEU) Chassot 99-11
Swertia membranifolia Franch. (NEU) Chassot & Yuan 99-186
Swertia minor (Griseb.) Knoblauch (NEU) Rao-1
Swertia multicaulis D. Don (NEU) Chassot 97-12
Swertia nervosa (G. Don) C. B. Clarke (NEU) Chassot & Yuan 99-67
Swertia patula Harry Sm. (NEU) Chassot & Yuan 99-211
Swertia perennis L. (NEU) Küpfer 01421
Swertia piloglandulosa Geesink (K) De Wild 15178
Swertia rosularis T.-N. Ho & S.-W. Liu (NEU) Chassot 99-228
Swertia striata Collett & Hemsl. (NEU) Chassot 99-240
Swertia swertopsis (Makino) (K) Makino H2000/OO139
Swertia tashiroi (Maxim.) Makino (NEU) Chassot 99-7bis
Swertia tenuis T.-N. Ho & S.-W. Liu (NEU) Yuan & Küpfer 92-216
Swertia teres (G. Don) J. Shah (NEU) Yuan & Küpfer 92-S4
Swertia tetraptera Maxim. (NEU) Yuan G016
Swertia yunnanensis Burkill (NEU) Chassot & Yuan 99-200



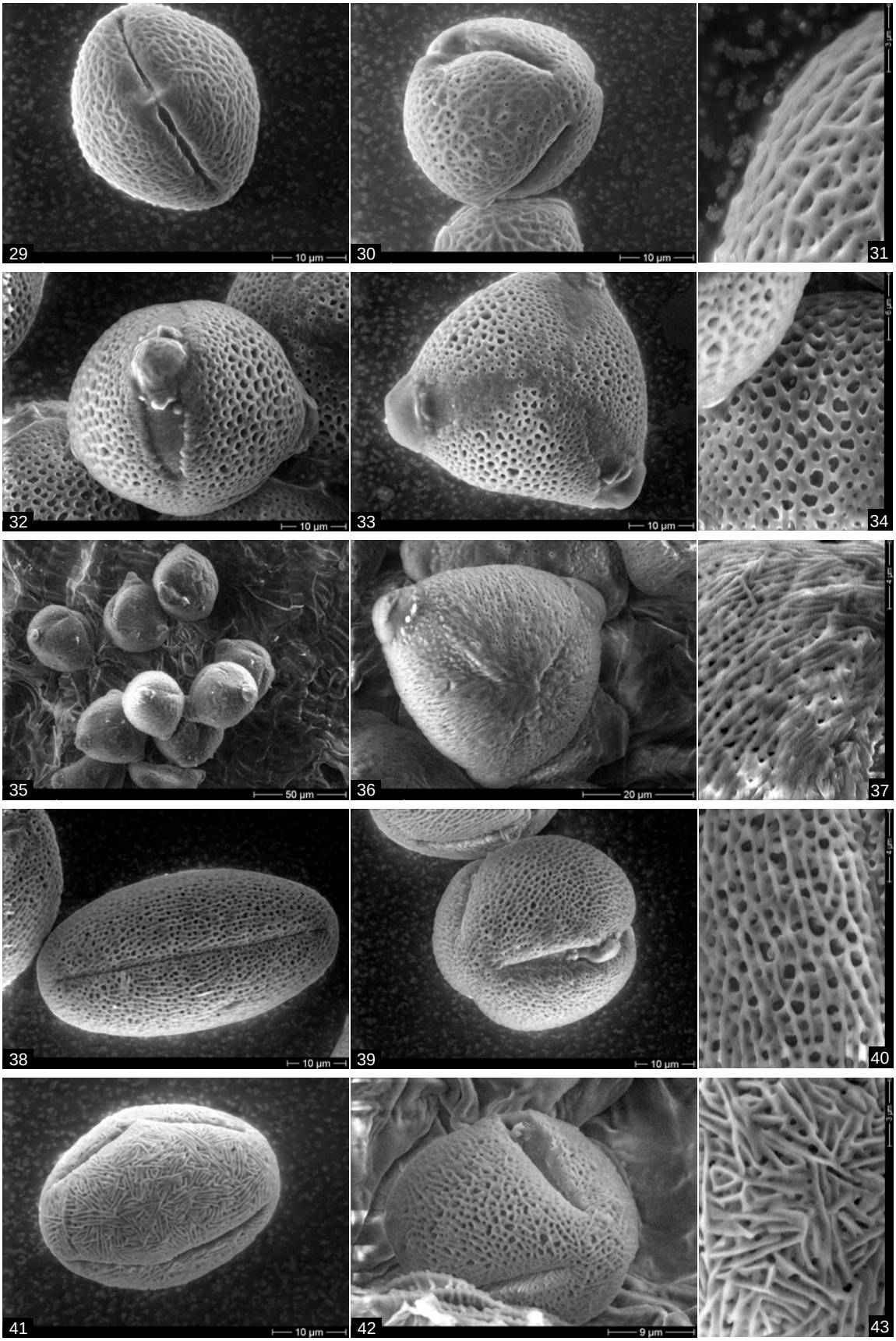
Figs. 1-13. Pollen grains of *Obolaria*, *Bartonia, Latouchea*, *Pterygocalyx* and *Gentianopsis*.

1-3 *Obolaria virginica*. 4-6 *Bartonia virginica*. 7 *Latouchea fokienensis*. 8-10 *Pterygocalyx volubilis*. 11-13 *Gentianopsis blepharophora*.



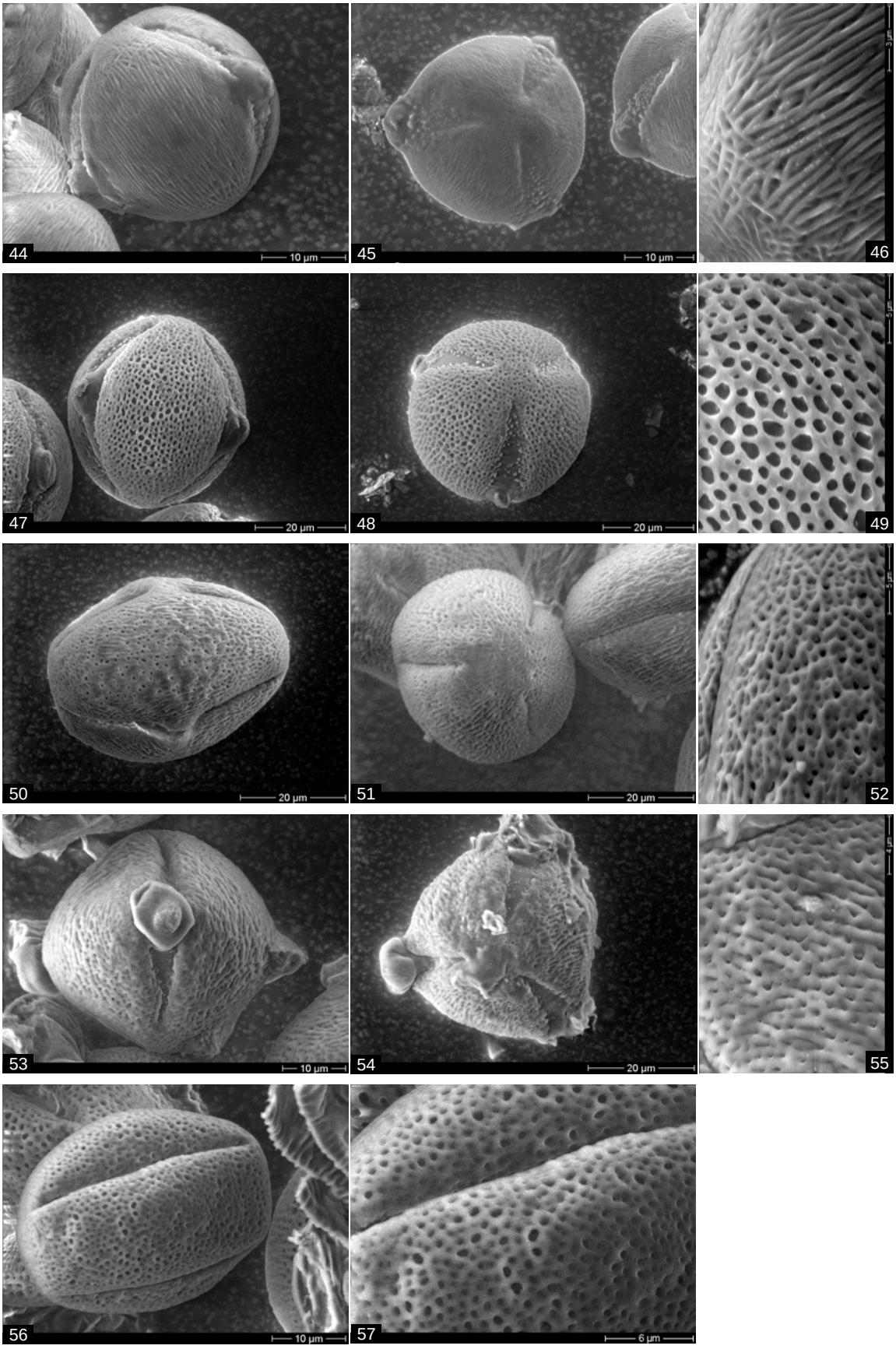
Figs. 14-28. Pollen grains of *Frasera*.

14-16 *Frasera speciosa*. 17-19 *Frasera umpquaensis*. 20 *Frasera caroliniensis*. 21 *Frasera coloradoensis*. 22 *Frasera gypsicola*. 23. *Frasera montana*. 24 *Frasera neglecta*. 25 *Frasera parryi*. 26 *Frasera puberulenta*. 27 *Frasera tubulosa*. 28. *Frasera paniculata*.



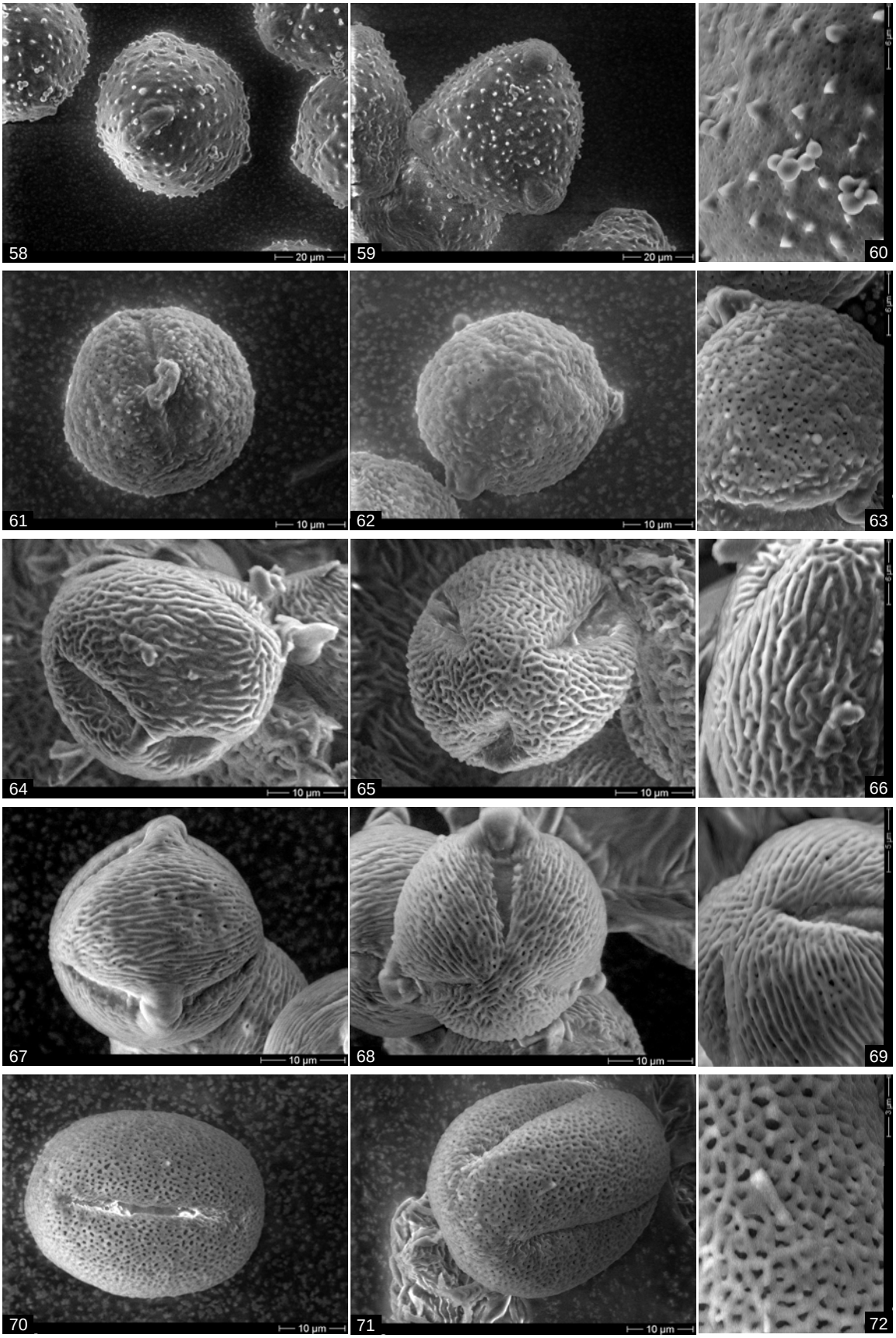
Figs. 29-43. Pollen grains of *Halenia* and *Gentianella*.

29-31 *Halenia elliptica*. 32-34 *Halenia weddelliana*. 35-37 *Gentianella rapunculoides*. 38-40 *Gentianella turkestanorum*. 41-43 *Gentianella arenaria*.



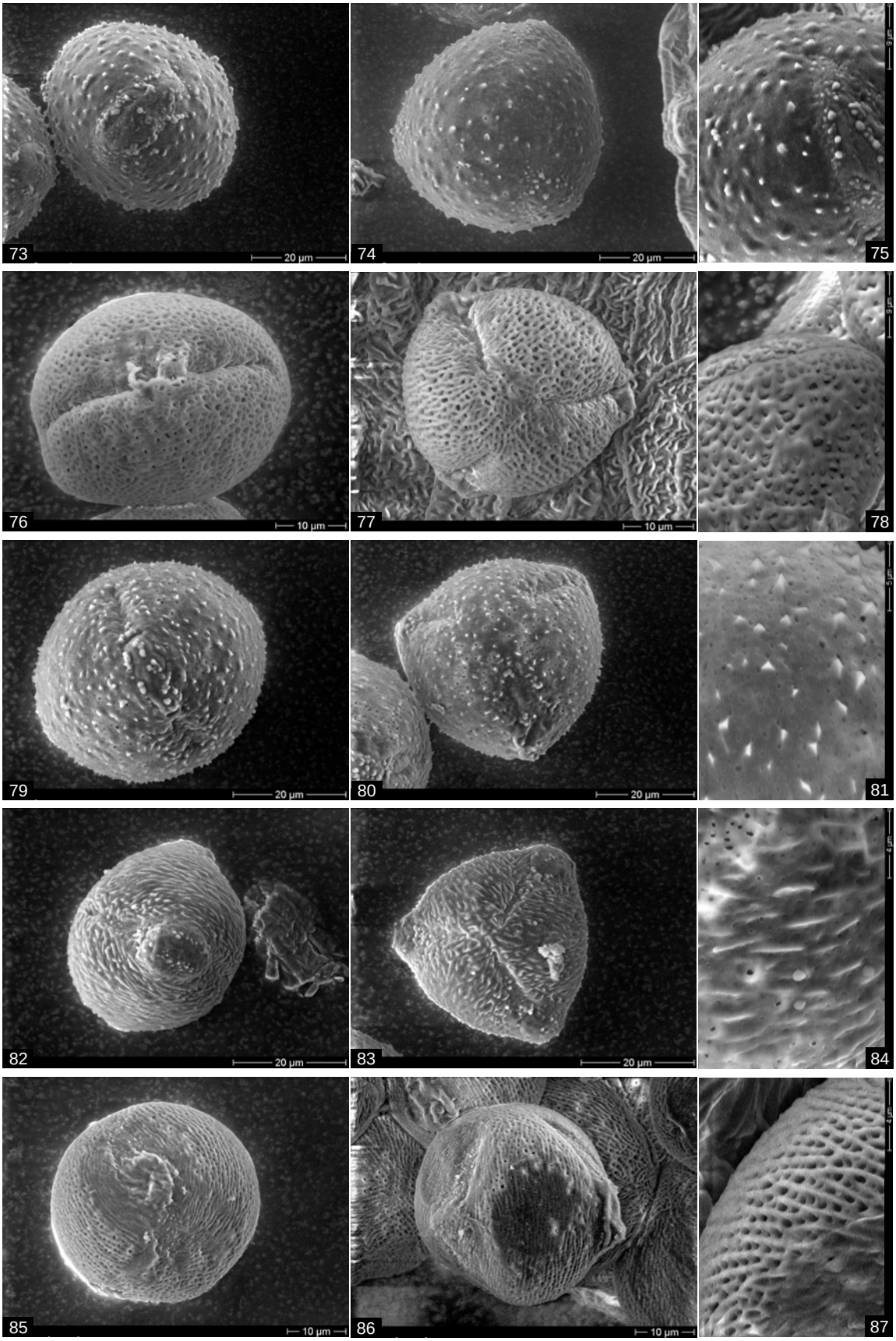
Figs. 44-57. Pollen grains of *Gentianella, Jaeschkea* and *Comastoma.*

44-46 *Gentianella gentianoides*. 47-49 *Gentianella moorcroftiana*. 50-52 *Gentianella azurea*. 53-55 *Jaeschkea microsperma*. 56-57 *Comastoma tenellum.*



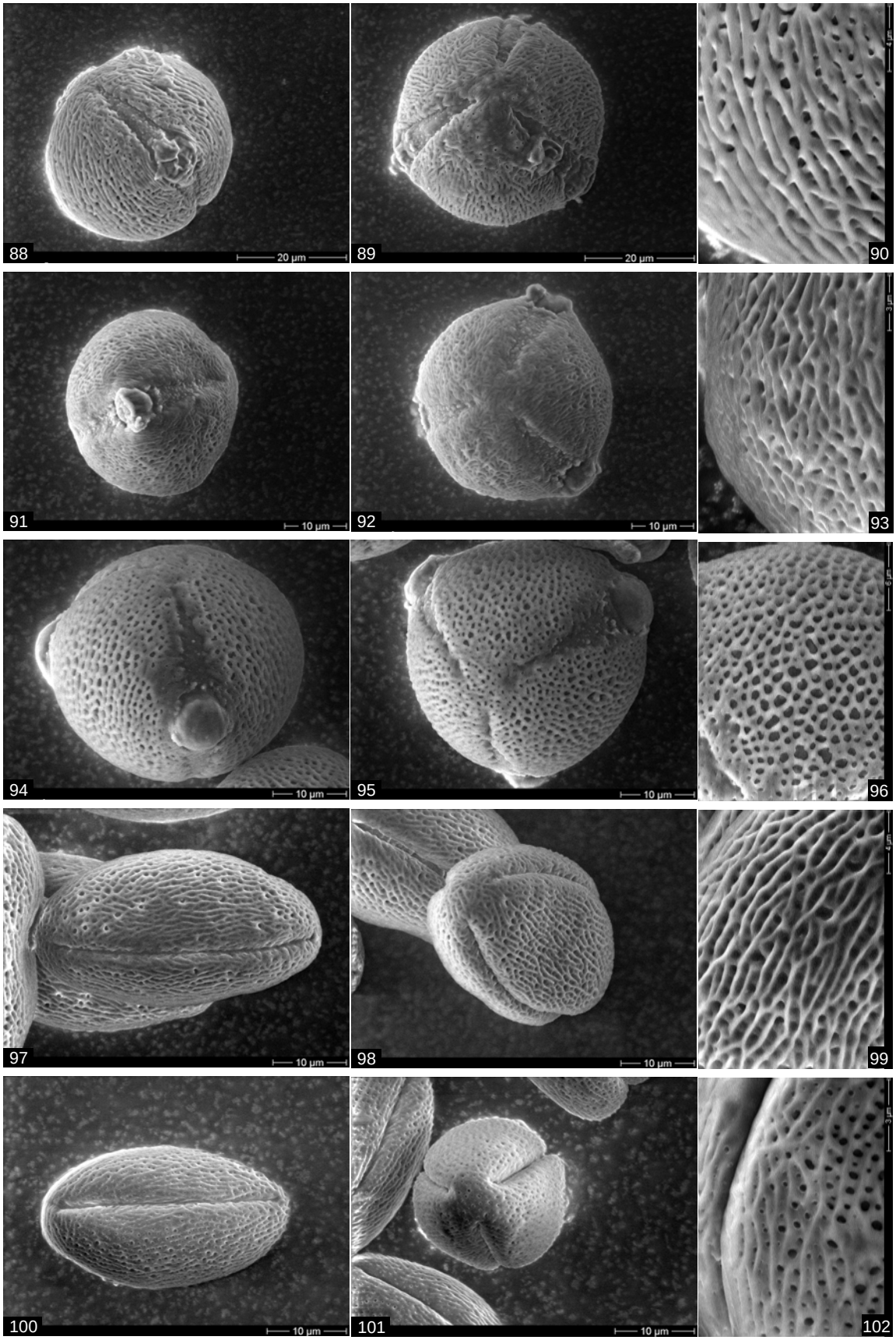
Figs. 58-72. Pollen grains of *Lomatogonium*.

58-60 *Lomatogonium oreocharis*. 61-63 *Lomatogonium longifolium*. 64-66 *Lomatogonium carinthiacum*. 67-69 *Lomatogonium micranthum*. 70-72 *Lomatogonium graciliflorum*.



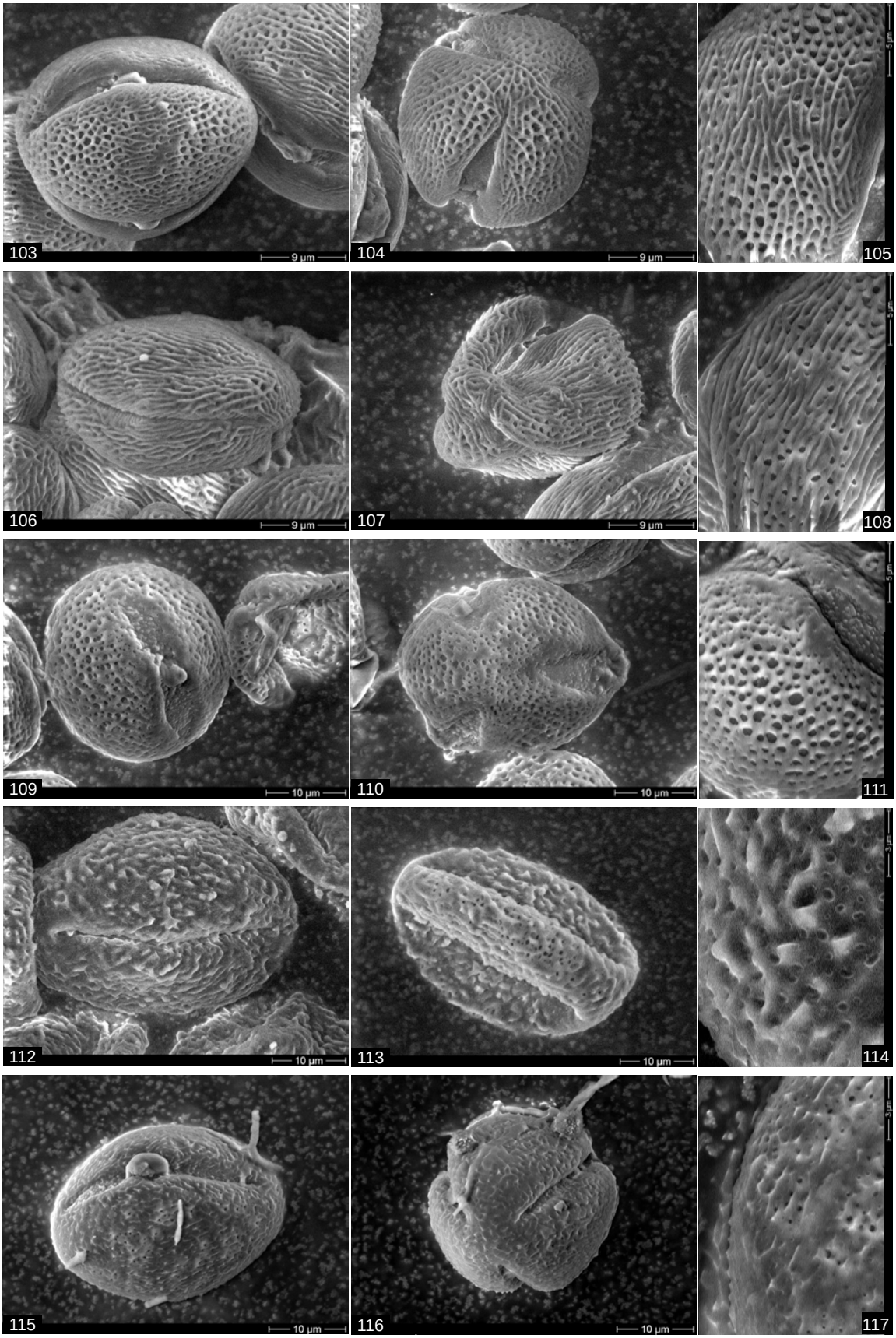
Figs. 73-87. Pollen grains of *Lomatogonium*, *Lomatogoniopsis* and *Swertia*.

73-75 *Lomatogonium gamosepalum*. 76-78 *Lomatogoniopsis alpina*. 79-81 *Swertia yunnanensis*. 82-84 *Swertia tenuis*. 85-87 *Swertia hispidicalyx*.



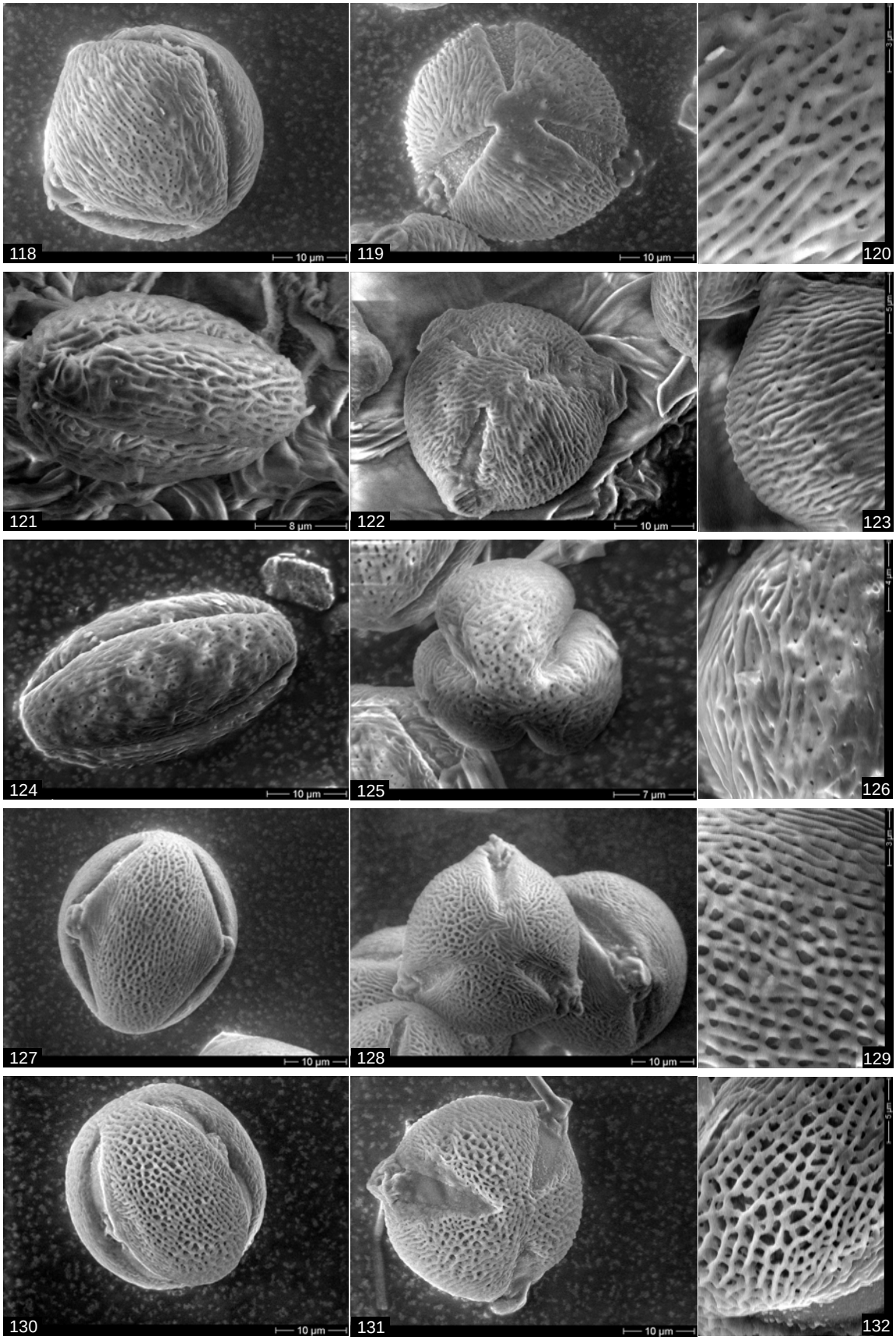
Figs. 88-102. Pollen grains of *Swertia*.

88-90 *Swertia kingii*. 91-93 *Swertia calycina*. 94-96 *Swertia erythrosticta*. 97-99 *Swertia perennis*. 100-102 *Swertia cordata*.



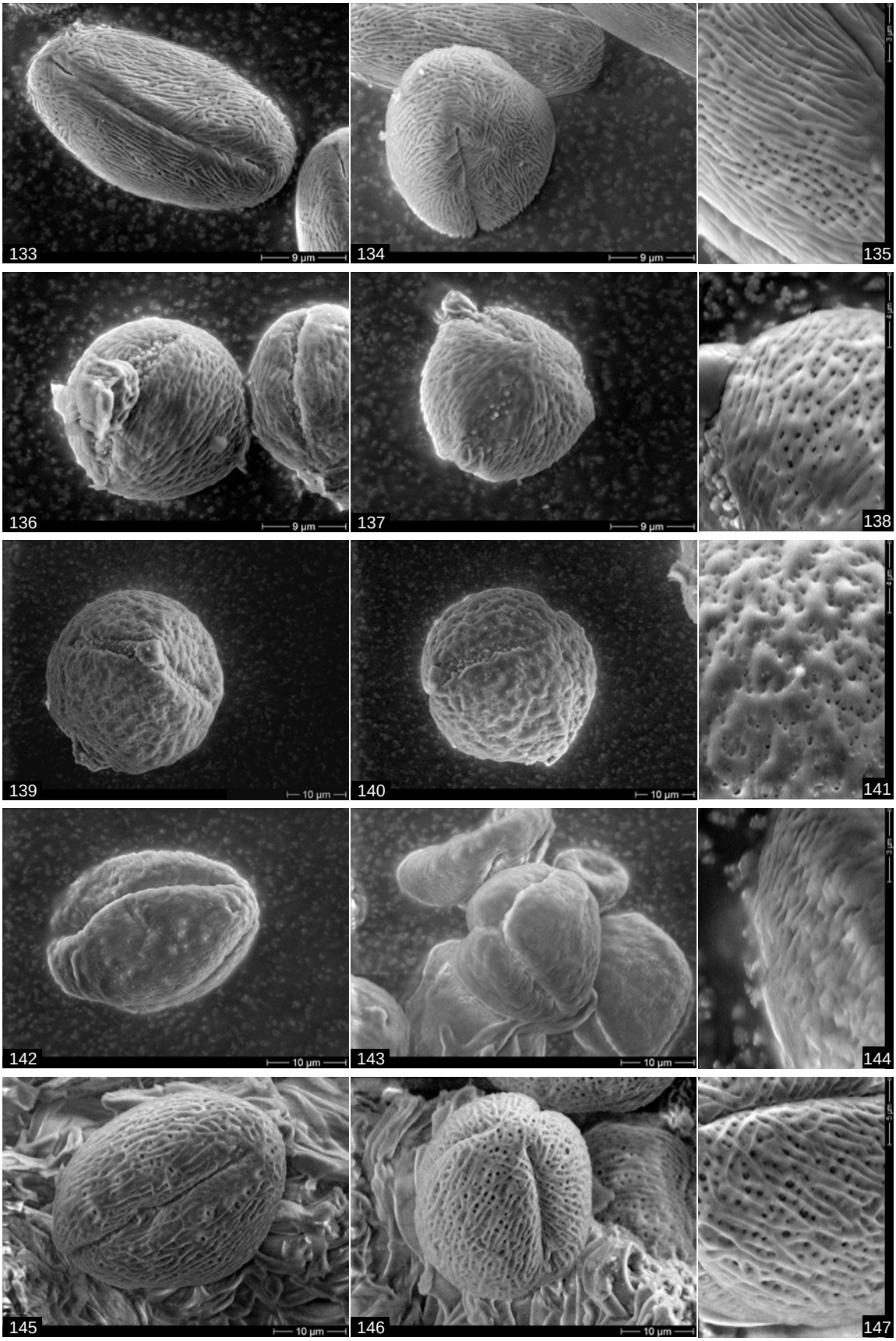
Figs. 103-117. Pollen grains of *Swertia*.

103-105 *Swertia angustifolia*. 106-108 *Swertia beddomei*. 109-111 *Swertia nervosa*. 112-114 *Swertia tashiroi* 115-117 *Swertia swertopsis*.



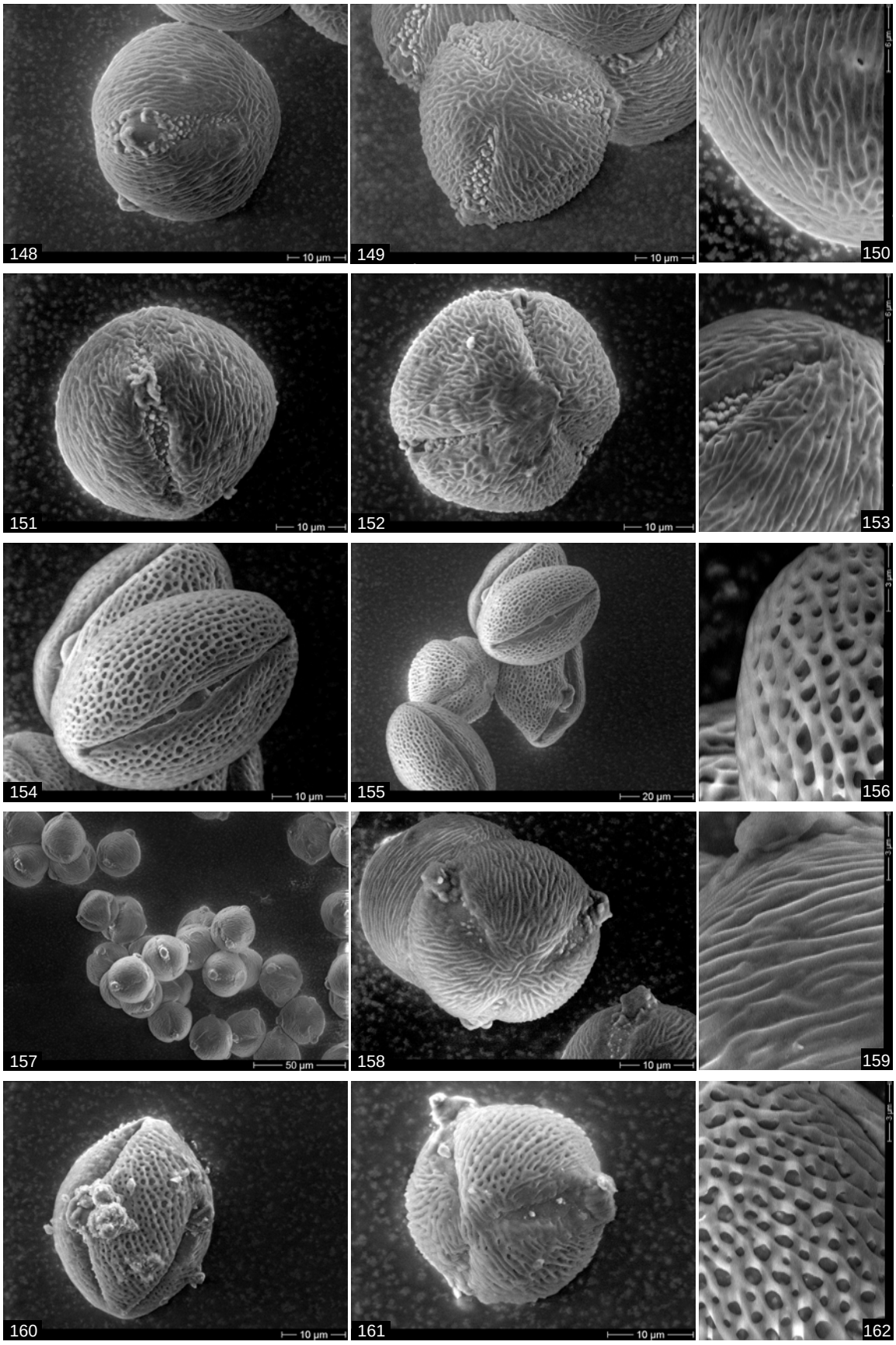
Figs. 118-132. Pollen grains of *Swertia*.

118-120 *Swertia bimaculata*. 121-123 *Swertia dichotoma*. 124-126 *Swertia tetraptera*. 127-129 *Swertia patula*. 130-132 *Swertia decora*.



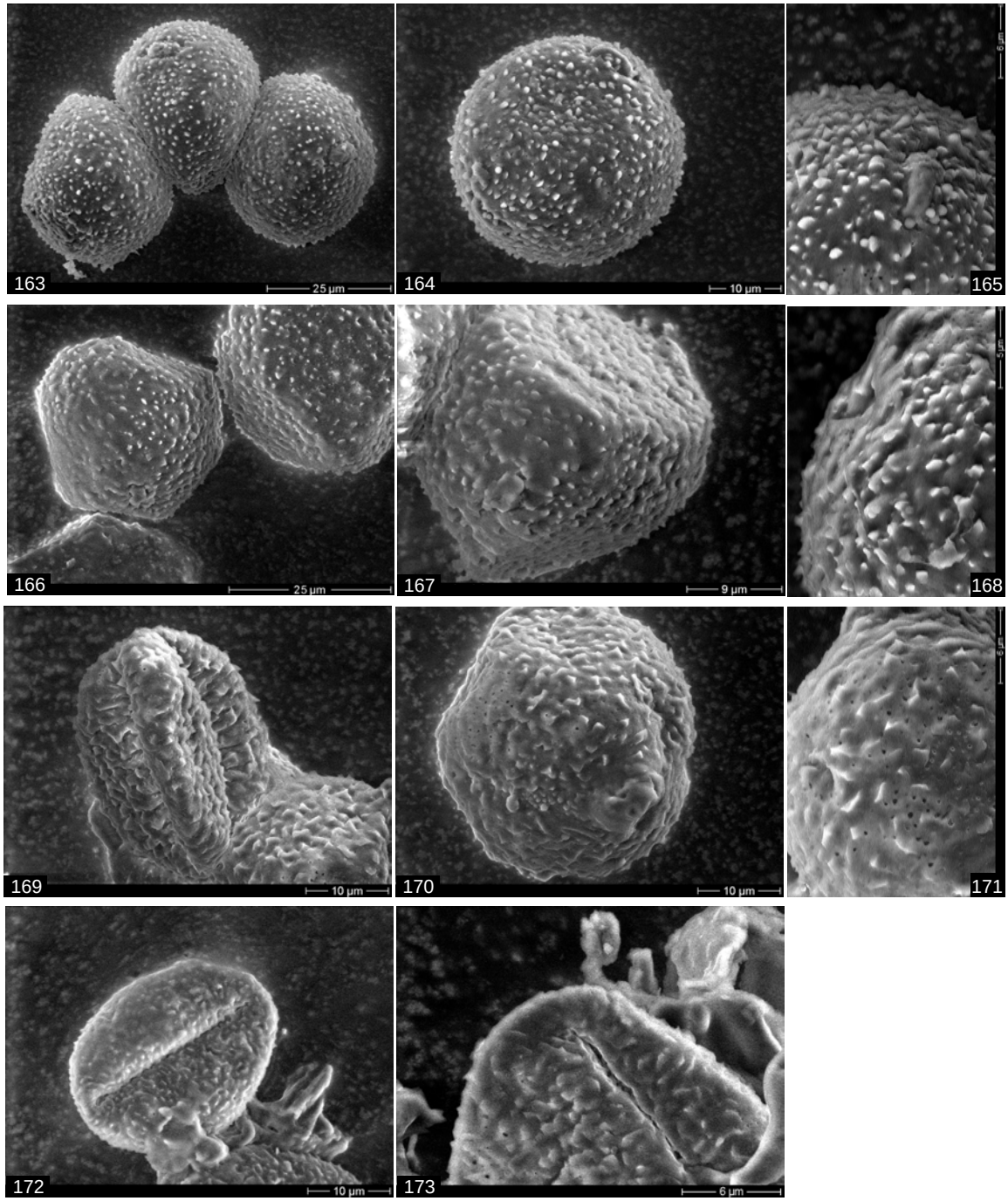
Figs. 133-147. Pollen grains of *Swertia*.

133-135 *Swertia rosularis*. 136-138 *Swertia membranifolia*. 139-141 *Swertia hookeri*. 142-144 *Swertia multicaulis*. 145-147 *Swertia ciliata*.



Figs. 148-162. Pollen grains of *Swertia*.

148-150 *Swertia laticalyx*. 151-153 *Swertia teres*. 154-156 *Swertia binchuanensis*. 157-159 *Swertia davidi*. 160-162 *Swertia striata*.



Figs. 163-173. Pollen grains of *Swertia*.

163-165 *Swertia macrosperma*. 166-168 *Swertia piloglandulosa*. 169-171 *Swertia cuneata*. 172-173 *Swertia minor*.

IV

Molecular phylogeny of the Swertiinae (Gentianaceae–Gentianeae): the lineages of *Swertia* L.

P. Chassot, Y.-M. Yuan & P. Küpfer

Abstract. Phylogenetic relationships among 128 species of 15 genera of subtribe Swertiinae (Gentianaceae-Gentianeae) were studied using chloroplast *trn*L intron, *trn*L-F and *trn*S-*ycf*9 intergenic spacer, and nuclear ITS sequences, as well as published *mat*K sequences for a reduced taxon sample. Phylogenies obtained by unweighted parsimony were similar to those inferred with the Bayesian method. Incongruent relationships inferred from nuclear and chloroplast data were explored in some detail, but found to be inconclusive. The phylogeny of the Swertiinae consisted of a basal grade comprising *Bartonia*, *Frasera*, *Gentianella* p. p. (1 lineage), *Gentianopsis*, *Halenia*, *Latouchea*, *Megacodon*, *Obolaria*, *Veratrilla* and *Swertia* p. p. (8 lineages), and of a more derived highly supported clade (clade A) including *Comastoma*, *Gentianella* p. p. (4 lineages), *Jaeschkea*, *Lomatogoniopsis*, *Lomatogonium* and *Swertia* p. p. (6 lineages). This main subdivision is also supported by chromosome numbers and seed morphology. The basic chromosome numbers of x = 13 and 14, and irregularly shaped or flattened seeds frequently with conspicuous exotestal outgrowths are characteristic of most basal taxa. Polyphyly was confirmed for *Gentianella*, *Jaeschkea*, *Lomatogonium* and *Swertia*. The North American *Obolaria* and Chinese *Latouchea* were disjunct sister taxa basal to all other Swertiinae. Other clades corresponding to genera and lineages of polyphyletic genera were individually well supported, but deeper level relationships among them were often not resolved confidently. Species of *Swertia* belonged to 14 different lineages, that were scattered across the phylogeny and either distantly or closely interrelate, or related to other genera. Of the lineages of *Swertia*, 13 are well supported by morphological and recent karyological and palynological data, but, for most of them, do not correspond to existing infrageneric taxa, or only partly. In one clade for which no corroboration from other data was found, 3 species of *Swertia* were related to species of *Gentianella* and *Lomatogonium*. All but a few species of *Swertia* not sampled here could be unequivocally related to one of the lineages defined for the genus, for which a simple key was provided. The taxonomic relevance of *Swertia* in its current circumscription is questioned, and the opportunity to define smaller genera is envisaged. The generic status of *Lomatogoniopsis* and *Pterygocalyx* is briefly touched upon, and some recent generic segregates of Asian binectariate species of *Gentianella* are discussed in a phylogenetic perspective. From a biogeographical point of view, the molecular phylogeny suggests that migrations between Asia and North America happened several times in different taxa, while an altitudinal vicariance was observed in 2 lineages of *Swertia*. A penalised likelihood estimation of divergence times led to the hypothesis that intense uplifting of the Himalaya and subsequent strengthening of the Asian monsoon at 8 ± 3 mya may have played a major role in the history of the Swertiinae, and that the last common ancestor of the Swertiinae could have lived in the early Neogene. In addition, migrations of *Swertia* from the Sino-Himalayan region to South India, Indonesia and Melanesia probably occurred only recently (Pleistocene), while migrations to Africa took place twice and at different dates.

Keywords: Bayesian, Gentianaceae, homoplasy, ILD, polyphyly, *Swertia*, taxonomy.

Introduction

Subtribe Swertiinae of the Gentianaceae-Gentianeae is a complex assemblage of polymorphic genera distributed almost world-wide in mostly temperate, alpine or arctic habitats. The centres of generic diversity are in Asia and North America, but species diversity is concentrated in the mountains of Eurasia and the southern hemisphere (*Gentianella* Moench, *Halenia* Bork.), with secondary centres in North America (*Frasera* Walter) and the mountains of tropical Africa (*Swertia* L.). Although based essentially on recent molecular phylogenetic results (Yuan & Küpfer, 1995; Struwe *et al.*, 1998, 2002; Thiv *et al.*, 1999ab), the current circumscription of the Swertiinae as the only subtribe sister to the Gentianinae does not differ much from the preceding treatment of Gilg (1895). Fourteen genera were recognised to belong to the Swertiinae in the most recent classification of Gentianaceae (Struwe *et al.*, 2002), but no unique morphological synapomorphy has yet been uncovered in support of this subtribe. All taxa included consist of annual, biennial or perennial delicate or robust herbs, occasionally twining (*Pterygocalyx* Maxim.) or mycotrophic (*Bartonia* Willd., *Obolaria* L.), but usually decumbent to erect and autotrophic. Dioecy is characteristic only of *Veratrilla* Baill., while other genera have hermaphroditic flowers. Corolla aestivation is usually convolute, but partly valvate in *Jaeschkea* Kurz, and imbricate in *Bartonia* and *Obolaria*. The flowers are gamopetalous, rotate and with a minute corolla tube in *Frasera*, *Lomatogonium* A. Braun and *Swertia*, but with a well developed tube in other genera, although not so in many species of *Gentianella*. In most genera the corolla bears nectaries at the base of the lobes, usually showy and accompanied by fimbriate appendages, or modified into spurs as in *Halenia*. However, *Latouchea* Franch., *Megacodon* (Hemsl.) Harry Sm. and *Obolaria* have nectaries in a whorl at the base of the ovary or on a distinct gynophore, as in species of subtribe Gentianinae. Yet all Swertiinae can be differentiated from the Gentianinae by the absence of a membrane between the bases of the calyx lobes (intracalycine membrane), and of elongated folds (plicae) between the corolla lobes, although a discontinuous intracalycine membrane exists in *Gentianopsis* Ma, of which some species additionally have corolla lobes fringed (but not plicate) at their base.

While molecular phylogenetics in the Gentianeae certainly clarified limits at suprageneric level, recent studies focusing on the Swertiinae (Chassot *et al.*, 2001; Hagen & Kadereit, 2002) have also demonstrated how weakly some genera are delimited, and how poorly relationships among lineages of the Swertiinae are understood. Most genera have been defined by combinations of flower morphological characters such as, i. a., the presence or

absence of a corolla tube or of a style, shape of the stigmata, ovary or petal nectaries, different types of appendages associated with the nectaries, and relative height of filament insertion. For a number of these, as *Bartonia*, *Comastoma*, *Gentianopsis*, *Halenia*, *Latouchea*, *Megacodon*, *Obolaria* and *Veratrilla*, these combinations are unambiguous and delineate homogeneous groups that were for most of them verified to be monophyletic. In other genera on the other hand, variation in these characters is often overlapping and limits are unclear. Moreover, a few genera, as for example *Swertia* (see Shah, 1984) and *Gentianella* (see Adams, 1995), altogether lack a proper definition and are merely recognised by not being one of the other well defined taxa. This has led to a proliferation of generic segregates and to considerable debate among taxonomists about their relevance.

From the same studies, it appeared that floral morphology had often undergone so radical changes between molecularly closely related lineages, that not a single supporting character could be found (e. g. *Gentianella* s. str. and its sister *Swertia* lineage, or *Comastoma*, *Lomatogonium* and binectariate species of *Gentianella*). Conversely, parallel evolution and/or morphological stasis between, e. g., some distantly related lineages of *Swertia* not only impeded the recognition of their faint affinities, but in some cases precluded a proper identification of these lineages. Variation in chromosome numbers (e. g. Toyokuni, 1965; Pringle, 1990; Yuan & Küpfer, 1993; Nemomissa, 1994; Yuan *et al.*, 1998; Ho *et al.*, 1999; Liu & Ho, 2002; Liu *et al.*, 2002), pollen morphology (e. g. Nilsson, 1967ab, 2002; Nilsson & Skvarla, 1969; Jonsson, 1973; Nemomissa, 1994), seed morphology and seedcoat structure (e. g. Maiti & Banerji, 1976; Shah, 1984; Yuan, 1993; Nemomissa, 1994) and flower anatomy (e. g. Lindsey, 1940; Gopal Krishna & Puri, 1962; Xue *et al.*, 2002) has been investigated by the past in the Swertiinae, but usually not on a sufficient number of species, and not in the perspective of transgressing the yoke of established generic concepts. Recent karyological and palynological results on more comprehensive taxon samples (Chassot & Hagen, in ms.; Chassot *et al.*, in ms.) were in good overall agreement with the existing preliminary molecular phylogenies of the Swertiinae.

In this study we have investigated relationships among 128 ingroup species representing 15 genera of the Swertiinae by using the chloroplast *trn*L-F intron and spacer and *trn*S-*ycf*9 spacer, and the nuclear ITS 1 and ITS 2 regions. Both parsimony and Bayesian approaches to phylogenetic inference were used to verify that the unexpected relationships and important level of polyphyly revealed for some genera were not due to a methodological bias. Emphasis was laid on relationships unresolved by anterior studies and on the reportedly non-

monophyletic genera *Gentianella*, *Lomatogonium* and *Swertia*. In particular for the latter, the aim was to assess the extent of polyphyly and to provide a review of morphological, karyological and palynological data supporting the lineages present in our phylogenetic hypothesis. It will be shown that each of these lineages can readily be characterised, in spite of the often important discrepancies with existing infrageneric classifications of *Swertia*, and that they probably deserve to be considered at generic rank. Relationships among genera and lineages of the Swertiinae are also discussed in a biogeographic perspective.

Materials and Methods

Taxon sampling. A total of 128 ingroup species were included in this study, representing all genera of the Swertiinae (Table 1). Considering the extensive non-monophyly revealed for certain genera of this complex by previous works (Chassot *et al.*, 2001; Hagen & Kadereit, 2002), considerable effort has been made to cover as much as possible of the potential diversity of these taxa. Special emphasis was thus laid on three genera: *Swertia*, *Lomatogonium* and the binectariate species of *Gentianella*. The sampling of *Swertia* species was defined with reference to the monograph of Shah (1984), in which the ca. 150 species were divided into 36 informal groups based on flower morphological characters essentially. 65 species are included in our sample, representing 30 of the groups recognised by Shah. The 6 groups that could not be obtained for this study in fact represent only 8 species and are not likely to influence the phylogenetic inferences at the subtribe level. For *Lomatogonium*, Liu & Ho (1992) provided a sectional classification which could be used as a guideline for sampling. Our observations on the species of *Lomatogonium* suggested that the characters used for the sectional classification are not as clear-cut as reported in Liu & Ho (1992). This has led us to include all the species for which suitable material was available. The concept of binectariate *Gentianella*, as opposed to *Gentianella* s. str. with a single nectary per corolla lobe, was first proposed by Hagen & Kadereit (2001). In their phylogeny of the genus *Gentianella*, these binectariate species were scattered across the trees and their affinities could not be assessed convincingly. Furthermore, a monophyletic binectariate *Gentianella* clade was rejected as alternative topology. It seemed that the only appropriate sample in this case was a near-exhaustive one, including one representative of each group of morphologically very closely related species. For the other genera, the sampling was conditioned essentially by the availability of adequate material for sequencing. However, in the aim of breaking down long

branches identified in Chassot *et al.* (2001), taxa were selected so as to span a maximum of the morphological and geographical diversity in each genus.

Because the level of resolution for deeper nodes was not satisfactory despite the sequencing of 3 chloroplast (*trn*L intron, *trn*L-*trn*F and *trn*S-*ycf9* intergenic spacers) and 2 nuclear (ITS 1 and ITS 2) regions, 33 *mat*K sequences available for taxa present in our sample were retrieved from Genbank and combined to our sequences (Table 2). These sequences were obtained by various authors and provide from different specimens, but we regard their combination as justified for the questions addressed here.

A preliminary analysis with 26 potential outgroup species of *Crawfurdia*, *Gentiana* and *Tripterospermum* using ITS sequence showed that the resolution of basal ingroup taxa were clearly conditioned by the species selected as outgroup. In order to break down the long branches between ingroup and outgroup taxa, 7 species of *Gentiana* and 1 species of *Crawfurdia* were selected as outgroup, representing basal and derived lineages of the 2 main sub-groups of the Gentianinae (Yuan & Küpfer, 1995).

DNA Extraction, Amplification, Sequencing and Sequence Alignment. Plant material was preferably taken from silica gel dried or fresh leaves. In some cases, herbarium specimens were used. Total Genomic DNA was extracted either following the CTAB procedure (Doyle & Doyle, 1987) or using the DNeasy Plant Mini Kit (QIAGEN AG, Basel). The leaf tissue was conventionally homogenised in the presence of liquid nitrogen or with a QIAGEN Mixer Mill MM 300 (QIAGEN AG, Basel). Extracted DNA for *Jaeschkea canaliculata* was kindly provided by Bernhard von Hagen (Halle). All DNA fragments were directly amplified by standard PCR in 25 µl reactions containing 2.5 µl 10X PCR buffer (with 1.5 mM $MgCl_2$), 0.5 µl 10mM dNTPs, 0.5 µl of 10mM each forward and reverse primers, 0.2 µl (1U) HotStar Taq polymerase (QIAGEN AG, Basel), 19.8 µl H_2O, and 1 µl (ca.10-20 ng) genomic DNA. PCRs were performed in a Biometra thermal cycler programmed for 15 min at 95°C for the activation of the HotStar Taq polymerase, followed by 35 cycles of 30 sec at 94°C, 45 sec at 55°C, 1 min at 72°C, with a final extension period of 5 min at 72°C. Primers ITS5 and ITS4 (White *et al.*, 1990) were used for the amplification of ITS of the nuclear ribosomal DNA for freshly dried samples. For herbarium samples a newly designed primer, ITSPAN (Yuan *et al.*, 2003) was used to replace the ITS5 for better amplification. The sequence of the ITSPAN primer is 5'-TCCGGTGAAGTGTTCGGATCGC-3'. It is located about 63 bp upstream of the original ITS5, and generally gave better amplification of ITS

when used in combination with ITS4 for the DNAs for which PCR amplifications failed with the primers ITS5 and ITS4. The primers "c" and "d" of (Taberlet *et al.*, 1991) were used for the amplification of the *trn*L (UAA) intron and “e” (5’-GGTTCAAGTCCCTCTATCCCC-3’) and “f” (5’-ATTTGAACTGGTGACACGAG-3’) were used for the spacer between *trn*L-(UAA)- 3’-exon and *trn*F- (GAA) gene. The spacer between *trn*S- (UGA) and ycf9 genes was amplified with primers *trn*S (5'-GAGAGAGAGGGATTCGAACC-3') and *trn*fM (5'-CATAACCTTGAGGTCACGGG-3') (Demesure *et al.*, 1995).

PCR products were checked on 1.2% agarose gel using a mini-gel applying a low voltage. There was always a single sharp band resolved on the gel for ITS, *trn*L intron and IGSs *trn*L-F and *trn*S-*ycf9* after each polymerase chain reaction. PCR products were purified using QIAquick™ PCR purification kit (QIAGEN AG, Basel) following the manufacturer's protocol prior to sequencing.

Cycle sequencing was performed using the dideoxy chain termination method using an ABI PRISM™ BigDye™ Terminator cycle sequencing kit with AmpliTaq DNA polymerase FS (Applied Biosystems) in a Biometra® Tgradient thermal cycler (5 µl reaction volumes). Cycling parameters were 25 cycles of 20 sec at 96°C for denaturation, 10 sec at 54°C for primer annealing and 4 min at 60°C for primer extension. The cycle sequencing products were cleaned by ethanol precipitation and applied to the ABI 310 automated sequencer (Applied Biosystems). Basecalling was checked on the chromatograms using Sequence Navigator (Applied Biosystems) and edited manually when necessary. Sequences have been deposited in Genbank. Their accession numbers are indicated in Table 1.

The boundaries of ITS and *trn*L intron in the study material were determined by comparison with *Gentiana* sequences (Yuan & Küpfer, 1995; Gielly & Taberlet, 1996). The limits of IGS *trn*L-F and IGS *trn*S-ycf9 were established by comparison with sequences of *Arabidopsis thaliana, Nicotiana tabacum* and *Zea mais* (Genbank numbers: AP000423, ZOOO44, X86563). Chloroplast sequences were aligned manually in order to respect indels otherwise not recognised. ITS sequences were initially aligned with ClustalX (Thompson *et al.*, 1997). A second, entirely manual alignment was also carried out, taking into consideration the conserved angiosperm motif in ITS 1 (Liu & Schardl, 1994) and the conserved and variable regions of ITS 2 (Hershkovitz & Lewis, 1996). Moreover, in doing so, no evidence was be found that any ITS sequence could represent paralogous pseudogenes as revealed for *Quercus* by Mayol & Rossello (2001). Potentially informative indels were recoded and added to the data matrix.

Maximum Parsimony Analyses. All data sets were analysed using PAUP* 4.0b10 (Swofford, 2002) and the maximum parsimony criterion using an Imac computer. For the analysis of the reduced taxa set including all genes plus *matK* sequences, the computing options were: Random taxon addition (100 replicates), TBR, steepest descent off, multiple trees on, zero length branches collapsed, gaps treated as missing, no maxtree limit. Due to computational constraints, the analysis of the complete taxa sample required a three-step approach: 1) An empirical minimum tree length was obtained with simple addition sequence and maxtree = 5000. 2) A search with 10000 random taxon addition replicates was carried out, keeping 1 tree ≤ to the length found in step 1. 3) These 10000 trees considered a fair estimate of the tree space, and were used as starting trees for a final search with maxtree = 50000 and other options as for the analysis of the reduced taxon sample. The 50000 trees were then filtered to keep only those with the best score (this is due to the "amb -" option; filtering has no incidence on the consensus topology and was done only for convenience). Different islands of trees are thus not considered and are merged in a strict consensus. The robustness of clades was evaluated using 500 jackknife replicates with 37% deletion (Farris *et al.*, 1996) (maxtree = 500), as implemented in PAUP*. Decay values were computed with AutoDecay (Eriksson, 1998) after performing heuristic searches (closest taxon addition, maxtree = 500) with converse constraints enforced in PAUP*. Trees were viewed with TREEVIEW (Page, 1996). Relative levels of homoplasy of the inferred phylogenies were evaluated using the consistency index (CI), retention index (RI) and rescaled consistency index (RC).

Bayesian Analyses. Phylogenetic inferences using full DNA substitution models were carried out using MRBAYES 2.01 (Huelsenbeck & Ronquist, 2001) on the ITS and chloroplast data separately and also combined. Substitution models were selected with MRMODELTEST (Nylander, 2002). Base frequencies were set to be estimated from the data, other options were the defaults. The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 500'000 generations with trees sampled every 10 generations for a total of 50'000 trees. Analyses were repeated twice with different starting trees to avoid local optima. Variation in the maximum likelihood scores was examined visually to determine the number of "burnin" trees. The proportion of "burnin" trees that had to be discarded varied, but a minimum of 10'000 trees with stable likelihood scores were obtained. Because the percent of the time that a branch occurs among the sampled trees is the posterior probability

of that branch existing (Huelsenbeck & Ronquist, 2001), a 50% majority rule consensus tree was computed in PAUP*, and the percent values were used as an estimate of the robustness of branches. But note that posterior probabilities tend to overestimate branch support in comparison to non-parametric resampling methods such as bootstrapping (see Whittingham *et al.*, 2002; Douady *et al.*, 2003).

Combining Data and Testing for Congruence. The incongruence length difference test (Farris *et al.*, 1994) was used to evaluate character congruence for the combined chloroplast data and the combined chloroplast and nuclear data (implemented in PAUP* as partition homogeneity test). 499 random partitions were used, with settings the same as for the main analyses, except that the closest addition sequence was used. Additionally, when the ILD test was significant at the P = 0.05 level, the test was rerun with various combinations of excluded taxa to identify those creating the conflict. The conflicting relationships suggested for these taxa were also evaluated using Templeton's (1983) significantly less parsimonious test (see below).

In view of recent results (Dolphin *et al.*, 2000) suggesting that the ILD test can erroneously indicate incongruence when homoplasy is distributed asymmetrically between data sets, the procedure suggested in Dolphin *et al.* (2000) was carried out to test the relative effect of noise on the length of trees obtained from random partitions. The method being extremely time-consuming, it was only applied to the combined ITS versus combined chloroplast data partition. It was proceeded as follows: 1) an ILD test was performed on the original partition. 2) For each data set, character states within each site were shuffled with MacClade (Maddison & Maddison, 1997) to create a series of 10 pure noise matrices. 3) The test was carried out between the first matrix of the original partition and the shuffled equivalents of the second matrix. 4) The same was repeated between the shuffled replicates of the first matrix and the original second matrix. 5) The P values and the mean difference in tree lengths (in S. D. units) between the original partition and the random partitions obtained in steps 2 and 3 were compared with those obtained in step 1 to estimate the level of incongruence.

Topological constraints. Templeton's (1983) significantly less parsimonious test (SLP test), as implemented in PAUP*, was used to test alternative topologies based on morphological, biogeographical or taxonomical considerations, and also to test some branches with discordant positions between the topologies obtained from different data sets. Constraint trees resolving only the branch to be tested were edited in Newick format and loaded as

backbone constraint (or reverse constraint) for parsimony analysis in PAUP* (10 random taxon additions, maxtree = 5000). All cladograms were then compared with 3 randomly chosen trees obtained from the unconstrained analyses (level of significance: P = 0.05).

Estimating Times of Divergence. The likelihood ratio (LR) test (Felsenstein, 1981) was used to evaluate whether the sequences of each data set evolved in a clock-like fashion. Because a molecular clock was rejected ($P_{LR} < 0.01$ for all data sets), the penalised likelihood (PL) method implemented in the program r8s (Sanderson, 2001a; Sanderson, 2002) was used to obtain at least a rough estimate of the age of some important nodes in the phylogeny of the Swertiinae. Penalised likelihood allows substitution rates to vary across branches, but a penalty is assessed when rates change from ancestral to descendant branches. The smoothing level, λ, is essential in this method, and was optimised through the data-driven cross-validation procedure implemented in r8s. The value associated with the lowest χ^2 error for a range of λ values tested was selected as the best fit for the data. Other options were truncated Newton (TN) algorithm, branch lengths with units of total numbers of substitutions, 3 different random combinations of starting divergence times, and 3 perturbed restarts after initial solution is found. Divergence times were computed for 3 trees randomly selected among those obtained from the parsimony analysis of combined data sets (1508 sites, recoded indels excluded). Outgroup taxa were pruned before further analyses (Sanderson, 2001b). Because fossil or unambiguous geological calibration points are scarce in the subtribe, the age of the root was arbitrarily set to 1 and subsequently scaled based on the estimated minimum (3.8 ma) and maximum (9.4ma) age of the split between *Gentianella* and its sister *Swertia* p. p. clade (Hagen & Kadereit, 2001). As this approach was only intended to provide approximate estimates of dating, the computationally heavy establishment of confidence intervals, via bootstrapping or analysis of likelihood surface as described in Sanderson (2001b) was not carried out, but the range of divergence time estimates obtained across the 3 analysed trees was mapped on the final tree for each node/lineage.

Table 1 Plant material and accession numbers. Sequences retrieved from the EMBL database that were not obtained from the same material as indicated here are marked with an asterisk. [(1)]Yuan & Küpfer (1995), [(2)]Gielly & Taberlet (1995), [(3)]Thiv *et al.* (1999b), [(4)]Hagen & Kadereit (2001), [(5)]Hagen & Kadereit (2002), [(6)]Chassot *et al.* (2001).

Taxon; Collector + N°; Herbarium; Locality	Genbank number				
	*trn*L(UAA) intron	*trn*S (UGA) - ycf9 spacer	*trn*L(UAA) –F (GAA) spacer	ITS1	ITS2
Bartonia virginica (L.) Britton, Stearns & Poggenb.; Strong 2394; NEU; USA, Virginia, Sussex county	AJ315185[(6)]	AJ315279[(6)]	AJ315231[(6)]	AJ318533[(6)]	AJ410312[(6)]
Comastoma cyananthiflorum (Franch.) Holub; Chassot & Yuan 99-141; NEU; China, Yunnan, Zhongdian,				Z48143*[(1)]	Z48125*[(1)]
Comastoma polycladum (Diels & Gilg) T.-N. Ho; Yuan & Küpfer 94-Cs1; NEU; China, Qinghai, Maqin					
Comastoma pulmonarium (Turcz.) Toyok.; Yuan & Küpfer 92-279; NEU ; China, Sichuan, Ganzi	AJ315225[(6)]	AJ315319[(6)]	AJ315271[(6)]	Z48108[(1)]	Z48121[(1)]
Comastoma tenellum (Rottb.) Toyok.; Favarger s. n. ; NEU; Switzerland, Grisons					
Comastoma traillianum (Forrest) Holub; Yuan & Küpfer 92-197; NEU ; China, Yunnan, Zhongdian	AJ315186[(6)]	AJ315280[(6)]	AJ315232[(6)]	AJ318534[(6)]	AJ410313[(6)]
Crawfurdia speciosa Wall.; Chassot 97-26; NEU; Nepal, Makalu	AJ242605*[(3)]			AJ294586*[(4)]	AJ294646*[(4)]
Frasera albicaulis Griseb.; Chassot 00-38; NEU; USA, California El Dorado Co.					
Frasera albomarginata S. Watson; Schweich 00-23; NEU; USA, California, San Bernardino Co.	AJ315187[(6)]	AJ315281[(6)]	AJ315233[(6)]	AJ318535[(6)]	AJ410314[(6)]
Frasera carolinensis Walter; Pringle 2727; NEU; Canada, Ontario, Halton Municip.					
Frasera coloradoensis Rogers; Chassot 00-28; NEU; USA, Colorado, Las Animas Co.					
Frasera fastigiata (Pursh) A. Heller; Chassot 00-41; NEU; USA, Idaho, Idaho Co.					
Frasera gypsicola Barneby; Chassot 00-31; NEU; USA, Nevada, Nye Co.					
Frasera montana Mulford; Chassot 00-39; NEU; USA, Idaho, Valley Co.					
Frasera neglecta Hall; Chassot 00-22; NEU; USA, California, Los Angeles Co.					
Frasera paniculata Torr.; Chassot 00-29; NEU; USA, Arizona, Navajo Co.					
Frasera parryi Torr.; Chassot 00-34; NEU; USA, California, San Diego Co.					
Frasera puberulenta Davidson; Chassot 00-37; NEU; USA, California, Inyo Co.					
Frasera speciosa Griseb.; Yuan 91-52; NEU ; USA, Colorado, Boulder	AJ315230[(6)]	AJ315324[(6)]	AJ315276[(6)]	Z48146[(1)]	Z48124[(1)]
Frasera tubulosa Coville; Chassot 00-36; NEU; USA, California, Inyo Co.					
Frasera umpquaensis Peck & Applegate; Chassot 00-42; NEU; USA, Oregon, Douglas Co.					
Gentiana frigida Haenke; Yuan 93-17; NEU ; Bulgaria, Mt. Rila	X77883*[(2)]	AJ315325[(6)]	AJ315277[(6)]	Z48063*[(1)]	Z48084*[(1)]
Gentiana leptoclada Balf. f. & Forrest; Chassot 99-239; NEU; Thailand, Chiangdao					
Gentiana lutea L.; Yuan Y91-S5; NEU ; Switzerland, Neuchâtel	X75702*[(2)]	AJ315326[(6)]	AJ315278[(6)]	Z48122[(1)]	Z48119[(1)]
Gentiana otophoroides Harry Sm.; Yuan cn2k2-66; NEU; China, Yunnan, Gongshan					
Gentiana phyllocalyx C. B. Clarke; Chassot 97-30; NEU; Nepal, Makalu	AJ315189[(6)]	AJ315283[(6)]	AJ315235[(6)]	AJ318537[(6)]	AJ410316[(6)]
Gentiana striata Maxim.; Chassot & Yuan s. n.; NEU; China, Yunnan					
Gentiana verna L.; Yuan Y93-12; NEU; Switzerland, Valais	X75704*[(2)]			Z48093[(1)]	Z48092[(1)]
Gentianella acuta (Michx) Hultén; Yuan & Küpfer 95-40; NEU; China, Xinjiang, Hanas					
Gentianella amarella (L.) Börner; Chassot 00-26; NEU; USA, New Mexico, Taos					

Taxon; Collector + N°; Herbarium; Locality	Genbank number				
	*trn*L(UAA) intron	*trn*S (UGA) - ycf9 spacer	*trn*L(UAA) –F (GAA) spacer	ITS1	ITS2
Gentianella anomala (Marquand) T.-N. Ho; Chassot & Yuan 99-144; NEU; China, Yunnan, Zhongdian					
Gentianella arenaria (Maxim.) T.-N. Ho; Yuan & Küpfer 92-76; NEU; Tibet, Karo Pass					
Gentianella azurea (Bunge) Harry Sm.; Yuan & Chassot 99-110; NEU; China, Yunnan, Deqin					
Gentianella bulgarica (Velen.) Holub; Yuan Glla-93.7.26; NEU; Bulgaria, Mt. Rhodop					
Gentianella campestris (L.) Börner; Yuan 93-G1; NEU; Italy, St. Bernard				Z48104(1)	Z48128(1)
Gentianella cerastioides (Kunth) Fabris; Chassot 00-2; NEU; Ecuador, Pichincha. Volcán Illiniza					
Gentianella fastigiata (Benth.) Fabris; R. Bu. & S. L. s. n. ; Herbarium of Stacion Scientifica Podocarpus, Zamora, Ecuador; Cajanuma, Ecuador					
Gentianella foliosa (Kunth) Fabris; Chassot 00-3; NEU; Ecuador, Pichincha. Volcán Illiniza	AJ315190(6)	AJ315284(6)	AJ315236(6)	AJ318538(6)	AJ410317(6)
Gentianella gentianoides (Franchet) Harry Sm.; Chassot & Yuan 99-201; NEU; China, Yunnan, Lijiang					
Gentianella gilioides (Gilg) Fabris; Chassot 00-16; NEU; Ecuador, Zamora, Nudo de Cajanuma					
Gentianella moorcroftiana (Wall ex G. Don) Airy Shaw ; Chassot 97-66; NEU; Nepal, Rara Lake					
Gentianella pygmaea (Regel & Schmalh.) Harry Sm. in S. Nilson; Yuan & Küpfer 95-Glla1; NEU; China, Xinjiang, Aksu					
Gentianella rapunculoides (Wild. Ex Schult.) J. S. Pringle; Chassot 00-10; NEU; Ecuador, Imbabura, laguna de Cuicocha					
Gentianella turkestanorum (Gandoger) Holub; Yuan & Küpfer Glla-95.8.23.2.2; NEU; China, Xinjiang, Aksu					
Gentianella umbellata (M. Bieb.) Holub; Küpfer 91-G3; NEU; Georgia, Mt. Caucasus	AJ315226(6)	AJ315320(6)	AJ315272(6)	Z48102(1)	Z48132(1)
Gentianopsis barbata (Froel.) Ma; Yuan & Küpfer 92-212; NEU; China, Yunnan, Lijiang					
Gentianopsis ciliata (L.) Ma; —; —; Switzerland, Neuchâtel					
Gentianopsis contorta (Royle) Ma; Chassot 97-69; NEU ; Nepal, Rara lake	AJ315191(6)	AJ315285(6)	AJ315237(6)	AJ318539(6)	AJ410318(6)
Gentianopsis grandis (Harry Sm.) Ma; Yuan & Küpfer 92-222; NEU; China, Yunnan, Lijiang	AJ315227(6)	AJ315321(6)	AJ315273(6)	Z48105*(1)	Z48130*(1)
Halenia brevicornis G. Don; Mansion & Zeltner 990115 ; NEU ; Mexico. Las Piedracitas	AJ315192(6)	AJ315286(6)	AJ315238(6)	AJ318540(6)	AJ410319(6)
Halenia corniculata (L.) Cornaz; Yuan cn2k-90; NEU; China, Jilin, Changbai Shan					
Halenia elliptica D. Don; Yuan & Küpfer 93-52; NEU; China, Sichuan, Shiqü	AJ315193(6)	AJ315287(6)	AJ315239(6)	AJ318541(6)	AJ410320(6)
Halenia weddeliana Gilg; Chassot 00-5; NEU ; Ecuador, Pichincha. Volcán Illiniza	AJ315194(6)	AJ315288(6)	AJ315240(6)	AJ318542(6)	AJ410321(6)
Jaeschkea canaliculata (Royle ex G. Don) Knobl.; J. Mugamab Sha 89; E ;	AJ408010(5)			AJ306089(5)	AJ306116(5)
Jaeschkea microsperma C. B. Clarke; Yuan & Küpfer 92-95; NEU; Tibet, Nyalam					
Latouchea fokienensis Franch.; Yuan cn2k-14; NEU; China, Fujian, Wuyishan	AJ315196(6)	AJ315290(6)	AJ315242(6)	AJ318544(6)	AJ410323(6)
Lomatogoniopsis alpina T.-N. Ho & S.-W. Liu; Yuan cn2k2-102; NEU; China, Sichuan, Batang					
Lomatogonium brachyantherum (C. B. Clarke) Fernald; Yuan & Küpfer 95-106; NEU; China, Xinjiang, Kunjerab					
Lomatogonium carinthiacum (Wulfen) Rchb.; Chassot 97-10; NEU; Nepal, Langtang					
Lomatogonium gamosepalum (Burkill) Harry. Sm. in S. Nilsson ; Yuan & Küpfer 92-83; NEU; Tibet, Karo pass					
Lomatogonium graciliflorum Harry Sm.; Chassot 97-73; NEU; Nepal, Rara Lake					
Lomatogonium lijiangense T.-N. Ho; Chassot & Yuan 99-156; NEU; China, Yunnan, Zhongdian					
Lomatogonium longifolium Harry Sm.; Chassot & Yuan 99-78; NEU; China, Yunnan, Deqin (as *L. perenne* in Chassot et al., 2001)	AJ315199(6)	AJ315293(6)	AJ315245(6)	AJ318547(6)	AJ410326(6)

Taxon; Collector + N°; Herbarium; Locality	Genbank number				
	*trn*L(UAA) intron	*trn*S (UGA) - ycf9 spacer	*trn*L(UAA) –F (GAA) spacer	ITS1	ITS2
Lomatogonium macranthum (Diels & Gilg) Fernald; Yuan 93-78; NEU; China, Sichuan, De Ge					
Lomatogonium micranthum Harry Sm.; Chassot & Yuan 99-151; NEU; China, Yunnan, Zhongdian					
Lomatogonium oreocharis (Diels) Marquand; Chassot & Yuan 99-219; NEU; China, Yunan, Lijiang					
Lomatogonium perenne T.-N. Ho & S.-W. Liu ex J.-X. Yang ; Yuan cn2k2-81; NEU; China, Sichuan, Daocheng					
Lomatogonium rotatum (L.) Fr. ex Nyman; Grant 97-02799 ; NEU; USA, Alaska					
Lomatogonium sikkimense (Burkill) Harry Sm.; Chassot 97-13; NEU; Nepal, Langtang (as *L. brachyantherum* in Chassot et al., 2001)	AJ315198[6]	AJ315292[6]	AJ315244[6]	AJ318546[6]	AJ410325[6]
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho; Chassot & Yuan 99-46; NEU; China, Yunnan, Zhongdian					
Megacodon stylophorus (C.B. Clarke) Harry Sm.; Chassot & Yuan 99-36; NEU; China, Yunnan, Zhongdian	AJ315200[6]	AJ315294[6]	AJ315246[6]	AJ318548[6]	AJ410327[6]
Obolaria virginica L.; Nicolson 24-IV-00; —; USA, Virginia	AJ315201[6]	AJ315295[6]	AJ315247[6]	AJ318549[6]	AJ410328[6]
Pterygocalyx volubilis Maxim.; Chassot & Yuan 99-100; NEU; ChinaYunnan, Deqin	AJ315202[6]	AJ315296[6]	AJ315248[6]	AJ318550[6]	AJ410329[6]
Swertia abyssinica Hochst.; Carvalho 3654; GE; Bioco, Mt. Basilè					
Swertia alata Royle ex D. Don; Chassot 97-61; NEU; Nepal, Jumla					
Swertia angustifolia Ham. ex D. Don; Chassot & Yuan 99-172; NEU; China, Yunnan, Binchuan	AJ315203[6]	AJ315297[6]	AJ315249[6]	AJ318551[6]	AJ410330[6]
Swertia atroviolacea Harry Sm.; Chassot & Yuan 99-131; NEU; China, Yunnan, Zhongdian					
Swertia barunensis P. Chassot; Chassot 97-28; NEU; Nepal, Makalu	AJ315219[6]	AJ315313[6]	AJ315265[6]	AJ318567[6]	AJ410346[6]
Swertia beddomei C. B. Clarke in Hook. f.; Piguet 98-3; NEU; South India					
Swertia bimaculata (Siebold & Zucc.) Clarke; Chassot 97-22; NEU; Nepal, Makalu	AJ315204[6]	AJ315298[6]	AJ315250[6]	AJ318552[6]	AJ410331[6]
Swertia binchuanensis T.-N. Ho & S.-W. Liu; Chassot & Yuan 99-160; NEU; China, Yunnan, Binchuan	AJ315205[6]	AJ315299[6]	AJ315251[6]	AJ318553[6]	AJ410332[6]
Swertia calcicola Kerr; Chassot 99-241; NEU; Thailand, Chiangdao					
Swertia calycina Franch.; Yuan & Küpfer 92-232; NEU; China, Yunnan, Lijiang	AJ315206[6]	AJ315300[6]	AJ315252[6]	AJ318554[6]	AJ410333[6]
Swertia chirayita (Roxb. Ex Fleming) H. Karst.; Chassot 97-2; NEU; Nepal, Langtang	AJ315207[6]	AJ315301[6]	AJ315253[6]	AJ318555[6]	AJ410334[6]
Swertia ciliata (D. Don ex G. Don) B. L. Burtt; Chassot 97-6; NEU; Nepal, Langtang	AJ315208[6]	AJ315302[6]	AJ315254[6]	AJ318556[6]	AJ410335[6]
Swertia cincta Burkill; Yuan & Küpfer 92-218; NEU; China, Yunnan, Lijiang					
Swertia cordata (Wall. ex D. Don) C. B. Clarke; Chassot 97-17; NEU; Nepal, Langtang	AJ315209[6]	AJ315303[6]	AJ315255[6]	AJ318557[6]	AJ410336[6]
Swertia crassiuscula Gilg; Wohlhauser 98-5; NEU; Kenya, Mt. Kenya	AJ315210[6]	AJ315304[6]	AJ315256[6]	AJ318558[6]	AJ410337[6]
Swertia cuneata Wall. ex D. Don; Chassot 97-14; NEU; Nepal, Langtang	AJ315211[6]	AJ315305[6]	AJ315257[6]	AJ318559[6]	AJ410338[6]
Swertia davidi Franch.; Hao 418; NEU; Cult. in Inst. For Traditional Medicine, Chongqing, China					
Swertia decora Franch.; Chassot & Yuan 99-176; NEU; China, Yunnan, Dali	AJ315212[6]	AJ315306[6]	AJ315258[6]	AJ318559[6]	AJ410339[6]
Swertia delavayi Franch.; Chassot & Yuan 99-66; NEU; China, Yunnan, Zhongdian	AJ315213[6]	AJ315307[6]	AJ315259[6]	AJ318561[6]	AJ410340[6]
Swertia dichotoma L.; Küpfer & Yuan 95-19; NEU; China, Gansu, Mingle	AJ408016*[5]				
Swertia diluta (Turcz.) Benth. & Hook. f.; Yuan G199; NEU; China, Gansu, Dangchang					
Swertia engleri Gilg;; Bingeli 02-1; NEU; Ethiopia, Mussolini tunnel					
Swertia erythrosticta Maxim.; Küpfer & Yuan S-94.9.15.2.1; NEU; China, Sichuan, Song Pan					
Swertia fimbriata (Hochst.) Cufod.; Bingeli 02-2; NEU; Ethiopia, Mussolini Tunnel					
Swertia franchetiana Harry Sm.; Yuan & Küpfer 92-S5; NEU; Tibet, Jitang					

Taxon; Collector + N°; Herbarium; Locality	Genbank number				
	*trn*L(UAA) intron	*trn*S (UGA) - ycf9 spacer	*trn*L(UAA) –F (GAA) spacer	ITS1	ITS2
Swertia hispidicalyx Burkill; Yuan & Küpfer 92-72; NEU; China, Tibet, Nagarze	AJ315215[6]	AJ315309[6]	AJ315261[6]	AJ318563[6]	AJ410342[6]
Swertia japonica Makino; Chassot 99-20; NEU; Japan, Kumamoto pref., Aso					
Swertia kilimandscharica Engl.; Sileshi Nemomissa 990725-2/1; ETH; Ethiopia, Bale mts.	AJ315216[6]	AJ315310[6]	AJ315262[6]	AJ318564[6]	AJ410343[6]
Swertia kingii Hook. f.; Yuan & Küpfer 92-111; NEU; Tibet, Nyalam					
Swertia kouitchensis Franch.; Chassot 99-226; NEU; China, Sichuan, Emei Shan					
Swertia laticalyx J. Shah. (as *S. pubescens*); Yuan & Küpfer 92-224; NEU; China, Yunnan, Lijiang	AJ315220[6]	AJ315314[6]	AJ315266[6]	AJ318568[6]	AJ410347[6]
Swertia macrosperma C.B. Clarke; Yuan & Küpfer 92-213; NEU; China, Yunnan, Lijiang	AJ315217[6]	AJ315311[6]	AJ315263[6]	AJ318565[6]	AJ410344[6]
Swertia membranifolia Franch.; Chassot & Yuan 99-186; NEU; China, Yunnan, Dali					
Swertia multicaulis D. Don; Yuan & Küpfer 92-112; NEU; Tibet, Nyalam					
Swertia mussotii ; Yuan & Küpfer 94-S9; NEU; China, Sichuan, Song Pan					
Swertia nervosa Wall.; Chassot & Yuan 99-67; NEU; China, Yunnan, Zhongdian					
Swertia papuana Diels; Hoogland & Pullen 6005; GE; New Guinea					
Swertia patula Harry Sm.; Chassot & Yuan 99-211; NEU; China, Yunnan, Lijiang					
Swertia pauciflora (Dalzell) J. Shah; Piguet 98-1; NEU; South India					
Swertia perennis L.; Küpfer 98-10; NEU; Switzerland, Jura	AJ315218[6]	AJ315312[6]	AJ315264[6]	AJ318566[6]	AJ410345[6]
Swertia af. *pinetorum* Kerr; Chassot 99-236; NEU; Thailand, Chomtong					
Swertia paniculata Wall.; Chassot 97-27; NEU; Nepal, Makalu					
Swertia pumila Hochst.; Sileshi Nemomissa s. n.; ETH; Ethiopia,					
Swertia punicea Hemsl.; Chassot & Yuan 99-63; NEU; China, Yunnan, Zhongdian	AJ315221[6]	AJ315315	AJ315267[6] [6]	AJ318569[6]	AJ410348[6]
Swertia randaiensis Hayata; Chassot 99-2; NEU; Taiwan, Kuanwu					
Swertia rosularis T.-N. Ho & S.-W. Liu; Chassot 99-228; NEU; China, Sichuan, Emei Shan					
Swertia rosulata (Baker) J. Klackenberg; Wohlhauser M023; NEU; Madagascar					
Swertia souliaei Burkill; Chassot & Yuan 99-218; NEU; China, Yunnan, Lijiang					
Swertia speciosa Hook. f.; Chassot 97-46; NEU; Nepal, Makalu					
Swertia striata Collett & Hemsl.; Chassot 99-240; NEU; Thailand, Chiangdao					
Swertia swertopsis Makino; Komatsu s. n.; NEU; Japan, Miyazaki pref.					
Swertia tashiroi (Maxim.) Makino; Chassot 99-7b; NEU; Taiwan, Hsinchi	AJ315222[6]	AJ315316[6]	AJ315268[6]	AJ318570[6]	AJ410349[6]
Swertia tenuis T.-N. Ho & S.-W. Liu; Yuan & Küpfer 92-216; NEU; China, Yunnan, Lijiang					
Swertia teres (G. Don) J. Shah; Yuan & Küpfer 92-117; NEU; Tibet, Nyalam					
Swertia tetrapetala Pall.; Pobidimovu et al. 5136; GE; Russia, Sachalin, Iturup Is.					
Swertia tetraptera Maxim.; Yuan & Küpfer 92-315; NEU; China, Gansu, Magu	AJ315229[6]	AJ315323[6]	AJ315275[6]	Z48115[1]	Z48139[1]
Swertia tozanensis Hayata; Chassot 99-7; NEU; Taiwan, Hualien					
Swertia volkensi Gilg; Wohlhauser 98-4; NEU; Kenya, Mt. Kenya	AJ315223[6]	AJ315317[6]	AJ315269[6]	AJ318571[6]	AJ410350[6]
Swertia welwitschii Engl.; Schlieben 662; GE ; Tanganyika, Lupembe					
Swertia younghusbandii Burkill; Yuan & Küpfer 92-130; NEU; Tibet, Dangxiong					
Swertia yunnanensis Burkill; Chassot & Yuan 99-200; NEU; China, Yunnan, Lijiang	AJ315224[6]	AJ315318[6]	AJ315270[6]	AJ318572[6]	AJ410351[6]
Veratrilla baillonii Franch.; Chassot & Yuan 99-37; NEU; China, Yunnan, Zhongdian	AJ315188[6]	AJ315282[6]	AJ315234[6]	AJ318536[6]	AJ410315[6]

Results

Alignment and Sequence Characteristics

***trn*L Intron.** 84 *trn*L intron sequences were new and 52 were retrieved from EMBL (Table 1). The intron length varied between 391 bp in *Gentianella umbellata* and *G. turkestanorum*, and 346 in *Gentiana striata*. The alignment for all taxa had 526 sites and was straightforward, except for 111 sites located mostly after the first ca 100 bp where multiple indels and slipped-strand mispairing occurred. This region was excluded because of alignment ambiguity. A total of 103 potentially informative sites and 16 potentially informative indels were used.

***trn*L - *trn*F Spacer.** 88 *trn*L - *trn*F spacer sequences were new and 48 were retrieved from EMBL (Table 1). The spacer length varied between 161 in *Gentianella arenaria* and 408 in *Crawfurdia speciosa*. The alignment for all taxa had 657 sites, and homology assessment was

Table 2 Accession numbers for *matK*1 and *matK*2 sequences retrieved from the EMBL database with references.

Taxon	Reference	Genbank number	
		***mat*K1**	***Mat*K2**
Bartonia virginica (L.) Britton, Stearns & Poggenb.	Struwe *et al.* (2002)	AJ388141	AJ388210
Comastoma cyananthiflorum (Franch.) Holub	Hagen & Kadereit (2001)	AJ406324	AJ406353
Comastoma tenellum (Rottb.) Toyok.	Struwe *et al.* (2002)	AJ388149	AJ388218
Crawfurdia speciosa Wall.	Thiv *et al.* (1999b)	AJ010512	AJ011441
Frasera albicaulis Griseb.	Hagen & Kadereit (2001)	AJ406325	AJ406354
Frasera parryi Torr.	Hagen & Kadereit (2002)	AJ408029	AJ408022
Frasera tubulosa Coville	Hagen & Kadereit (2002)	AJ408030	AJ408023
Gentiana frigida Haenke	Struwe *et al.* (2002)	AJ388166	AJ388236
Gentianella amarella (L.) Börner	Hagen & Kadereit (2001)	AJ406326	AJ406355
Gentianella arenaria (Maxim.) T. N. Ho	Hagen & Kadereit (2001)	AJ406328	
Gentianella azurea (Bunge) Harry Sm.	Hagen & Kadereit (2001)	AJ406331	406359
Gentianella cerastioides (Kunth) Fabris	Thiv *et al.* (1999b)	AJ010518	AJ011447
Gentianella moorcroftiana (Wall ex G. Don) Airy Shaw	Hagen & Kadereit (2001)	AJ406338	AJ406366
Gentianopsis ciliata *(L.) Ma*	Struwe *et al.* (2002)	AJ388164	AJ38823
Halenia corniculata (L.) Cornaz	Struwe *et al.* (2002)	AJ388168	AJ388238
Jaeschkea canaliculata (Royle ex G. Don) Knobl.	Hagen & Kadereit (2002)	AJ408031	AJ408024
Lomatogonium carinthiacum (Wulfen) Rchb.	Hagen & Kadereit (2001)	AJ406346	AJ406374
Lomatogonium oreocharis (Diels) Marquand	Struwe *et al.* (2002)	AJ388174	AJ388244
Lomatogonium rotatum (L.) Fr. ex Nyman	Hagen & Kadereit (2002)	AJ408032	AJ408025
Megacodon stylophorus (C.B. Clarke) Harry Sm.	Struwe *et al.* (2002)	AJ388177	AJ388247
Obolaria virginica L.	Struwe *et al.* (2002)	AJ388180	AJ388250
Swertia abyssinica Hochst.	Struwe *et al.* (2002)	AJ388191	AJ388261
Swertia bimaculata (Siebold & Zucc.) Clarke	Hagen & Kadereit (2002)	AJ408033	AJ408026
Swertia cordata (Wall. ex D. Don) C. B. Clarke	Hagen & Kadereit (2002)	AJ408034	AJ408027
Swertia crassiuscula Gilg	Hagen & Kadereit (2001)	AJ406347	AJ406375
Swertia dichotoma L.	Hagen & Kadereit (2002)	AJ408035	AJ408028
Swertia japonica Makino	Hagen & Kadereit (2001)	AJ406348	AJ406376
Swertia macrosperma C.B. Clarke	Hagen & Kadereit (2001)	AJ406349	AJ406376
Swertia perennis L.	Thiv *et al.* (1999b)	AJ010528	AJ011457
Swertia punicea Hemsl.	Hagen & Kadereit (2001)	AJ406350	AJ406378
Swertia teres (G. Don) J. Shah (as *S. racemosa* (Wall. ex Griseb.) C. B. Clarke)	Hagen & Kadereit (2001)	AJ406351	AJ406379
Swertia volkensi Gilg	Hagen & Kadereit (2001)	AJ406352	AJ406380
Veratrilla baillonii Franch.	Struwe *et al.* (2002)	AJ388196	AJ388266

difficult. The *trn*L - *trn*F spacer contained many hotspots where multiple and sometimes homoplastic indels occurred. In some cases the large taxon sample allowed to identify how sequences evolved across taxa, which in turn helped identifying reliable sites. Nevertheless, only 335 sites were kept for the analysis. A total of 137 parsimony informative characters and 34 potentially informative indels were used. One deletion appeared to be specially informative, as it is a synapomorphy for nearly half of the taxa (*S. rosularis* and upward species in Figs. 1 and 2). This deletion is covered by a larger one and thus not informative in *Gentianella arenaria*, *G. pygmaea*, *Gentianella moorcroftiana*, *Jaeschkea microsperma* and *Swertia cuneata*.

***trn*S - *ycf*9 Spacer.** 88 *trn*S - *ycf9* spacer sequences were new and 48 were retrieved from EMBL (Table 1). The spacer length varied between 220 bp in *Gentiana phyllocalyx* and 364 bp in *Frasera montana* and *Gentianella moorcroftiana*. The alignment for all taxa had 486 sites and was unambiguous, except for 2 poly-N regions and 3 regions with multiple indels. A total of 121 potentially informative sites and 27 potentially informative indels were kept for analysis.

Internal Transcribed Spacer. 83 sequences of the ITS 1 and 2 regions were new and 53 sequences were obtained from EMBL (Table 1). ITS 1 and 2 sequences were mostly 229 ± 2 bp long, with very rare indels of more than 1 bp. The alignment of the combined ITS 1 and 2 regions had 555 sites. A total of 324 parsimony informative characters and 11 potentially informative indels were used. The manual alignment was preferred over the ClustalX alignment for 2 reasons: 1) Alignments generated by ClustalX for a range of gap penalties were always sensibly shorter than the manual alignment. Too few gap insertions was shown to be a common error of automated alignments in presence of long branches (Simmons & Freudenstein, 2003). Although a large taxon sample, such as is the case here, is expected to break down long branches, several lineages including one or few species only, that apparently diverged rather early in the history of the subtribe, necessarily have long branches that cannot be broken down (see Discussion). 2) The manual alignment produced phylogenetic trees more in agreement with those based on chloroplast data, and, to some extent, with morphological and karyological data.

***mat*K and other Sequence Data.** A reduced matrix was build including 33 species for which *mat*K sequences were available in addition to the other regions sequenced (for

Gentianella arenaria, only *mat*K 1 was available). The aligned *mat*K sequences were 697 bp long and had 90 parsimony informative characters. One potentially informative indel was recoded and added to the matrix.

Parsimony Analyses

ITS Data. The analysis of ITS 1 sequences yielded a relatively well resolved strict consensus tree but with very low branch support, whereas that of ITS 2 sequences resulted in a largely polytomic consensus tree, also with very low support (not shown). The analysis of combined ITS data resulted in an unknown number of trees (26653 kept) of 2059 steps (HI = 0.65, RI = 0.64 RC = 0.42; strict consensus in Fig. 1B). Several small clades were well resolved and received good jackknife and decay support, but, within clade A and the basal grade, relationships between clades were highly variable and involved very short branches that all collapsed in the strict consensus.

Chloroplast Data. The individual strict consensus trees resulting from the analysis of the three chloroplast data sets separately, although poorly resolved, were similar to each other as regards their overall topology and the few well-supported clades (not shown). The analysis of the combined chloroplast data sets resulted in an unknown number of trees (5879 kept) of 1345 steps (HI = 0.33, RI = 0.84, RC = 0.28; strict consensus in Fig. 1A). The chloroplast phylogeny was similar to the ITS one as regards shallower clades (see Table 3 for differences) and overall topology (clade A and basal grade), and resolved deeper nodes slightly better, although 2 large polytomies were still present. Contrary to the ITS phylogeny, the polytomies in clade A and the basal grade are mostly due to 0-length branches. Additional resolution in the basal polytomy compared to the ITS strict consensus was generally not well supported, excepted for the *Latouchea* - *Obolaria* clade, the *Gentianopsis* - *Pterygocalyx* clade, and the position of the *Swertia decora* - *S. rosularis* clade as basal to clade A. Similarly, in the upper polytomy, differences with the ITS phylogeny are not supported, with exception of the broad *Gentianella anomala* - *Comastoma polycladum* clade that was not found by the analysis of ITS data but well supported by the chloroplast data.

Chloroplast and ITS Data Combined. When all 4 data sets were combined, the parsimony analysis resulted in an unknown number of trees (25433 kept) of 3464 steps (HI = 0.53, RI = 0.71, RC = 0.38). This represents 60 steps more than the sum of the shortest trees

obtained from the ITS and chloroplast data separately. The strict consensus (Fig. 2) was rather well resolved compared to the strict consensus trees obtained from the nuclear and chloroplast data prior to combination. Jackknife and decay index values were generally increased, particularly for clade A, reflecting the overall agreement for all major clades between the chloroplast and ITS topologies (Figs. 1A and 1B). The branching pattern along the backbone was similar to the one observed in the chloroplast phylogeny, although with lower support for the deepest branches, whereas the resolution of more terminal clades resembled more the ITS one. Apart from the broad *Lomatogonium longifolium* - *Comastoma polycladum* clade, most new branches recovered by the combined analysis were only poorly supported. For

Table 3 Results of Templeton's SLP test for conflicting relationships between ITS and chloroplast data (cf. Fig. 1). The first column shows pairs of alternative relationships tested (in Newick syntax when too complex). The second and third column indicate in which data set each relationship is found, and the P value of the SLP test on the other data set. The last columns shows which relationship is recovered in the analysis of the combined data sets (chloroplast = chloroplast). Constrained cladograms were compared with 3 randomly chosen unconstrained ones. Asterisks indicate significance at the P = 0.05 level.

Relationship tested	Supporting data set and max. P value in alternate data set		Relationship in combined analysis
	ITS	Cp	
Comastoma tenellum sister to other *Comastoma* spp.	0.81	√	√
C. polycladum sister to other *Comastoma* spp.	√	0.28	
Gentianella umbellata + *G. turkestanorum* sister to other *Gentianella* s. str. spp.	0.69	√	√
G. acuta, *G. amarella*, *G. bulgarica*, *G. campestris* sister to other *Gentianella* s. str. spp.	√	0.38	
Swertia multicaulis, *S. barunensis*, *S. chirayita*, *S. membranifolia* monophyletic	0.86	√	√
only *S. barunensis*, *S. chirayita*, *S. membranifolia* monophyletic	√	0.04*	
Frasera albicaulis, *F. coloradoensis*, *F. gypsicola*, *F. montana*, *F. puberulenta* monophyletic	0.56	√	√
only *Frasera albicaulis*, *F. coloradoensis*, *F. montana* monophyletic; *F. gypsicola*, *F. utahensis* monophyletic; *F. albomarginata*, *F. parryi* monophyletic	√	0.06	
Lomatogonium graciliflorum in *Comastoma* and *Lomatogonium* p. p. clade	√	0.82	√
L. graciliflorum in *Lomatogonium* p. p. and *Lomatogoniopsis* clade	0.79	√	
S. dichotoma, *S. tetrapetala*, *S. tetraptera* monophyletic and sister to *Halenia* spp.	√	0.36	√
(*S. tetraptera*, (*S. dichotoma*, *H. elliptica*, (*S. tetrapetala*, *H. brevicornis*, *H. corniculata*, *H. weddeliana*)))	0.03*	√	
Bartonia virginica and *S. tashiroi* monophyletic	√	0.41	√
Bartonia virginica sister to *G. arenaria* , *G. pygmaea*	0.46	√	
S. beddomei, *S. nervosa*, *S. pauciflora* sister to *S. angustifolia*, *S. alata*	√	0.61	√
(*S. pauciflora*, (*S. nervosa*, *S. alata*, (*S. beddomei*, *S. angustifolia*)))	0.30	√	
S. decora, *S. delavayi*, *S. patula*, *S. rosularis* not monophyletic	√	0.76	√
S. decora, *S. delavayi*, *S. patula*, *S. rosularis* monophyletic	0.61	√	

conflicting branches (Table 3), the topology retained was the same as the best supported one in separate analyses, but with a slight decrease in branch support except for the position of *C. tenellum* and *L. graciliflorum*, for which support was ameliorated.

***mat*K and other Sequence Data.** The analysis of all available chloroplast data for the reduced taxon sample resulted in a single most parsimonious tree of 1016 steps (HI = 0.23, RI = 0.67, RC = 0.51) that did not provide additional resolution, nor additional support (Fig. 3). When ITS sequences were combined to the chloroplast data for inference, the strict consensus tree was more resolved, but the new branches were only weakly supported (not shown).

Congruence Tests and Data Combination. The ILD test detected no incongruence between *mat*K sequences and any other data set (P = 0.30 - 0.88 for all tests) for the reduced taxon sample. For the complete sample, the ILD test suggested significant incongruence between the 3 chloroplast data sets (P < 0.01). It was found that the *trn*L and the *trn*L - *trn*F data sets showed no conflict (P = 0.27), but that both independently were in disagreement with *trn*S - *ycf9* sequences (P < 0.01 for both). However, various combinations of as few as 3 species could be excluded to obtain P values ≥ 0.05. No significant conflict was detected between the ITS 1 and ITS 2 partitions, although the ILD test was only marginally non-significant (P = 0.05).

The ILD test also showed significant incongruence between combined chloroplast and combine ITS data sets (P < 0.01). No sum of tree lengths for the random partitions smaller or equal to that for the original partition was found. Non-significant P values could be achieved by excluding various combinations of taxa occupying conflicting positions in the chloroplast and nuclear topologies, or by excluding combinations of taxa from the basal grade subtended by long phylogenetic branches. In addition, for all data partitions, largely non-significant P values could be obtained by excluding all but 1 representative species within a clade of closely related taxa (e. g. *Comastoma*, *Gentianella* s. str., *Gentianopsis*, *Frasera*, groups of *Swertia* and *Lomatogonium* etc.), but keeping all other taxa with unresolved or conflicting positions.

A strong asymmetrical distribution of homoplasy was revealed between the ITS (HI = 0.65) and the chloroplast (HI = 0.33) data sets, that was suspected to bias the ILD test (see *Materials and Methods*, Dolphin *et al.*, 2000). When the ILD test was run on partitions of one original and one shuffled (purely homoplastic) matrix, the P value was the same as for the original data, and all random partitions also yielded longer sums of tree lengths than the

original partition. However, the comparison of the average difference in tree lengths for the original data and the shuffled data (Table 4) suggests that the amount of incongruence revealed for the nuclear and chloroplast data sets (5.68 S. D.) is small in regard to the level of incongruence attributable to homoplasy alone (17.81 S. D. and 22.46 S. D.).

Table 4 Results of ILD test for original ITS and chloroplast data compared to shuffled data (see text). Each data set was tested against 10 shuffled equivalents of the competing data set. P=0.002 for all analyses.

Data sets tested	Mean tree length difference between original and random partitions	s. d.	Mean tree length difference in s. d. units
ITS and Chloroplast	31.37	5.52	5.68
ITS and shuffled Chloroplast	278.23	15.62	17.81
Chloroplast and shuffled ITS	315.57	14.05	22.46

Table 5 Results of Templeton's SLP test on the combined data sets. The first column shows the group for which monophyly is tested (*Lomatogoniopsis* is treated as part of *Lomatogonium*). The second column shows the additional number of steps of constrained trees in comparison to unconstrained trees. The third column gives the highest P value of the SLP test. Constrained cladograms were compared with 3 randomly chosen unconstrained ones. Asterisks indicate significance at the P = 0.05 level.

Constrained monophyletic clade	Additional N° of steps	max. P value	
Swertia s. l.	90	< 0.01	*
Swertia of basal grade	26	< 0.01	*
Swertia of basal grade and *Frasera*	28	< 0.01	*
Swertia of clade A	50	< 0.01	*
Swertia of clade A excl. *S. hispidicalyx*, *S. tenuis*, *S. yunnanensis*	5	0.39	
Lomatogonium	49	< 0.01	*
Lomatogonium excl. *L. oreocharis*, *L. zhongdianense*	8	0.409	
Gentianella s. l	41	< 0.01	*
G. arenaria,*G. pygmaea* and *Gentianella* s. str.	12	0.16	
Gentianella spp. not in *Gentianella* s. str. clade	33	< 0.01	*
Gentianella spp. not in *Gentianella* s. str. clade excl. *G. arenaria*, *G. pygmaea*	23	0.02	*
Gentianella spp. not in *Gentianella* s. str. clade excl. *G. arenaria*, *G. pygmaea*, *G. azurea*	6	1.00	
Bartonia and *Obolaria*	16	0.10	
Latouchea, *Megacodon*, *Obolaria*	8	0.34	
Latouchea, *Megacodon*, *Obolaria*, *Bartonia*	18	0.02	*
Taxa with petal nectaries (*Bartonia* unconstrained)	12	0.18	
Bartonia and *Obolaria* sister to all other taxa, with *Latouchea* and *Megacodon* basal (i. e. taxa with ovary nectaries basal, but with N. American - Asian vicariance)	25	0.01	*

The results of Templeton's SLP test on various pairs of conflicting relationships (Table 3) also showed that constraining a competing partial topology found in one data set on the other data set usually did not result in significantly less parsimonious trees. Significant P values were only found when a discordant constraint was enforced on the *Swertia multicaulis* clade (*Swertia* 8 in Fig. 2) for chloroplast data, and *S. dichotoma* clade (*Swertia* 4 in Fig. 2) for ITS data.

Alternative Topologies. The results of the SLP test for alternative topologies based on morphological, biogeographical or taxonomical considerations are given in Table 5. The tests were carried out on the combined data set because it contained the maximum phylogenetic signal. Only the highest P values were considered, as these indicated those comparisons between constrained and unconstrained analyses that conflict least. Constrained monophyly of *Swertia* in its current circumscription was clearly less parsimonious than polyphyly. So was the constrained monophyly of the basal lineages of *Swertia*, irrespective of the inclusion or exclusion of *Frasera*. However, with exception of *S. hispidicalyx*, *S. tenuis* and *S. yunnanensis*, the monophyly of the species of *Swertia* in clade A (Fig. 2) was not rejected. Monophyly of *Lomatogonium* was also not significantly less parsimonious when *L. oreocharis* and *L. zhongdianense* were excluded. Forced monophyly of *Gentianella* s. l. was strongly rejected, although the inclusion of *G. arenaria* and *G. pygmaea* in the *Gentianella* s. str. clade did not result in significant P values. Among the taxa with nectaries on the ovary, i. e. *Bartonia* (?, see discussion), *Latouchea*, *Megacodon* and *Obolaria*, monophyly was not rejected when *Bartonia* was excluded, but this result is somewhat contradictory with the non-significant P value for the constrained monophyly of *Bartonia* and *Obolaria* only.

Bayesian Analyses

The model selected for the ITS data was the General Time Reversible with rate heterogeneity (site specific substitution rates drawn from the gamma distribution). The same model but with a proportion of invariable sites (some sites are invariant and the rates of others are drawn from the gamma distribution) was chosen for the chloroplast and the combined data. As the 50% majority rule consensus of the trees obtained from each data set was similar to the strict consensus of the parsimony trees, posterior probabilities were indicated in percent values on Figs. 1 A & B and 2 A to maintain a reasonable number of figures (second number

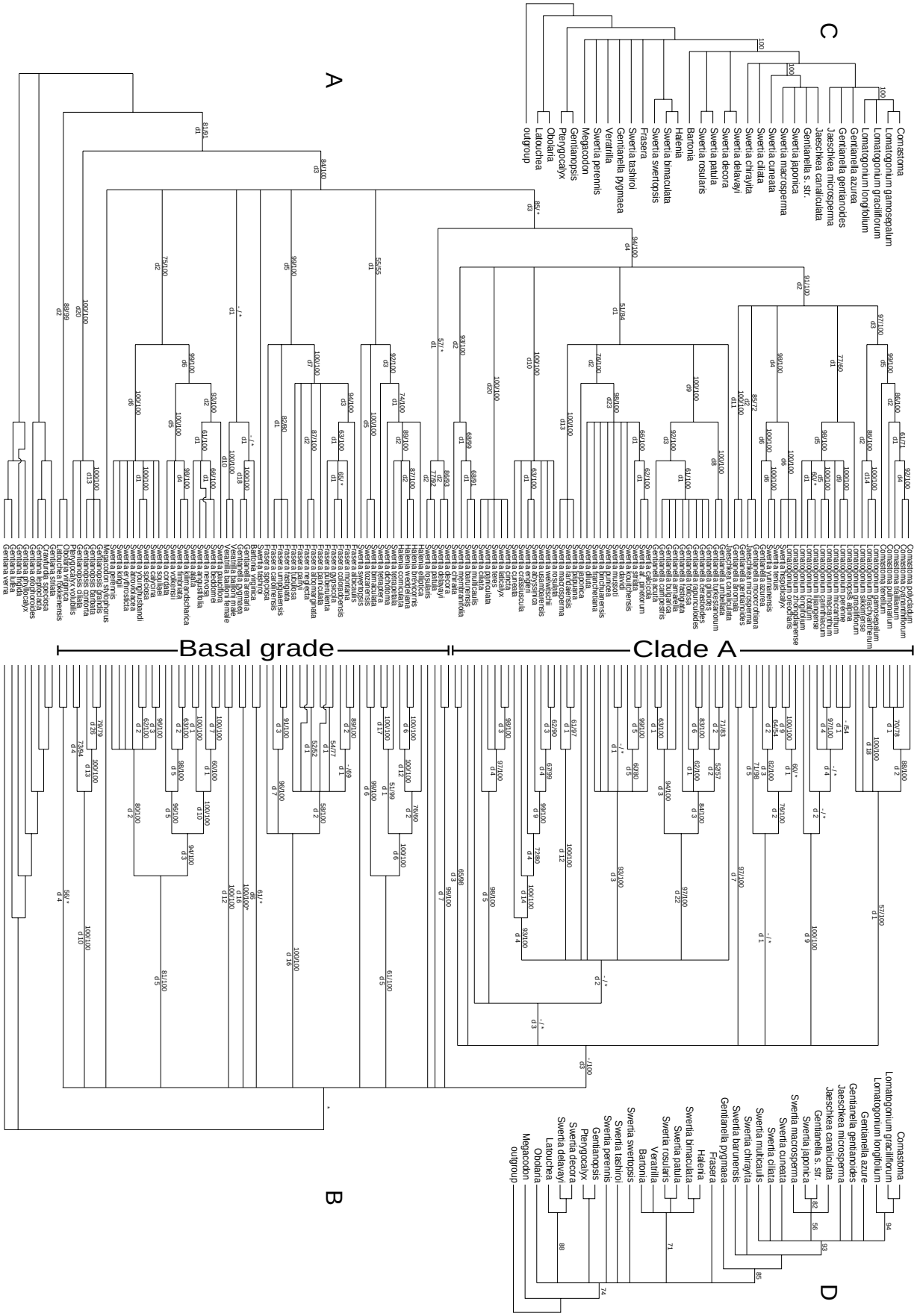


Fig. 1 Strict consensus of most parsimonious trees of **A**, chloroplast sequences (L = 1345, HI = 0.33, RI = 0.84, RC = 0.28) and **B,** ITS sequences (L=2059, HI = 0.65, RI = 0.64 RC = 0.42). Above branches: jackknife values if > 50% / 50% majority rule consensus values of Bayesian analysis (see text for explanations). Below branches: decay index.

above branches). Simplified trees (Figs. 1 C & D and 2 B) were build to indicate the position and probability of branches resolved differently in the Bayesian analyses (indicated with an asterisk in Figs 1 A & B and 2 A; Taxa/clades related by a branch marked with an asterisk but not detailed in the simplified trees formed a polytomy at the next higher level). For the chloroplast data alone, the Bayesian phylogeny only differed from the parsimony one in the position of *Bartonia* and the polytomic relationship of the *Swertia rosularis* clade and the *S. decora* clade. Increased resolution for the *S. cuneata* clade and for the broad *Comastoma - Lomatogonium* clade was also achieved with high probabilities. For the ITS data, additional resolution of the basal polytomy present in the parsimony analysis was obtained but with generally low probabilities. Notably, *Megacodon* was basalmost in the Swertiinae, *Latouchea* was related to the *S. decora - S. delavayi* clade, and the *Halenia - S. bimaculata* and *S. patula - S. rosularis* clades were related to *Bartonia* and *Veratrilla*. Within clade A, the *S. macrosperma - Jaeschkea canaliculata* clade found in all other analyses was also present but with a low probability. The Bayesian phylogeny inferred from the combined data differed from the parsimony one only by branches with low probabilities, notably the position of the *S. patula - S. rosularis* clade, and was less resolved as regards relationships of *Bartonia virginica*, *S. tashiroi* and the *Gentianella arenaria - G. pygmaea* clade, which were resolved but poorly supported in the parsimony analysis.

Times of Divergence

The LR test strongly rejected a clock-like behaviour of the sequence data ($P < 0.01$ for all data sets). Visual inspection of MP phylograms obtained from all data sets showed an extraordinarily long branchfor *Bartonia virginica* only (see Fig. 3, and also Hagen & Kadereit, 2002). However, the exclusion of this species had no incidence on the negative result of the LR test. Because the branch leading to *B. virginica* was so long, it was excluded for the PL analyses and added to the topology a posteriori according to its approximate maximum age deduced from its position in Fig. 2. The important rate heterogeneity suggested by the LR test was also reflected by the small smoothing parameter λ defined for the PL analyses. The cross-validation test performed with r8s showed the minimum χ^2 error for the smallest λ tested, 0.01. PL estimates of divergence times (Fig. 4) were carried out on the entire taxon sample, but were mapped on a simplified version of the strict consensus tree depicted in Fig. 2. Branches are proportional to the time scales below based on the 2

calibration dates. Shaded boxes show the minimum and maximum age of nodes or lineages calculated for the 3 trees analysed, and dashed lines indicate artificial branches.

Estimates for the approximate age of the last common ancestor of the Swertiinae were the most variable, and ranged from ca. 12 to 17 mya (calibration 1) or from ca. 31 to 42 mya (calibration 2). Differences for the age of other nodes/clades were sensibly smaller. Disregarding unresolved relationships (dashed lines) within the polytomies of the basal grade, it appeared that most major clades of the Swertiinae diverged within a short lapse of time, ca 4.5 to 6.5 mya (calibration 1) or 11.5 to 16 mya (calibration 2).

Discussion

Phylogenetic Inference

Incongruence Between Data Sets. In view of the increasing amount of literature criticising the reliability of the ILD test (e. g. Dolphin *et al.*, 2000; Yoder *et al.*, 2001; Barker & Lutzoni, 2002; Darlu & Lecointre, 2002; Dowton & Austin, 2002), and the results obtained here with respect to the effect of homoplasy and number of included taxa per clade on the test results, appraising the significance of the ILD test results between the data sets utilised seemed somewhat awkward. As regards the significant ILD test values revealed between the *trn*S and other chloroplast sequences, which have a monoparental inheritance and may have only one evolutionary history, incongruence was attributed to random error for 2 reasons: 1) Numerous unsupported and dubious branches were found exclusively in the poorly resolved phylogenies inferred from the individual data sets. Yet, the taxa concerned by these branches formed more coherent and better supported clades when chloroplast sequences were combined for analysis, and the overall resolution and branch support were greatly improved. 2) Non-significant ILD P values were obtained by excluding variable combinations of taxa, suggesting that significant P values resulted from the cumulative effect of minor conflicts due to random error (Johnson & Soltis, 1998). Likewise, the high level of homoplasy in the ITS 1 and ITS 2 data sets (HI = 0.64 and 0.65 respectively) may also account for the only marginally non-significant ILD test value obtained for this partition.

On the other hand, as suggested by the ILD test, incongruence between the ITS and chloroplast phylogenies might be expected, as the nuclear genome may have experienced an evolutionary history different from that of the plastid genome. However, several observations suggested that this was not the case. Firstly, as indicated by the tests performed on pure noise matrices (Table 4), the asymmetrical distribution of homoplasy between the chloroplast data

(HI = 0.33) and the nuclear data (HI = 0.65) was likely to bias the ILD test (Dolphin *et al.*, 2000). Secondly, by visual inspection, the nuclear and plastid phylogenies appeared to be globally congruent (e. g. Fig. 1: clade A, identical or nearly identical shallower clades both in the basal grade and in clade A), but note that the weak resolution of deeper nodes, in particular in the ITS phylogeny prevented a more extensive comparison of these topologies. Thirdly, none of the conflicting relationships recovered from the separate analysis of nuclear and plastid sequences submitted to the SLP test were found to be less parsimonious in both data sets. Yet, these relationships had a major incidence on the significant ILD test results, as the exclusion of the taxa involved resulted in non-significant P values. And lastly, long-branch attraction, frequently associated with saturated data (Sanderson & Shaffer, 2002) has probably not been overcome in the ITS data (HI = 0.65) despite the large number of ingroup and outgroup species sampled to break these down. Indeed, several lineages of the basal grade including 1 or few species (e. g. *Bartonia*, *Gentianella pygmaea* and *G. arenaria*, *Latouchea*, *Megacodon*, *Obolaria*, *Swertia swertopsis*, *S. tashiroi*, *Veratrilla*) apparently diverged early and rapidly in the history of the Swertiinae (see *Biogeography* below and Figs 3 & 4), and necessarily have long phylogenetic branches. In addition, the mycotrophic *B. virginica* has a sharply increased substitution rate for ITS, *trn*L and *mat*K sequences (Hagen & Kadereit, 2002), also observed in our other plastid sequences (see Fig. 3). Long-branch attraction could account for part of the incongruence suggested by the ILD test, as removing the taxa concerned led to non-significant P values, and also for the decrease in support observed for some deeper branches in the phylogeny inferred from combined data compared to the chloroplast phylogeny, as well as for the differences in the resolution of basal lineages in the Bayesian analyses of separate and combined data.

Differences Between Parsimony and Bayesian Inferences. The parsimony based phylogenetic hypotheses proposed for the Swertiinae at date have revealed unexpectedly high levels of para/polyphyly in *Gentianella*, *Lomatogonium* and, in particular, in the genus *Swertia*, that strongly contrast with the apparent morphological homogeneity of these taxa (Chassot *et al.*, 2001; Hagen & Kadereit, 2002). Facing this hiatus, a legitimate criticism would be that the method of phylogenetic inference, based solely on the maximum parsimony criterion, may be inappropriate and the deduced phylogenies misleading. Bayesian inference, which incorporates complex models of evolution and branch lengths in a probabilistic estimation of the branching history, provides a credible alternative to parsimony. Although

the two methods yielded phylogenies that differed in some parts (relationships between lineages of the basal grade, Figs. 1 & 2), these were largely in agreement as regards the question addressed, i. e. the non-monophyly of *Gentianella*, *Lomatogonium* and *Swertia*.

Compared to the parsimony trees, the Bayesian phylogenies inferred from the nuclear and plastid data sets separately (Fig. 1) contained additional resolution within clade A (e. g. the *Lomatogonium longifolium* - *Comastoma* clade in both data sets, the *Swertia cuneata* - *Jaeschkea canaliculata* clade in the chloroplast data set, the *Swertia macrosperma* - *J. canaliculata* clade in the nuclear data set), that was also achieved in the parsimony and Bayesian analyses when all data were combined, indicating that both different data sets and different methods converge to the same phylogenetic hypothesis in this clade. Conversely, among taxa of the basal grade, although shallower clades were consistent across the methods or data sets utilised except for the *Latouchea* - *Obolaria* clade in the Bayesian ITS phylogeny (Figs. 1 & 2), the relationships between these clades, when resolved, were variable, and the additional or different resolution found in the separate analyses was not retained when the data were combined, regardless of the method employed. However, considering the hard polytomy revealed by the analysis of the extended sequence set including *mat*K data for several of these clades (Fig. 3), it is likely that the discrepancies in the relationships among these lineages observed across methods and data sets reflect the lack of synapomorphies in our data that would enable a reliable identification of the correct branching history between lineages that apparently diverged extremely rapidly. In this context, relationships inferred from the rather homoplasious ITS data may be particularly sensible to random error and long-branch attraction, as exemplified by the grouping of *Latouchea* with the *S. decora* - *S. delavayi* clade (although with only 88% posterior probability) in the Bayesian ITS phylogeny, which is incoherent from a morphological point of view. Although *Latouchea* and *Obolaria* are both monotypic and morphologically difficult to relate, their sister relationship, as suggested by all other analyses, is at least supported by the shared ovary nectaries. Nonetheless, when all data were combined, the parsimony and Bayesian phylogenies (Figs. 1 A & B) were largely congruent as regards clade A and the resolution of basal lineages up to the *S. perennis* clade, and the relationships between the remaining lineages of the basal grade were either unresolved (Bayesian analysis) or more resolved but unsupported (parsimony analysis). The only supported difference, albeit weakly, concerned the position of the *S. decora* - *S. delavayi* clade. In sum, the overall consistency of molecular phylogenies to the method of inference clearly supports previous results based on parsimony only.

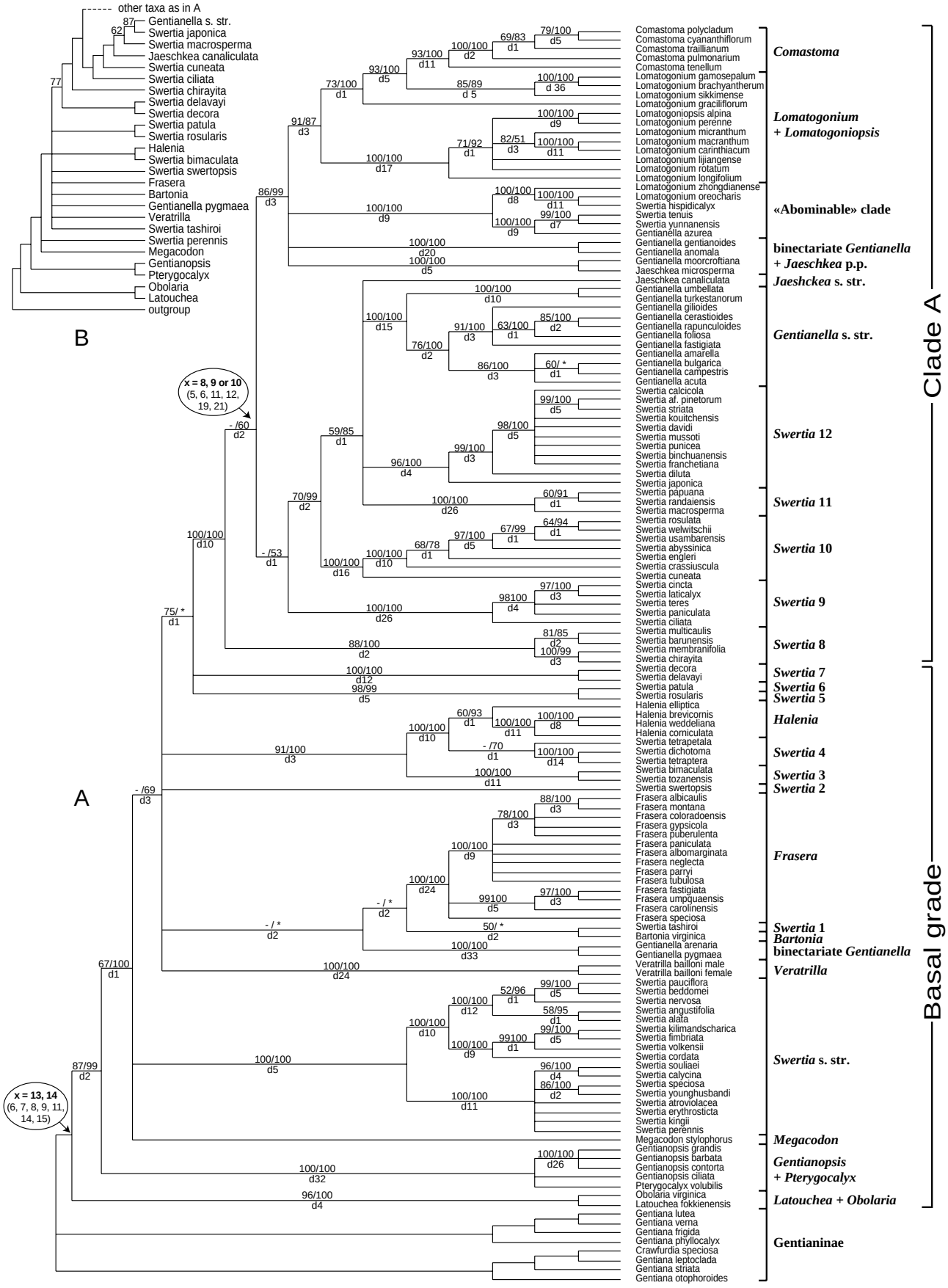


Fig. 2 Strict consensus of most parsimonious trees from the analysis of combined chloroplast and ITS sequences (L = 3464, HI = 0.53, RI = 0.71, RC = 0.38). Above branches: jackknife values if > 50% / 50% majority rule consensus values of Bayesian analysis (see text for explanations). Below branches: decay index. Circled: basic chromosome numbers reported for taxa of the basal grade and clade A (most frequent numbers in boldface).

Phylogeny of the Swertiinae

Although some level of incongruence between the nuclear and chloroplast sequence data is evident, their combination for analysis resulted in phylogenies showing a global increase of resolution and branch support. Ambiguous relationships among the basal lineages, as revealed by the separate analysis of nuclear and plastid sequences, were respectively unresolved or poorly supported in the Bayesian and parsimony phylogenies inferred from the combined data, which represent rather conservative estimates of the evolutionary relationships in the Swertiinae such as they may be deduced from the sequence data available. These trees (Figs 1 A & B) were thus used as final phylogenetic hypotheses. Compared to preceding molecular phylogenies of the Swertiinae (Chassot *et al.*, 2001; Hagen & Kadereit, 2001, 2002), our results do not alter previous conclusions except in a few cases, but provide further details on the number and relationships of the various lineages of non-monophyletic genera. As the focus of this work was mainly to explore the extent of polyphyly in the genus *Swertia*, relationships of other genera in the Swertiinae will not be discussed in detail, except in cases where the present results differed from previous ones.

Basal Grade and Clade A. At the level of the entire subtribe, a major partitioning was revealed between the lineages of the highly supported clade A, and those of the basal grade (Fig. 2). Yet, morphological characters in agreement with this subdivision are scarce. Only the exomorphic seed structure was found to be rather well correlated with molecular results. Taxa of the basal grade have irregularly shaped or flattened seeds, often conspicuously winged or cristate, or presenting various exotestal outgrowths (see Fig. 5 A-J), whereas those of clade A have globose or ovoid seeds with a smooth or moderately alveolate testa, except in the basal lineage of this clade (*Swertia* 8, Fig. 2) where the seeds of some species may be winged and, rarely, flattened. (see Fig 6 K, M, N). Notable exceptions were found in the basal grade in the *Swertia* 3 - *Halenia* and the *Gentianella arenaria* - *G. pygmaea* clades, where the seeds are similar to those of clade A (see Fig. 5 L), and in *Bartonia* and *Obolaria*, that have minute seeds with a slightly striate or reticulate testa. Other flower-morphological traits commonly referred to for taxonomic delimitation such as the presence of a well developed corolla tube, place of insertion of the filaments, presence of a style or gynophore, number of nectaries per corolla segment and type of their appendages, etc. were found to have undergone a parallel evolution in several lineages (see Chassot *et al.*, 2001 and Hagen & Kadereit, 2001, 2002 for details). Parallel evolution may have also occurred in seed types among basal

lineages. For example, flattened seeds with an annular wing are found in species of e. g. *Gentianopsis*, *Veratrilla*, and perennial species of *Swertia* s. str and *Swertia* 8, corrugate seeds in e. g. *Latouchea*, *Megacodon* and *Swertia* s. str., and spinose ones in e. g. *Gentianopsis* and annuals of *Swertia* s. str.

A detailed survey of chromosome numbers in the Swertiinae (Chassot *et al.*, in ms.) showed a good overall agreement with the molecular phylogeny as regards the distinction between taxa of the basal grade, and those of clade A. The basic chromosome numbers x = 13 and x = 14 confine to lineages of the basal grade and only to the basal lineage *Swertia* 8 within clade A. Other basic chromosome numbers also occur in some taxa of the basal lineages (i. e. x = 6 and 7 in *S. tetraptera*, x = 11 in *Halenia* and *Gentianopsis*, x = 12 and 13 in *Pterygocalyx*), but, according to molecular results, the taxa in which these numbers occur

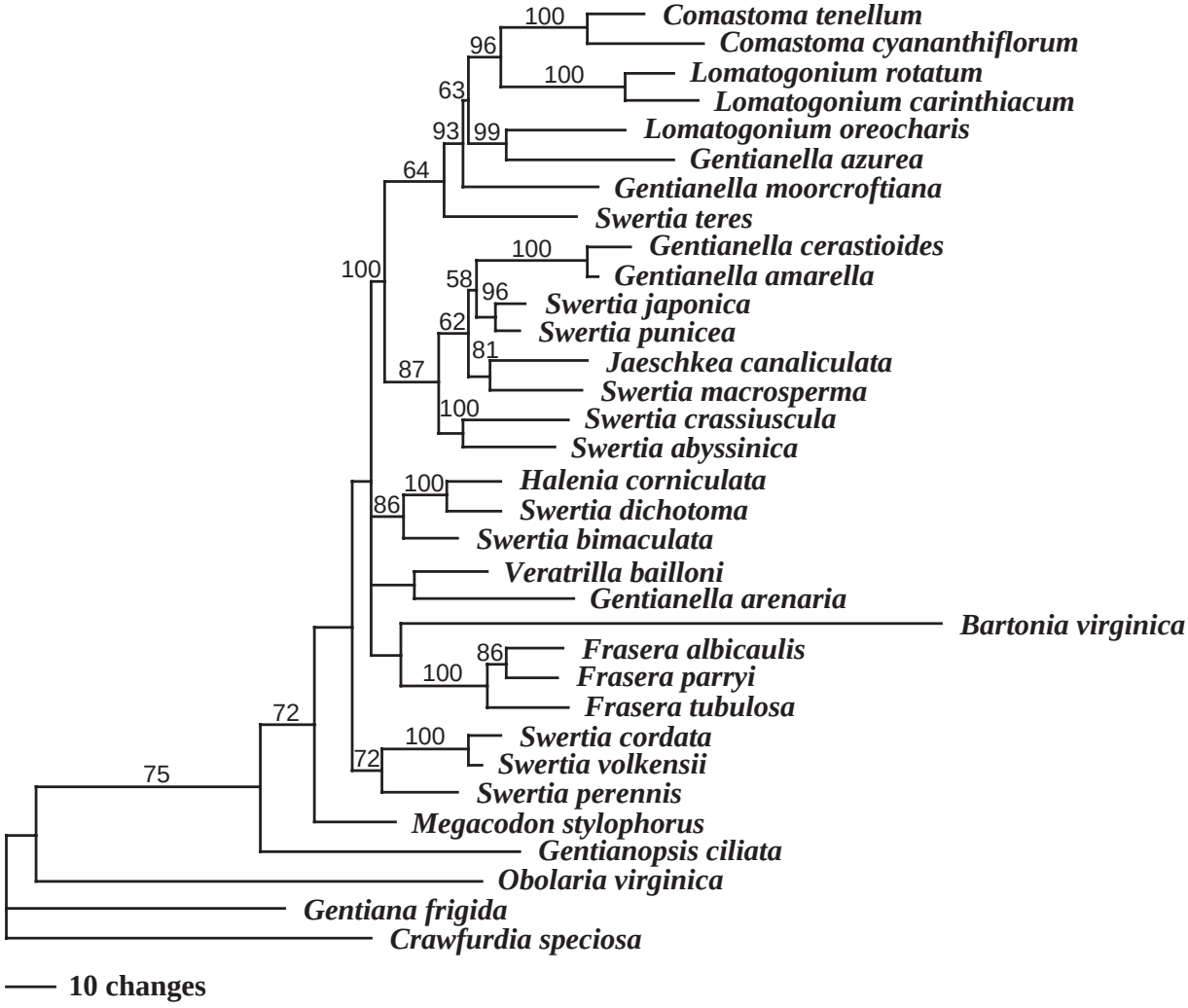


Fig. 3 Single most parsimonious phylogram (L = 1016, HI = 0.23, RI = 0.67, RC = 0.51) obtained from the reduced taxon sample for which *mat*K sequences retrieved from EMBL were added to our data (see text). Above branches: jackknife values if > 50%.

are all clearly related to species or lineages with x = 13 and may have been derived repeatedly from x = 13 (see Chassot *et al.*, in ms.). Within clade A, and disregarding the basal lineage *Swertia* 8, not a single taxon was found to have a basic chromosome number of x = 13 or 14. The most common basic numbers are x = 8, 9 and 10, and a wide range of subsidiary basic numbers were also found (x = 5, 6, 11, 12, 19 and 21). In the light of the molecular phylogeny, all the basic numbers of clade A (exclusive of *Swertia* 8) may have experienced multiple origins (e. g. x = 5 and 8 in species of *Comastoma* and *Lomatogonium*, x = 9 in species of *Comastoma*, *Lomatogonium* and *Swertia* 9, 11 and 12, x = 10 in species of *Swertia* 9, 10 and 12; See also Table 6 and Chassot *et al.*, in ms.). In addition, basic numbers such as x

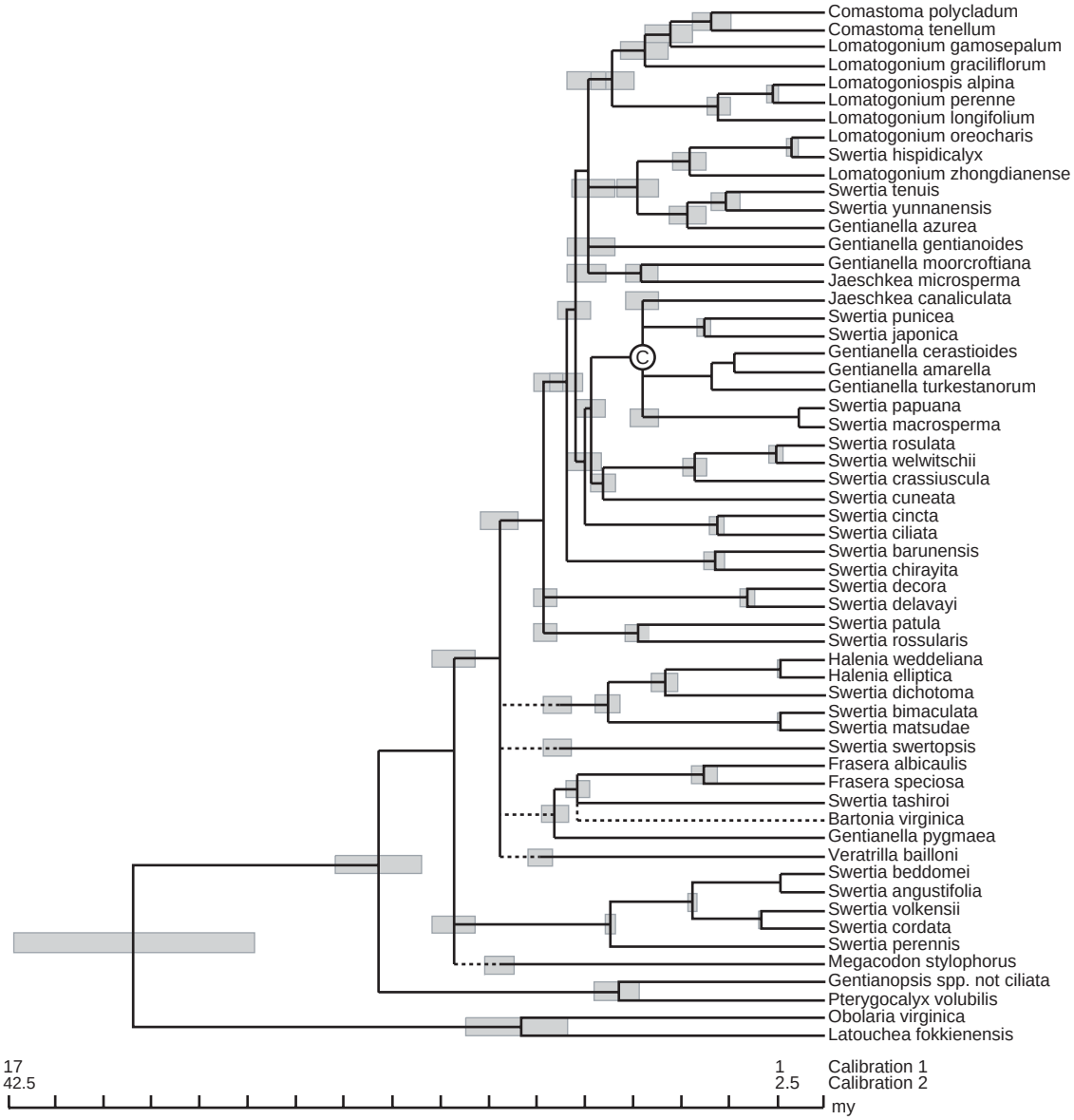


Fig. 4 Simplified chronogram of the Swertiinae obtained from penalised likelihood rate estimation. Time scaled according to 2 calibration dates for node c (see text). Shaded boxes indicate maximum and minimum age estimates of a node or clade. Dashed lines indicate artificial branches.

= 6, 8, 9, 11 and 12 apparently originated independently both in the basal grade and in clade A. The important level of parallel evolution in karyotypes, concentrated in clade A, but also the relative constancy of x = 13 in the basal grade, generally precluded the use of chromosome numbers in inferring phylogenetic relationships with more detail than the broad distinction between taxa of the basal grade plus *Swertia* 8, and those of the rest of clade A. However, combined with other characters, karyology was particularly useful in discriminating between morphologically closely resembling lineages of *Swertia*.

Basal Taxa of the Swertiinae. One difference with anterior results (Hagen & Kadereit, 2002) concerns the assumption that the taxa with nectaries at the base of the ovary, (i. e. *Latouchea*, *Megacodon* and *Obolaria*), as also found in the Gentianinae, are basal in the Swertiinae, and that all other taxa of the subtribe sharing epipetalous nectaries are monophyletic. According to our results, the basal position of *Latouchea* and *Obolaria* could be confirmed, but taxa with ovary and petal nectaries were not found to form monophyletic groups considering the position of *Megacodon* and of the *Gentianopsis* - *Pterygocalyx* clade, although monophyly for *Latouchea*, *Megacodon* and *Obolaria* was an acceptable alternative according to Templeton's SLP test (). Moreover, on grounds of their imbricate corolla aestivation and mycotrophic habit, *Bartonia* was related to *Obolaria* (Hagen & Kadereit, 2002) and thus to the putative basal genera of the Swertiinae. Contrary to *Obolaria* (Lindsey, 1940), ovary nectaries have never been reported for *Bartonia*, and our results do not support such a relationship, although here again, Templeton's SLP test did not reject monophyly. However, monophyly for all 4 genera was clearly found to be less parsimonious. Even though strong evidence is wanting, a polyphyletic origin of epipetalous nectaries in the Swertiinae seems plausible in view of our results and of the morphological homoplasy displayed throughout the subtribe (Chassot *et al.*, 2001; Hagen & Kadereit, 2002).

***Jaeschkea*.** Species of this genus were found to be polyphyletic with respect to *J. microsperma*, that grouped with binectariate species of *Gentianella* in the broad *Lomatogonium* and *Comastoma* clade (Fig. 2), whereas *J. canaliculata* was related to species of *Gentianella* s. str. and of *Swertia* 12. *Jaeschkea oligosperma* (= *J. gentianoides* Kurz [Garg, 1987]), the type of the genus, was related to *J. canaliculata* (Hagen & Kadereit, 2002). As other species of *Jaeshckea*, *J. microsperma* has filaments adnate to the long corolla tube and becoming free near or at the sinus of corolla lobes, and corolla lobes valvate at their base,

and was only found to differ by its more numerous seeds and pollen (Chassot & Hagen, in ms.). Our results thus suggest that *J. microsperma* is misplaced in the genus *Jaeschkea*, and that additional studies are necessary to identify more reliable characters defining this genus.

***Lomatogoniopsis, Lomatogonium* and *Comastoma*.** Species of *Lomatogoniopsis*, *Lomatogonium* (except *L. oreocharis* and *L. zhongdianense*) and *Comastoma* included in the sample formed a well supported clade (Fig 2). Monophyly for all species of *Lomatogonium* (including *Lomatogoniopsis*) was clearly rejected by Templeton's SLP test, but not when *L. oreocharis* and *L. zhongdianense* were excluded (Table 5). Nevertheless, the paraphyletic relationship of some species of *Lomatogonium* with respect to *Comastoma* had rather good branch support (Fig. 2). As in previous works (Liu *et al.*, 2001; Hagen & Kadereit, 2002), *Lomatogoniopsis alpina* was nested deeply within a clade of *Lomatogonium* species, and was highly supported as sister to *L. perenne*. These two species differ remarkably as regards ovary shape, type of nectary appendages and life habit. In addition, *L. alpina* has a chromosome number of 2n = 12 (Liu *et al.*, 2002), which is unknown from *Lomatogonium* (Chassot *et al.*, in ms.). *Lomatogoniopsis alpina* was thought to be morphologically and palynologically intermediate between *Comastoma* and *Lomatogonium* (Nilsson, 1967b), but its close relationship with *L. perenne* supported by both nuclear and plastid phylogenies (Fig. 1) suggests that the peculiarity of this taxon resulted from a recent (Fig. 4) and brutal shift of its morphology (and possibly also of its karyotype) rather than from hybridisation. No other species of *Lomatogoniopsis* could be included in our sample, but the nectary appendages in those species are galeate and similar to those of e. g. *L. gamosepalum* but not to the straight, petaloid scale of *L. alpina*. As a genus, *Lomatogoniopsis* does not seem to be well defined, and in view of our results, its recognition at generic rank is not supported.

The large number of *Lomatogonium* species included in our analysis (14/19 in the genus) allowed for a comparison with the 3 sections proposed in the classification of Liu & Ho (1992). Annual species were divided up into two sections, sect. *Pleurogynella* (Ikon.) T.-N. Ho based on conspicuous corolla and calyx tubes, and ovate to lanceolate ovary shape, and sect. *Lomatogonium* with very short corolla and calyx tubes, and ensiform to oblanceolate ovary shape. Sect. *Sarcorhizoma* S.-W. Liu & T.-N. Ho was based chiefly on the perennial habit of the species included, but apart from their perennial habit, species of sect. *Sarcorhizoma* have all the characteristics of sect. *Lomatogonium*, except for *L. oreocharis* and

L. stapfii (Burk.) Harry Sm., in which the nectaries appendages are fimbriate rather than laciniate (see Hagen & Kadereit, 2002 for illustrations). From a molecular phylogenetic point of view, species of *Lomatogonium* belonged to 3 groups. The first one consists of the well supported *L. longifolium* - *Lomatogoniopsis alpina* clade (Fig. 2) and corresponds more or less to sect. *Lomatogonium* (*L. carinthiacum, L. lijiangense, L. macranthum* and *L. rotatum*) and sect. *Sarcorhizoma* (*L. longifolium* and *L. perenne*). The second group was related to *Comastoma* and consists of the *L. graciliflorum* - *L. gamosepalum* grade including in the majority species of sect. *Pleurogynella* (*L. brachyantherum, L. gamosepalum* and *L. graciliflorum*). The last group pertains to *L. oreocharis* and *L. zhongdianense*, that were placed in the «abominable» clade (Fig. 2). These are discussed below. Disregarding perennial species of sect *Sarcorhizoma*, two species were related by molecular data to a group not corresponding to the section that they were ascribed to. These are *L. sikkimense* (sect. *Lomatogonium*) and *L. micranthum* (sect. *Pleurogynella*). This was surprising, as both species correspond rather well to the criteria of their respective sections, suggesting convergent evolution for characters such as the length of calyx and corolla tube, ovary shape and type of nectary appendages (the latter only for *L. sikkimense*, as *L. micranthum* has naked nectaries). In sum, our results do not entirely support the infrageneric classification of *Lomatogonium*, in particular with respect to sect. *Sarcorhizoma*. It is also noteworthy that the relationships revealed here between species of *Lomatogonium* and *Lomatogoniopsis* are not in agreement with pollen morphology (see Nilsson, 1967b; Liu & Ho, 1992; Chassot & Hagen, in ms.), and that in view of the position of *L. oreocharis*, *L. zhongdianense* and *Lomatogoniopsis alpina*, additional work seems necessary in order to achieve a classification reflecting evolutionary relationships.

«Abominable» Clade. This clade was present in the analyses of both separate (Fig. 1) and combined (Fig. 2) data, where it received very high support. Yet, the species of this clade present an amount of gross-morphological, karyological (Chassot *et al.*, in ms.), and palynological (Chassot & Hagen, in ms.) diversity unprecedented in any other lineage of the Swertiinae, and not a single character was found to be shared by all these species, hence the epithet abominable. *Swertia hispidicalyx*, *S. tenuis* and *S. yunnanensis* present each morphological, palynological and karyological combinations of characters unique in the genus (see Table 6), and *Lomatogonium zhongdianense* and *L. oreocharis* were placed in different sections (Liu & Ho, 1992). Moreover, the belonging of the species of this clade to their respective genera has never been questioned. For some taxa, evidence from morphology

and palynology suggests connections with other lineages of the Swertiinae and possibly a hybrid origin or introgression, although both nuclear and plastid phylogenies resulted in identical relationships (Fig. 1). For example, *L. zhongdianense* closely resembles *L. brachyantherum* and related species (sect. *Pleurogynella*) and *L. oreocharis* has close affinities with *L. perenne* (sect. *Sarcorhizoma*). As regards the species of *Swertia, S. hispidicalyx* has all the characteristics of *Swertia* 9, in particular the inverted nectaries and flattened, pubescent filaments, and was placed in the same group by Shah (1984) (see Table 6). But it has 2 nectaries per corolla segment, as do *L. oreocharis* and *L. zhongdianense* to which it was related, whereas species of *Swertia* 9 have only one. Likewise, *S. yunnanensis* closely resembles species of *Lomatogonium* in its habit, laciniate scales covering the nectaries, and in particular in its pollen grains very similar to those of e. g. *L. gamosepalum* or *L. oreocharis* (Chassot & Hagen, in ms.). Yet, the stigmata of this species are not decurrent on the carpelar suture (the distinguishing feature of *Lomatogonium*; but note that according to our observations, the stigmata are not clearly decurrent in all species of the genus, e. g. *L. gamosepalum* and akin species, *L. oreocharis*, *L. stapfi*), and Shah (1984) thought it to be related to species of *Swertia* 12. In view of its morphology and palynology, and in agreement with molecular results, *S. yunnanensis* shows closer affinities with lineages of *Lomatogonium* than with lineages of *Swertia*, from which it should be excluded. *Swertia tenuis* is more difficult to relate to other lineages of the Swertiinae. From a floral morphological point of view, it closely resembles *S. rosularis* (*Swertia* 5), in particular as regards the single watch-pocket shaped nectary per corolla lobe and compound fimbriae. However, its pollen grains are very different from those of *S. rosularis*, and present similarities in the exine structure with *L. forrestii* (Chassot & Hagen, in ms.). Regarding *Gentianella azurea*, its long corolla tube and paired nectaries suggest affinities with other binectariate species of *Gentianella*. Yet this species was found to have a row of sparse, compound fimbriae above each nectary somewhat similar to the fimbriae of *S. tenuis* with which it had molecular affinities (Fig. 2), and the shape of its ovary and stigmata is similar to that of species of *Comastoma*.

Binectariate Species of *Gentianella* and *Jaeschkea microsperma.* Both in the phylogeny (Fig. 2) and according to Templeton's SLP tests (Table 5), the monophyly of these species was not supported, and these may roughly be divided into two groups. The first one relates to the very similar *G. arenaria* and *G. pygmaea*, that formed a strongly supported clade related to lineages of the basal grade (Fig. 2). The position of this clade is however contradicted by seed morphology and chromosome numbers. Seeds of *G. arenaria* and *G. pygmaea* have a

smooth testa, which is more characteristic of the species of clade A, although also present in some basal lineages such as *Halenia*, *Swertia* 3 and *Swertia* 4. Chromosome numbers are only documented for *G. arenaria* (2n = 16, 18) (see Chassot *et al.*, in ms.), and here again it is a number more typical of clade A. In addition, Templeton's SLP test did not reject the inclusion of these two species in the *Gentianella* s. str. clade (Table 5).

The second group consists of Asian species of *Gentianella* and of *Jaeschkea microsperma*, showing molecular affinities with taxa of a broad *Lomatogonium*, *Comastoma* and *Swertia* p. p. clade (see also Hagen & Kadereit, 2001, 2002 for *G. angustiflora* Harry Sm. not included here). These affinities are difficult to understand from a morphological point of view because the species of this group lack the most obvious features of *Lomatogonium* (decurrent stigmata, dichromatic corolla lobes, rotate corolla) and of *Comastoma* (well developed fimbriate scale fused to the corolla tube, absence of style). They also share no particular resemblance with species of *Swertia* included in the «abominable» clade. With exception of *G. azurea* that clearly belonged to the «abominable» clade (Fig. 2), these species of *Gentianella* and *Jaeschkea* were closely related, and their monophyly was not rejected by Templeton's SLP test (Table 5). Yet *J. microsperma* (2n = 16), *G. gentianoides* (2n = 22, 44) and *G. moorcroftiana* (2n = 12, 18) all have different chromosome numbers (Chassot *et al.*, in ms.). In addition, the nectaries of the first 2 species are perfectly naked, whereas those of *G. gentianoides* are associated to a fimbriate scale (Hagen & Kadereit, 2002, pers. obs.). *G. anomala* was thought to be related to *G. arenaria* and *G. pygmaea* based on its tetramerous flowers (Hagen & Kadereit, 2002), but it was found to have angular seeds and nectary appendages similar to those of the pentamerous *G. gentianoides*, in agreement with molecular results. Whether such morphological diversity not only among binectariate species of *Gentianella* and *Jaeschkea microsperma*, but also between these taxa and *Lomatogonium* and *Comastoma* is attributable to convergent evolution, or to processes such as hybridisation, or structural rearrangements of the genome as suggested by karyology (Chassot *et al.*, in ms.), is still far from clear and will not be discussed further here.

Table 6 Characteristics of the lineages of *Swertia*, with indication of a representative species and corresponding informal group in Shah (1984). Groups of which no species was sequenced are marked with an asterisk. Chromosome numbers: see Chassot *et al.* (in ms.) for references. +: presence, -: absence; Distribution: Eurasia is exclusive of Malaysia and Papuasia New Guinea, Himalaya is inclusive of the Tibetan plateau and mountains of South-western China; Habit: p: perennial, a: annual or biennial; Leaves: o: opposite, a: alternate, v: verticillate; Style: s: short, st: stout, sl: slender; Pollen: s-r : common striate-reticulate pattern, r: reticulate, s: spinose, ru: rugulate, v: verucate, c: cristate, p: punctate.

Swertia clade (Fig. 1)	Informal group (Shah,1984)	Representative species and spp. sequenced / spp. in group	Chromosome number (2n)	Distribution & habitat a: alpine m: montane l: lowland	Habit	Leaves	Roots	Merosity	N° of nectaries	Nectary type +: fimbriae present -: fimbriae absent	Style	Seeds	Pollen
Frasera	10	*F. caroliniensis* 4/4	78	N America, m-a	p	v	thick fleshy taproot	4	1 (2)	saccate or with membranous rim +	+ s /sl	flat annular winged	s-p (r)
	11	*F. albicaulis* 5/5	26, 52	N America, l-m	p	o (v)	thick woody taproot	4	1	saccate + (membranous tube lacerate at top)	+ sl	oblong, slightly trigonate, warted	s-p
	12	*F. albomarginata* 5/5	26, 52	N America, l	p	o (v)	thick woody or fleshy taproot	4	1	saccate, oblong or bilobed at base or apex +	+ sl	trigonate or reniform, warted or corrugate (oblong with minute annular wing)	s-p
s. str.	1	*S. perennis* 4/11	26 (28)	temperate Eurasia (N America), m-a	p	o (a)	short rhizomes	5 (4)	2 (1)	cupular (cushion shaped) +	-	flat annular winged	s-r to r
	2	*S. bifolia* 1/12	26 (28)	temperate Eurasia, m-a	p	o (a)	short (bulbous) rhizomes	5	2 (1)	saccate (cushion or cup shaped) + (-)	-	corrugate-cristate	s-r
	3	*S. calycina* 3/7	26	temperate Eurasia, m-a	p	o (a)	short (bulbous) rhizomes	5	2 (1)	cupular + (-)	-	polyhedral winged	s-r
	13	*S. volkensii* 1/4	26	C & E trop. Africa, a	p	o	rhizomes, stem stoloniferous	5 (4)	1	naked spot -	-	corrugate-cristate	s-r
	15	*S. kilimandscharica* 2/4	26	E & NE trop. Africa, m-a	p	o	rhizomes (stoloniferous)	5 (4)	1	saccate (naked spot)+ (-)	+	corrugate-cristate, corrugate-winged	s-r
	31	*S. cordata* 1/1	26	S Himalaya, m	a	o	fibrous roots	5	1	naked spot -	+	corrugate-cristate	s-r
	29	*S. alata* 4/6	26 (24)	S temperate Eurasia, m	a	o	fibrous roots	4	1	watch-pocket shaped +	+ s	echinate	s-r
	30	*S. beddomei* 1/4	26	S India, m	a	o	fibrous roots	4	1	sub-orbicular (pouch shaped) +	+ s st	polyhedral, echinate	s-r
	*32	*S. zeylanica* 0/1	—	Ceylon, m	a	o	fibrous roots	4	1	sub-orbicular +	+ s st	corrugate-cristate	—
1	21	*S. tashiroi* 1/0	52, 60	Taiwan, SW Japan, l-m	a	o	fibrous roots	5	1	broad, naked patch -	-	echinate	s (t)-p

Swertia clade (Fig. 1)	Informal group (Shah,1984)	Representative species and spp. sequenced / spp. in group	Chromosome number (2n)	Distribution & habitat a: alpine m: montane l: lowland	Habit	Leaves	Roots	Merosity	N° of nectaries	Nectary type +: fimbriae present -: fimbriae absent	Style	Seeds	Pollen
2	24	*S. swertopsis* 1/1	52	SW Japan, l	a	o	fibrous roots	5	2	V-shaped, saccate +	+	ovoid, large, shallowly corrugate	s-p
3	21	*S. bimaculata* 2/3	26 (22, 24)	SE temperate Eurasia, m	a	o	fibrous roots	5	2	naked spot -	-	rounded, finely warted	s-r
	20	*S. dichotoma* 3/3	12, 14	N & E Eurasia, S C China, l-a	a	o	fibrous roots	4	2 (1)	elongated or elliptic & protruding abaxially, scale attached on longitudinal edges +(-)	- (+)	rounded, smooth	s-r
4	—	*S. rosularis* 1/0	—	Mt. Emei, China, m	p	o	short rhizomes & fibrous roots	5	1	watch-pocket shaped +	+ s st	rounded, smooth	s-r
5	25	*S. patula* 1/3	26	SW China, m	a	o	fibrous to woody roots	4	2	+- triangular, scale attached at bottom and interior longitudinal edge +	+ s st	polyhedral warted	s-r
6	22	*S. delavayi* 2/2	26	SW China, m	a	o	fibrous roots	5	2	oblong saccate +	+ sl	polyhedral warted	s-r
7	8	*S. multicaulis* 1/2	26	C & E Himalaya, a	p	o	long fleshy taproot	4	1	sub-orbicular (rhomboid) + -	-	rounded, smooth	ru-p to smooth
	9	*S. hookeri* 1/12	—	C & E Himalaya, a	p	v (o)	long fleshy taproot	4	1	rounded (triangular, rhomboid) margin with raised scale or not (naked) + (-)	+ s st	rounded (flat annular winged/ winged at 1 or 2 ends) (-)	ru-p
8	26	*S. chirayita* 2/4	26	Himalaya, m (-a)	a	o	fleshy taproot (small)	4	2	oblong saccate +	+	rounded, smooth	s-r lirae wide
	*5	*S. handeliana* 0/1	—	SW China, a	p	o	short, thick rhizomes	4 ?	1	naked spot -	+ s st	rounded,smooth	nearly smooth
	*7	*S. ramosa* 0/1	—	E Himalaya, m-a	p	o	?	4	1	watch-pocket shaped +	+ s st	rounded, smooth	—
9	27	*S. ciliata* 5/13	16, 20, 24, 36	Asia (incl. Indonesia and Papuasia, m-a	a	o	fibrous roots, often fasciculate	5	1	oblong (rounded), scale attached at top and partly on longitudinal edges (naked) + (-)	+	rounded, smooth	s-r, lirae sharp
10	4	*S. cuneata* 1/1	20	Himalaya, a	p	o	short rhizomes	5	2	oblong saccate +	-	rounded, smooth	s-p
	14	*S. crassiuscula* 3/8	20	E & NE trop. Africa, Arabian peninsula, m-a	p	o	rhizomes	5	2	oblong +	-	rounded, smooth	s-p
	33	*S. welwitschii* 1/3	—	S & trop Africa, m	a	o	fibrous roots	5	2	oblong +	-	rounded, smooth	s-p
	34	*S. abyssinica* 2/4	20 (42)	trop. Africa, Madagascar l -m	a	o	fibrous roots	5	2	oblong +	-	rounded, smooth	s-p
	35	*S. intermixta* 1/3	20	E & NE trop. Africa, m	a	o	fibrous roots	4	2	sub-oblong +	-	rounded, smooth	s-p
	*19	*S. minor* 0/1	20	W India, m	a	o	fibrous roots	4	2	V-shaped, saccate + (dentate)	-	rounded, smooth	s-p

Swertia clade (Fig. 1)	Informal group (Shah,1984)	Representative species and spp. sequenced / spp. in group	Chromosome number (2n)	Distribution & habitat a: alpine m: montane l: lowland	Habit	Leaves	Roots	Merosity	N° of nectaries	Nectary type +: fimbriae present -: fimbriae absent	Style	Seeds	Pollen
11	23	*S. macrosperma* 3/5	18	Himalaya, E New Guinea m (-a)	a	o	fibrous roots	5 (4)	2	oblong +	(-)	(1-) 4 large (many small), smooth	v-p, colpi very short
	*6	*S. piloglandulosa* 0/1	—	N Sumatra, a	p	o	short rhizome, lower branches stoloniferous	4	2	oblong +	(-)	1-4 rounded, smooth	v-p, colpi very short
	17	*S. punicea* 8/11	20 (18, 21, 24)	Asia, m (- h)	a	o	fibrous roots	5 (4)	2	oblong (saccate) +	-	rounded, smooth	s-r
12	18	*S. striata* 3/3	20	E end of Himalaya, m	a	o	fibrous roots	5 (4)	2	+- V-shaped, saccate +	-	rounded, smooth	s-r
«abominable» clade	27	*S. hispidicalyx* 1/0	—	Tibetan plateau, a	a	o	fibrous roots	5	2	oblong saccate, opening towards receptacle +	+	rounded, smooth	s-r, lirae sharp
	28	*S. tenuis* 1/1	22	SW China, m	a	o	fibrous roots	5	1	watch-pocket shaped +	+ st	rounded, smooth	s to c-p
	16	*S. yunnanensis* 1/1	22	SW China, m	a	o	fibrous roots	5	2	oblong, opening laterally +	-	rounded, smooth	s to v-p, colpi short

Lineages of Swertia

Only two taxonomic treatments cover the whole genus. In his monograph, Shah (1984) proposed 35 informal groups, whereas Ho *et al.* (1994) recognised 11 sections and 16 series. In both treatments *Frasera* was considered as congeneric with *Swertia* and for this reason will be discussed in this section. These classifications are contradictory in many parts (see Chassot *et al.*, 2001 for examples), and sequence data corroborated almost exactly Shah's morphological groups. However, the definition of these groups was rather narrow, and a number of them were related in larger clades in our phylogeny (see Table 6). Combining information on the distribution (Shah, 1984; Nemomissa, 1994; Ho & Pringle, 1995), chromosome numbers (Chassot *et al.*, in ms.) and palynology (Nilsson, 1967b; Chassot & Hagen, in ms.) to gross morphological characters commonly used in the taxonomy of *Swertia* (Table 6), the clades of *Swertia* can be delineated as discussed below.

***Frasera*.** The *Frasera* clade was highly supported in all analyses (Figs. 1, 2 & 3) but the affinities of this exclusively North American genus with other, mostly Asian, lineages of the basal grade remained essentially unresolved (but see *Swertia* 2 below). Distinguishing features of this genus were never brought out convincingly and it has been repeatedly argued in favour or against its inclusion in *Swertia* (e. g. St. John, 1941; Shah, 1984; Pringle, 1990). Toyokuni (1965) even transferred some Japanese species of *Swertia* to *Frasera* on grounds of their distinct style, but his views were mostly not followed. From a flower morphological point of view, species of *Frasera* conform to the general bauplan observed in most species of *Swertia*, except for the presence of a variably dissected coronal scale (membrane between filaments) present in some species (e. g. *F. caroliniensis*, *F. albicaulis*) but absent in others. Vegetatively, xerophilous species have conduplicate papillous leaves with a silvery-white margin unknown from any species of *Swertia*, while more mesophilous species (*F. speciosa* - *F. fastigiata* grade) have flat leaves with a green margin. In addition, the latter species have verticillate leaves and a thick fleshy taproot, much like perennial species of *Swertia* 8 (see below), to which they were, however, not related. Yet, the few seeds per capsule, and their larger size separate species of *Frasera* from nearly all species of *Swertia*. Only taxa of *Swertia* 11 and *Swertia* 2 have a small number of seeds, although these are considerably smaller in the former (compare Fig. 5 D & N). On the other hand, seeds of *S. umbellata* (*Swertia* 2) are not only comparable in number, but also in size and exotestal structure (see below and Fig. 5 E). Shah (1984) divided species of *Swertia* (including *Frasera*) into 35

groups (Table 6). Group 10 corresponds to the *F. speciosa* - *F. fastigiata* grade and is also morphologically unambiguously defined. Groups 11 and 12 were separated on grounds of inflorescence type (dense and interrupted paniculate, and lax and corymbose paniculate respectively). These groups were not supported by the phylogeny, but no character was found in support of the *F. puberulenta* - *F. albicaulis* clade and *F. tubulosa* - *F. paniculata* polytomy. Considering the large sample of *Swertia* species and the exhaustive one of *Frasera*, the monophyly of *Frasera* in its current circumscription is quasi-certain. Thus, in view of molecular results, of the distribution, and of the seed characteristics of *Frasera*, we believe that it should be kept as a separate genus.

***Swertia* s. str.** It is by far the most speciose clade, containing ca. one third of the species of *Swertia*, and the type of the genus, *S. perennis*. *Swertia* s. str. was found to be related to *Megacodon*, and also to be the basalmost of all *Swertia* lineages in both Bayesian and parsimony phylogenies, although with low branch support. This group includes rather heterogeneous species, which, however, consistently lack the traits found to be characteristic of the other clades of *Swertia* (see Table 6). That is, seeds are variously shaped and sculptured (Fig. 5 F, G, H, I) but never rounded and smooth (difference with most other clades of *Swertia* in Fig. 2), roots never thickened and fleshy (difference with some species of *Swertia* 8 in Fig. 2), pollen grains never spinose (difference with *Swertia* 1, 2 and 10 in Fig. 2), etc., the main distinguishing feature being the seeds. Only in *Swertia* 1, 2 and 6 are the seeds also not rounded with a more or less smooth testa, but species of these clades differ from *Swertia* s. str. by a number of other characters (see below). Species of *Swertia* s. str. grouped into two broad and highly supported sub-clades, one of them further divided into 2 equally well supported lineages. These subdivisions apparently suggest a clear correlation between habit and habitat. In the first sub-clade (species of groups 1 to 3 in Table 6), the plants are perennial, mostly robust high altitude dwellers, widespread in the mountains of temperate Asia, with only a few species in eastern Europe, and one, *S. perennis*, also present in North America. In opposition, the second sub-clade consists of one lineage including Asian montane annuals or biennials growing along the southern margin of temperate Asia and in the southern mountains of the Indian sub-continent (groups 29, 30 & 32), and of a second lineage composed of the Asian annual and montane *S. cordata*, which is basal to all African perennial and mostly alpine species of *Swertia* s. str. (groups 13 & 15). Our results thus suggest that within the *Swertia* s. str. clade, the perennial versus annual or biennial habit, also the character most consistently retained in the infrageneric taxonomy of *Swertia*, reflects

ecological adaptation rather than phylogenetic affinities. The number of nectaries, on the other hand, coincides well with the phylogeny. In the *S. cordata* - *S. pauciflora* sub-clade, nectaries are constantly one per corolla lobe, whereas in the *S. perennis* sub-clade, nectaries are two per lobe, although confluent or altogether fused in a few species. Taxonomically, species of the *S. perennis* - *S. souliaei* sub-clade correspond to sect. *Swertia* and sect. *Rugosa* T.-N. Ho & S.-W Liu, but the inadequate resolution (Fig. 2) and limited number of species included did not allow to compare Shah's (1984) division into 3 groups with the 2 sections retained by of Ho *et al.* (1994). Species of the *S. alata* - *S. pauciflora* clade correspond to sect. *Spinosisemina* T.-N. Ho & S.-W Liu proposed in Ho *et al.* (1994), and were previously included in sect. *Ophelia* ser. *Ramosae* T.-N. Ho & S.-W Liu, along with most annual or biennial Asian species of *Swertia*. Although invalid according to art. 36.1 of the ICBN, sect. *Spinosisemina* is in agreement with molecular results. African species of *Swertia* s. str. were placed in sect. *Montana* T.-N. Ho & S.-W Liu (nom. invalid. according to art. 36.1 of the ICBN) in Ho *et al.* (1994), along with other perennial African species of *Swertia* 10. *Swertia cordata* belongs to sect. *Ophelia* ser. *Maculatae* T.-N. Ho & S.-W Liu, as do species of *Swertia* 1 and 3. In the context of our phylogenetic results, sect. *Montana* and sect. *Ophelia* ser. *Maculatae* and ser. *Ramosae* are largely polyphyletic.

***Swertia* 1.** *Swertia tashiroi* is the only species found to belong to this lineage, and is endemic of Taiwan and south-western Japan. This species was placed by Shah (1984) in the same group as species of *Swertia* 3 (group 21) on grounds of its Asian distribution and naked nectaries. The same characters were retained to justify its inclusion in sect. *Ophelia* ser. *Maculatae* (Ho *et al.*, 1988). But *S. tashiroi* combines spinose seeds (see Wang & Lu, 1998 for illustrations), a single broad nectary per corolla lobe devoid of any appendage, spinose pollen grains (Nilsson, 1967b; Chassot & Hagen, in ms.), nodding flowers and a robust, somewhat fleshy habit, distinguishing this species from all others. *Swertia tashiroi* was related to *Bartonia virginica* with low support in the parsimony analysis (Fig. 2 A), but its affinities were unresolved in the Bayesian one (Fig. 1 B), Apart from chromosome numbers (2n = 52 was found in both taxa, 2n = 60 only in *S. tashiroi*), we could not name a single gross morphological or palynological character supporting this relationship. Considering that *S. tashiroi* has probably experienced a long and isolated evolution (see Fig. 4), and also the accelerated substitution rate of *Bartonia virginica*, the sister relationship between these species (Fig. 2A) is most probably due to long-branch attraction.

***Swertia* 2.** *Swertia swertopsis*, the only species in this lineage, is morphologically unique in the genus, and was also first described as a monotypic genus, *Swertopsis umbellata* Makino. This species is a rare endemic of south-western Japan, and, much like *S. tashiroi*, represents a lineage of long isolated history (see Fig. 4). Characterised by its fasciculate flowers disposed in an umbel-like inflorescence, V-shaped nectaries, distinct slender style, shallowly corrugate and rather large seeds (Fig. 5 E), and spinose pollen, this species is clearly separable from all other clades of *Swertia*. Although chloroplast phylogenies (Fig. 1) suggested a relationship with species of *Swertia* 3 and 4 and *Halenia*, sequence data did not allow to reliably establish its position in the Swertiinae. Also from a morphological point of view, the relationship suggested by the chloroplast phylogeny is not convincing, as these taxa share none of the characteristics of *S. swertopsis* listed above. On the other hand, among the taxa of the basal grade (Fig. 2), some species of *Frasera* share a distinct and slender style such as that of *S. swertopsis*, petal nectaries, and similar seeds (Fig. 5 D & E).

***Swertia* 3.** This lineage consists of 3 species only. *S. bimaculata* is common from Nepal eastward to Japan, *S. oculata* Hemsl. is known only from the type gathering in China (Sichuan), and *S. tozanensis* (= S. matsudae Satake sensu Shah, 1984; see Wang & Lu, 1998) is endemic to Taiwan. All are annual or biennial, and distinguished by their paired naked nectaries situated relatively high on the corolla lobes (just below the middle). Species of *Swertia* 3 were sister to the *Halenia* - *Swertia* 4 clade with high branch support (Fig. 2), although hardly any morphological correspondence could be established, apart from the position of the nectaries (see Hagen & Kadereit, 2002), and seeds that are smooth to finely warted, which is rather unusual among taxa of the basal grade, that mostly have irregular seeds (e. g. winged, corrugate or echinate). According to chromosome numbers (2n = 26, rarely 22 or 24), the position of *Swertia* 3 in the basal grade of the Swertiinae seems justified, but the relationship with *Swertia* 4 (2n = 12, 14) and *Halenia* (2n =22) is not supported. Taxonomically, with exception of *S. cordata* (*Swertia* s. str.) and *S. tashiroi* (*Swertia* 1), this clade corresponds to sect. *Ophelia* ser. *Maculatae* sensu Ho & Liu (1980).

***Swertia* 4.** This lineage also consists of 3 species only, that differ from all other species of *Swertia* by their tetramerous flowers, deltoid-ovate leaves and nectaries that are bordered on their longitudinal edges only by 2 scales bearing short compound fimbriae (see Hagen & Kadereit, 2002 for illustrations). Seeds are globose and of a very light yellowish colour, and the testa is extremely smooth (Fig. 5 L). *Swertia tetraptera* and *S. tetrapetala* are very similar,

in particular as regards their floral morphology which varies only in the number of nectaries on each corolla lobe (Shah, 1984). *Swertia dichotoma,* on the other hand, differs by its dichotomous habit and nectaries, that are sub-rounded to elliptic, protruding on the abaxial side of the corolla lobes as miniature spurs, and bordered by much reduced appendages. The relationship of *Swertia* 4 with *Halenia* suggested by sequence data (Fig. 2) may seem surprising, considering that these species lack the most obvious features of *Halenia* such as a sinistrorse corolla aestivation, well-developed nectariferous spurs, a corolla tube and stamens inserted at the sinus of corolla lobes, and chromosome numbers are also different (2n = 12, 14 in *S. tetraptera*, 2n = 22 in *Halenia*). However, the embryology of *S. tetraptera* (Xue *et al.*, 1999) was shown to be similar to that of *Halenia*, and the protruding nectaries of *S. dichotoma* may be viewed as an early stage of the spurs observed in a majority of species of *Halenia*. Taxonomically, *S. dichotoma* is the type species of *Anagallidium* Griseb., and of the monotypic sect. *Ophelia* ser. *Dichotomae* T.-N. Ho & S.-W Liu. *Swertia tetrapetala* and *S. tetraptera* were placed in sect. *Heteranthos* T.-N. Ho & S.-W Liu (Ho *et al.*, 1994). Our results agree with Shah's(1984) placement of these 3 species in a narrow group (group 20), and do not support the separation of *Swertia* 4 into two different taxa. Moreover, the molecular monophyly and morphological particularities of this lineage may be viewed as an argument in favour of its recognition at generic rank under *Anagallidium*.

***Swertia* 5.** The single species of this clade, *S. rosularis*, was described recently (Ho & Liu, 1980) and is known only from the type locality. Its floral morphology is nearly identical to that of *S. tenuis* (a species of the «abominable» clade), and was considered by Shah (1984) as synonymous with it. Despite this floral similarity, *S. rosularis* is a perennial plant with rosulate, somewhat leathery basal leaves, clearly distinguishing it from *S. tenuis*. Furthermore, it is the only rhizomatous species of *Swertia* to have pentamerous flowers, smooth seeds, and a single nectary per corolla lobe. All molecular analyses related *S. rosularis* to *S. patula* (*Swertia* 6), and with high support in the combined analyses (Fig. 2), but not a single morphological character was found to support this relationship (see Table 6). However, the divergence time between these lineages was found to be comparable with that between *Halenia* and *Swertia* 3 or between *Gentianella* s. str., *Jaeschkea* s. str., *Swertia* 11 and *Swertia* 12 (Fig. 4), all lineages for which morphology was not found to reflect the relationships suggested by molecular data. In addition, adaptation to sensibly different habitats between species of *Swertia* 6 (xerophilous) and *Swertia rosularis* (hygrophilous) may have had a repercussion on their morphology. Upon its description (Ho & Liu, 1980), *S.*

rosularis was included in sect. *Ophelia* ser. *Perennes* T.-N. Ho & S.-W Liu, along with species of *Swertia* 6 (*S. patens* Burkill), *Swertia* 12 (*S. davidi*), and *Swertia* 8 (*S. pianmaensis*). Being based uniquely on the perennial habit of those Asian species obviously not related to other Asian perennials of *Swertia* s. str. (sects. *Swertia* and *Rugosa*), ser. *Perennes* has no phylogenetic relevance.

***Swertia* 6.** According to Shah (1984), three species (group 25) are closely related to *S. patula*, the only species included in our sample. These species can be distinguished from other Asian species of *Swertia* by their patulous habit, polyhedral warted seeds, stout style and nectary type (Table 6). The whole plant is often profusely branched from the base and forms a spreading inflorescence of showy reddish to pinkish-white tetramerous flowers borne on long pedicels. Each corolla lobe bears two more or less triangular nectaries covered by a fimbriate scale attached to the inner longitudinal edge and bottom of the nectariferous tissue. Despite the remarkable homogeneity of this group, *Swertia patens* was separated from the other two species (sect. *Ophelia*, ser. *Ramosae*) and included in sect. *Ophelia* ser. *Perennes* on grounds of its purportedly perennial habit (Ho *et al.*, 1988). Some specimens of this species studied for the purpose of this work presented well-developed and woody roots, probably owing to the dry environment in which the plants were growing, but all were annuals, as reported in Shah (1984).

***Swertia* 7.** The two species of this small lineage, *S. decora* and *S. delavayi*, resemble *Swertia* 6 in their habit, showy perianth, seed morphology (Fig. 5 J), and pollen grains (Chassot & Hagen, in ms.), but differ in having pentamerous flowers with two oblong saccate nectaries per corolla lobe and compound fimbriae, and a slender style topped by broad orbicular stigmas. *Swertia* 7 was related to the *Swertia* 5 - *Swertia* 6 clade in the combined parsimony phylogeny. This was not the case in the combined Bayesian one, but, as suggested by the ITS Bayesian analysis, the breaking down of this relationship is likely to be due to the attraction of the *Latouchea* branch with the *Swertia* 7 one. Considering the ovary nectary, fused corolla lobes forming a tube, long style and linear stigmas, corrugate seeds, spinose pollen and rhizomatous habit of *Latouchea*, the parsimony topology seems far more plausible than the Bayesian one. Taxonomically, both species were placed in the heterogeneous sect. *Ophelia* ser. *Ramosae* (Ho *et al.*, 1988).

***Swertia* 8.** This clade groups annual or biennial (group 26) and perennial (groups 8 and 9) species that are rather different in their gross morphology and palynology (see Table 6). However, their root system was found to be similar. Whether acaulous (group 8) or with a tall, robust stem (group 9), perennials have a thick and fleshy taproot (Fig. 5A) unique among Asian and African species of *Swertia* but also present in some species of the North American genus *Frasera* (see Chassot, 2003), to which they were molecularly not related. Among the annuals or biennials of *Swertia* 8, this type of taproot is less obvious in blooming plants (e. g. *S. chirayita*, Fig 5B), but distinct in vegetative rosettes (e. g. *S. membranifolia*, Fig. 6C, compare with *S. bimaculata*, Fig. 6D). Rosettes are seldom present in herbarium material, but out of the 38 annual or biennial species collected and observed in the field for this study, a such taproot was only found in *Swertia* 8. Two species not included in our phylogeny and corresponding to two monospecific groups in Shah (1984), may also be related to *Swertia* 8. *Swertia handeliana* Harry Sm. (group 5) lacks the typical taproot of this clade, but agrees in flower symmetry, number and structure of nectaries, seed shape, and exine ornamentation with other perennial species of this clade (see Table 6). In the original description (Smith, 1926) and in Chinese floras (Ho *et al.*, 1988; Ho & Pringle, 1995), *S. handeliana* is tetramerous, at contradiction with Shah's (1984) description and illustration of a pentamerous *S. handeliana*, otherwise in exact agreement with the other descriptions. *Swertia handeliana* is the only Asian dwarf perennial species of *Swertia* with a single naked nectary per corolla lobe, and smooth, rounded seeds, precluding confusion with any other taxon. We have not seen the type of this species, but all specimens studied were tetramerous, and we therefor consider tetramery to be the correct state. The second species is *S. ramosa* W. W. Smith. As no specimens were available to us for this study, its relationship with *Swertia* 8 follows from Shah's (1984) statement that it closely resembles species of group 9. Although poorly supported, the basal position of *Swertia* 8 in clade A (Fig. 2) is coherent with chromosome numbers and seed morphology. The few karyologically documented species of *Swertia* 8 all have x = 13 (2n = 26), which is typical for the taxa of the basal grade, whereas all other taxa of clade A have basic numbers different from 13, and usually smaller (Chassot *et al.*, in ms.). Likewise, the winged seeds of e. g. *S. barunensis* (Fig. 5K), *S. hookeri* C. B. Clarke, or *Swertia multicaulis* are reminiscent of the seeds found in some lineages of the basal grade, as *Swertia* s. str. (group 1) (Fig. 5F) or *Veratrilla bailloni* (Fig. 5C), whereas most species of *Swertia* 8 have more or less rounded seeds devoid of wings, as found in all other taxa of clade A. Moreover, constraining the monophyly of the species of *Swertia* in clade A (*S. hispidicalyx*, *S. tenuis*, *S. yunnanensis* excluded, see Table 5) resulted in not significantly less

parsimonious cladograms according to Templeton's SLP test (P = 0.39), but in which the *Swertia* 8 clade was consistently placed as most derived (not shown). Such topologies are particularly at discrepancy with chromosome numbers and thus unlikely, suggesting that paraphyly of *Swertia* in Clade A, as inferred without constraint, may be more probable than monophyly. Within the clade *Swertia* 8, and as it was the case in Asian species of *Swertia* s. str., the annual or biennial and perennial habit appears to be correlated with the adaptation of the plants to medium or high altitude habitats respectively (see Table 6). Moreover, this correlation was also reflected in the phylogeny, where plants of similar habit and altitude range formed sister lineages (Fig. 2). Taxonomically, no infrageneric taxon corresponds to *Swertia* 8, but perennial species of groups 8 and 9 belong to rather the well delineated sect. *Poephila* (C. B. Clarke) Gilg and sect. *Macranthos* T.-N. Ho & S.-W Liu respectively. Annuals of group 26 were placed in sect. *Ophelia* ser. *Ramosae* (Ho *et al*., 1988).

***Swertia* 9.** Species of this clade form a morphologically homogeneous group (group 27) delineated by flattened, pubescent, often monadelphous filaments, a slender style, and a single nectary per corolla lobe. In most species, the scale bordering or covering the nectariferous tissue is attached to its upper and longitudinal edges, the nectary thus appearing inverted (i. e. opening towards the receptacle). *Swertia* 9 was related to the *S. cuneata* - *Jaeschkea canaliculata* clade (Fig. 2), but a relationship with *Comastoma*, *Lomatogonium* and binectariate species of *Gentianella* was also suggested by the analysis of the reduced taxon sample including all chloroplast sequences (Fig. 3). Neither topologies were highly supported, and no morphological, karyological or palynological character was found that could reliably favour one or the other hypothesis. Interestingly, *Swertia* 9 is one of the few lineages of *Swertia* to have expanded its distribution range south-eastwards into the mountains of the Malaysian islands. Taxonomically, *Swertia* 8 corresponds to sect. *Platynema* T.-N. Ho & S.-W Liu. *Swertia teres* was placed in a separate section (sect. *Kingdon-Wardia* T.-N. Ho & S.-W Liu) on grounds of its distinct calyx tube. This was rejected by Shah (1984), and molecular results confirmed the close relationship of *S. teres* with other species of *Swertia* 8.

***Swertia* 10.** This clade consists of one Asian species (*S. cuneata*, group 4), and of the second lineage of African species of *Swertia* (groups 14, 33, 34, 35), characterised by their spinose-punctate pollen grains, smooth, rounded seeds, and chromosome number of 2n =20 (2n = 42 in *S. rosulata*). *Swertia cuneata* is perennial, but both perennial (e. g. *S. crassiuscula, S. engleri*) and annual (e. g. *S. abyssinica, S. rosulata, S. welwitschii*) African species belong

to *Swertia* 10. Phylogenetically, the situation in *Swertia* 10 parallels that in the *S. cordata* - *S. kilimandscharica* clade in *Swertia* s. str. (Fig. 2), with one Asian species sister to an African lineage. According to the basal position of perennial species, this African lineage may have evolved from perennial ancestors, contrary to the African lineage of *Swertia* s. str., *Swertia* 10 was related with moderate support to a broad and poorly supported clade comprising *Jaeschkea* s. str., *Gentianella* s. str. *Swertia* 11 and *Swertia* 12. No character was found to support this relationship between taxa of 3 rather different genera. Between lineages of *Swertia*, species of *Swertia* 10 and *Swertia* 12 share similar chromosome numbers (2n = 20 in most species), but not *Swertia* 11 (2n = 18). However, all 3 lineages have a markedly different pollen exine ornamentation (spinose-punctate, striate-reticulate and verrucate-punctate respectively, see Table 6). One additional Asian species not included in our sample, *S. minor* (Griseb.) Knobl. (group 19), could belong to this lineage, as it has all of its karyological, palynological and seed morphological characteristics. Taxonomically, no infrageneric taxon coincides with *Swertia* 10. *Swertia cuneata* was placed in sect. *Rugosa* T.-N. Ho & S.-W Liu (Ho *et al.*, 1988) on grounds of its perennial habit and despite its smooth, rounded seeds. Some perennial African species were placed in sect. *Montana*, others in sect. *Ophelia* ser. *Perennes*, and annual species were placed in sect. *Ophelia* ser. *Ramosae*.

***Swertia* 11.** Only few species belong to this lineage, corresponding to Shah's (1984) group 23 (but see Wang & Lu, 1998 for nomenclatural details on Taiwanese species included). Shah (1984) based this group on characters such as the presence of a very short style, sub-orbicular stigmas, oblong, fimbriate but not saccate nectaries, only 1-4 seeds per capsule and with a smooth testa (Fig. 5 N), and an annual habit. In addition, species of *Swertia* 11 differ from all other species of *Swertia* by their verrucate-punctate pollen grains with very short colpi (Chassot & Hagen, in ms.). The only species of group 6, the perennial *S. piloglandulosa* Geesink, was not included in our sample, but has pollen grains identical to those of *Swertia* 11 (Chassot & Hagen, in ms.), as well as only 1-4 seeds per capsule. On the other hand, *S. zayüensis* T.-N. Ho & S.-W Liu, known from the holotype only, has numerous small seeds, but was included by Shah (1984) in group 23. From the illustration (Ho & Liu, 1980), this species has saccate, radially elongated nectaries, and an ovoid-oblong ovary with sessile sub-orbicular stigmata, suggesting a more probable relationship with *Swertia* 12. Its pollen morphology is unknown, but would allow to assess its affinities with more certainty. As discussed for *Swertia* 10, no convincing correlation could be found between the relationships suggested for *Swertia* 11 by molecular results, and gross-morphology, karyology or

palynology. As regards the distribution of *Swertia* 11 (inclusive of *S. piloglandulosa*), its range extends from the mountains of mainland Asia south-eastwards as far as East New Guinea, presenting similarities with the distribution of *Swertia* 9. No infrageneric taxon confines to the species of *Swertia* 11. Annuals were placed in sect. *Ophelia* ser. *Ramosae*, and the perennial *S. piloglandulosa* in sect. *Ophelia* ser. *Perennes* (Ho *et al.*, 1994).

***Swertia* 12.** This clade corresponds to Shah's (1984) groups 17 and 18, between which the distinction was based only on the outline of the nectary. Plants of this lineage are all annual or biennial, rarely rooting at lower nodes on ascending branches (e. g. *S. davidi*), and are recognised from other Asian annual or biennial species by the paired fimbriate or dentate nectaries, ovoid to oblong ovary with sessile sub-orbicular stigmata, numerous rounded seeds with a more or less smooth testa, and chromosome numbers usually of 2n = 20 (Chassot *et al.*, in ms.). Relationships between *Swertia* 12 and the lineages included in the same polytomy (Fig. 2) are still unclear, and gross morphology and karyology did not allow to address the problem in more detail. However, species of *Gentianella* s. str., *Jaeschkea* s. str. and *Swertia* 12 share a common pollen type with a striate-perforate to striate-reticulate or reticulate sexine pattern, much different from the pollen of *Swertia* 11 (Nilsson, 1967b; Chassot & Hagen, in ms.). Within clade *Swertia* 12, relationships were unresolved, except for the basal position of *S. japonica* and *S. diluta*. Both species have an Eastern Asian distribution, suggesting that this lineage may have originated in Eastern Asia and subsequently colonised the Sino-Himalayan region along a westward route. Taxonomically, all the species of *Swertia* 12 were placed in sect. *Ophelia* ser. *Ramosae*, except for *S. davidi* that was placed in sect. *Ophelia* ser. *Perennes* on grounds of its occasionally rooting lower nodes (Ho *et al.*, 1988).

Molecular Phylogeny and Taxonomy

A majority of genera in the Swertiinae, i. e. *Bartonia*, *Comastoma*, *Gentianopsis*, *Halenia*, *Latouchea*, *Megacodon*, *Obolaria*, and *Veratrilla*, are morphologically unambiguously defined, and, from a molecular phylogenetic perspective and within the limits of the taxon sample, formed distinct monophyletic lineages. This was not the case for the remaining genera, that were either polyphyletic (*Gentianella*, *Jaeschkea*, *Lomatogonium* and *Swertia*), or very closely related to other genera and probably not deserving recognition at generic rank (*Lomatogoniopsis* and *Pterygocalyx*). Morphological observations on the latter two genera were in part on erroneous, and, when corrected for, leave only very few diagnostic characters.

Lomatogoniopsis was segregated from *Lomatogonium* chiefly on grounds of a single nectary per corolla lobe (Ho & Liu, 1985), but *L. alpina* clearly has two nectaries (Hagen & Kadereit, 2002, pers. obs.), and one species retained in *Lomatogonium*, *L. graciliflorum*, also has a single nectary. As discussed above, the actual circumscription of *Lomatogoniopsis* does not seem to be satisfactory, and our results concerning *L. alpina* suggest that it should be merged with *Lomatogonium*.

Similarly, *Pterygocalyx volubilis* was believed to differ from *Gentianopsis* in having, i. a., nectaries on the ovary and not on the petals (Ho & Pringle, 1995), but detailed studies revealed that this was not the case and that only few characters separated the two genera (Chen & Ho, 1998). Moreover, the affinity of *P. volubilis* with *G. ciliata*, as suggested by ITS data (Fig. 1 B), is also supported by the shared flattened seeds with an annular wing. In view of the very high support for the *Gentianopsis* - *Pterygocalyx* clade (Fig. 2), and of the weak morphological and molecular differences between both taxa, it may be better to consider *Pterygocalyx* at a section level.

Gentianella and *Swertia* are particular genera from a taxonomic point of view, as they may be viewed as leftovers of larger genera from which several smaller taxa were segregated in the recent past. For example, *Gentianella* has seen the number of its species increase drastically as former species of *Gentiana* were transferred to it following the generalised acceptance of a *Gentianella* lineage, as opposed to the *Gentiana* lineage (see Adams, 1995; Chassot *et al.*, 2001). This resulted in a large and heterogeneous genus without a more precise circumscription than its differences with *Gentiana*. Later on, *Comastoma*, *Gentianopsis* and *Megacodon* were separated from *Gentianella* on grounds of solid morphological (Smith, 1936; Ma, 1951; Toyokuni, 1961), anatomical (Lindsey, 1940) and palynological (Nilsson, 1967b) characters, and these genera were also well supported by molecular phylogenetic results. Of the ca 250 species left in *Gentianella*, a vast majority were shown to be monophyletic (Hagen & Kadereit, 2001), but molecular evidence suggests that our understanding of the binectariate species is not yet satisfactory. New genera pertaining to these species have been recently described or reinstated (Omer *et al.*, 1988; Omer & Qaiser, 1991, 1992), but only regional floras were taken into consideration. Of these, the monotypic *Aliopsis* Omer & Kaiser relates to *Gentianella pygmaea*. Yet, the original description (Omer & Qaiser, 1991) mentions a single nectary per corolla segment, but in all specimens of this species examined, 2 greenish nectariferous folds were observed at the point of insertion of the filament, much higher on the corolla tube than in the original illustration. The closely related

Gentianella arenaria also has two nectaries but at the base of the corolla tube. Our results support the recognition of these two species at generic rank under *Aliopsis*, but the description would need corrections, as it relies much on the number of nectaries. In a similarly confusing manner, based essentially on paired nectaries, binectariate species of *Gentianella* from Pakistan and Kashmir (e. g. *G. moorcroftiana* in our sample) were all accommodated under the name *Aloitis* Rafin. (Omer *et al.*, 1988), along with species of *Gentianella* s. str (e. g. *G. umbellata* in our sample, *G. holosteoides* and *G. stoliczkai* in Hagen & Kadereit, 2001), that may have bilobed but not distinctly paired nectaries (Hagen & Kadereit, 2001). Moreover, *G. umbellata* was included in *Aloitis* but illustrated with a single nectary per corolla segment! Likewise, Omer & Kaiser (1992) described *Kurramiana* based on *Gentiana micrantha* Aitch. & Hemsl. with tetramerous flowers and a single gland per corolla segment, while Garg (1987) listed this species as a synonym of *G. gentianoides*, which is pentamerous and has 2 nectaries (Ho & Pringle, 1995, pers. obs), and Ho & Pringle (1995) considered *Kurramiana* to be synonymous with *Jaeschkea*. Thus, molecular data strongly suggest that binectariate species of *Gentianella* have not been sorted out yet, but it also seems that the morphology and diversity of Asian species of *Gentianella* and in particular of the binectariate ones are still poorly understood, leading to premature taxonomic undertakings.

In the case of *Swertia*, the extent of polyphyly revealed by the present results is only moderately surprising. In its current acceptation, the genus *Swertia* consists of a rather heterogeneous assemblage of polymorphic species (Shah, 1984). Numerous generic segregates were proposed (e. g. *Agathotes* Griseb., *Anagallidium* Griseb., *Monobothrium* Hochst., *Ophelia* Griseb., *Sczukinia* Turcz., *Swertopsis* Makino), but diagnostic characters were never brought out convincingly, and limits became ever fainter as new species were discovered and described (see Shah, 1984 for a historical survey of *Swertia*). The only genera separated from *Swertia* to have been widely accepted are *Halenia*, *Lomatogonium* and *Veratrilla*. Other generic names were abandoned, and the circumscription of *Swertia* widened to fit the increasing morphological diversity. Consequently, according to Shah (1984), the definition of *Swertia* (inclusive of *Frasera*) is a plant with a rotate corolla (as opposed to the genera with a long corolla tube), epipetalous nectaries (as opposed to the genera with nectaries on the ovary), not dioecious (i. e. not a *Veratrilla*), spurless (i. e. not a *Halenia*), and without decurrent stigmata (i. e. not a *Lomatogonium*). In sum thus, *Swertia* is simply defined as not being anything else in the Swertiinae. But the fact that the various lineages of *Swertia* do not show particularly striking characteristics as may other lineages recognised at generic rank does not justify, in our opinion, that they be denied of an equivalent taxonomic rank. The

recent advances in molecular phylogenetics (present results), palynology (Nilsson, 1967b; Chassot & Hagen, in ms.) and karyology (Chassot *et al.*, in ms.) in *Swertia* have allowed to establish affinities between the numerous and narrow groups defined by Shah (1984), and to define a more reasonable number of larger but coherent groups. The question is thus not whether our results support one or the other of the numerous segregates proposed for *Swertia*, as none were found to be congruent with our results excepted for *Frasera* and partly for *Anagallidium*, but whether or not this large group of species should continue to be regarded as a single nomenclatural entity. If the idea of splitting *Swertia* into a dozen smaller genera may seem daring, one should nevertheless consider that each of the lineages presently identified by molecular data can readily be characterised by unambiguous combinations of characters. Moreover, and in spite of the fact that several lineages of *Swertia* are delineated by karyological or palynological characters, all these lineages may be keyed out relatively easily using gross-morphological character only (see determination key). It must be pointed out, however, that, as detailed in the description of the lineages of *Swertia*, these are often at conflict with the existing infrageneric classifications of Ho *et al.* (1988, 1994). As these classifications were often based on a single or very few gross-morphological characters only, such as annual or perennial habit (e. g. sect. *Ophelia* and sect. *Ophelia* ser. *Perennes*), naked or appendaged nectaries (e. g. sect. *Ophelia* ser. *Maculatae*), more or less developed calyx and corolla tube (sect. *Kingdon-Wardia*), floral dimorphism (sect. *Heterathos*), etc., it is not unsafe to consider that they were inadequately defined, and probably for this reason also not retained in the more recent account of the Flora of China (Ho & Pringle, 1995).

Biogeography

Time Scale. The 2 calibration dates utilised resulted in rather large intervals of estimated divergence times (Fig. 4), and it is difficult to identify particular climatic or geological events that may have caused the lineage splits observed in our phylogeny and thus provide reliable dates for their cladogenesis. Fossils enabling a calibration of divergence events are lacking, except for recent pollen of *Gentianella* in South America (see Hagen & Kadereit, 2001), according to which our calibration was based. In another biogeographic interpretations (Ho *et al.*, 1994), it was postulated that *Swertia* initially diverged in the Cretaceous. This seems highly unlikely, as according to the present chronogram (Fig. 4), it would set the radiation of *Gentianella* s. str. in the Andes some 25 mya. Moreover, the Gentianaceae probably originated only 50 mya (Yuan *et al.*, 2003). It could be suggested, however, that the rapid

multiplication of lineages both in the basal grade (from *Veratrilla* upward in Fig. 4) and in clade A, that occurred between roughly 4.5 and 6 mya or 11.5 and 15 mya depending on the calibration, resulted from profound and equally rapid environmental changes. Within this time span and coeval with a global vegetation change (Cerling *et al.*, 1997), at 8 ± 3 mya, the Tibetan plateau was thrust up 1000 to 2500 m in only a few million years, accentuating the Asian monsoon and thereby creating a considerable climatic disruption in Asia (Molnar *et al.*, 1993). As a consequence of changing altitude, temperature and hydric conditions, plants may have migrated and/or adapted to these new conditions relatively fast, resulting in the very short and uncertain (Fig. 2 A & B), or 0-length branches (Fig. 3) relating these lineages, and in the mosaic distribution pattern observed at present (but this pattern may have been considerably influenced by later migrations in the late Tertiary and Quaternary). If this hypothesis were to be correct, the subdivision between the basal lineage *Latouchea* - *Obolaria* and the remaining Swertiinae would roughly coincide with the onset of the Himalayan orogeny in the early Miocene (Molnar, 1986; Valdiya, 2002), and, by deduction since Gentianinae were not considered for this analysis, the split between Swertiinae and Gentianinae could have occurred before the Miocene. Yet, prior to the main Tibetan uplift, a global temperature deterioration and aridification beginning at ca 13 mya (Wolfe, 1985) or possibly as early as 22 mya (Guo *et al.*, 2002) may also have played a role in the history of lineages such as *Gentianopsis*/*Pterygocalyx*, *Megacodon* and *Swertia* s. str.

Distribution patterns. It was postulated (Struwe *et al.*, 2002) that the original range of Swertiinae was Laurasia-wide, and that, as inferred from the distribution of putative basal genera, an early split occurred between an eastern North American lineage (*Bartonia* and *Obolaria*) and an East Asian lineage (*Latouchea* and *Megacodon*). According to this scenario, the remaining taxa of the Swertiinae would have originated from the basal Asian lineage, but subsequent migrations between the continents may have masked this ancient split (e. g. *Gentianopsis, Frasera*). However, a such primary North American - Asian subdivision was not supported by the phylogeny, as *Bartonia* and *Obolaria* were not found to be monophyletic, and *Latouchea* and *Megacodon* not basal to the other Swertiinae (see above and Fig. 2). This hypothesis was also rejected as alternative topology by Templeton's SLP test (Table 5). The basal lineage of the Swertiinae consisting of the two monotypic genera *Latouchea* and *Obolaria* showed an East Asian - eastern North American disjunct distribution and did not allow to decide of the place of origin of the subtribe. Yet, considering the

probable Asian origin (Yuan & Küpfer, 1995; Struwe *et al.*, 2002) of Gentianinae, and also the fact that the remaining Swertiinae diversified mainly or exclusively in Asia, and from there to other continents (see below), an Asian origin for this subtribe seems reasonable but remains hypothetical.

Most genera/lineages in the Swertiinae have an exclusively Asian distribution. This is the case for *Jaeschkea*, *Latouchea*, *Megacodon*, *Pterygocalyx*, *Veratrilla*, the binectariate species of *Gentianella*, and all but 2 lineages of *Swertia* (*Swertia* s. str. and *Swertia* 10). Some genera/lineages such as *Comastoma*, *Lomatogonium* and Asian perennials of *Swertia* s. str. are Asian in their majority but count a few species of broader distribution (e. g. the circumboreal *C. tenellum*, *L. rotatum* and *S. perennis*). All these genera/lineages clearly have a maximum species diversity in the Sino-Himalayan region, and for most of them a peak of species richness in Southwest China. Within these Asian lineages, biogeographical history is difficult to reconstruct, although a few patterns emerged from the phylogeny, as the possible colonisation of the Sino-Himalayan region from the East by species of *Swertia* 12 (see above). The rather recent split (Fig. 4) between the Asian *S. angustifolia* and the South Indian *S. beddomei* (*Swertia* s. str. clade) suggests that southward migrations to the montane habitats of South India were governed by Pleistocene glaciations, in agreement with the views on the origin of Himalayan floristic elements of this region (Ashton & Gunatilleke, 1987). Similarly, the divergence in the lineage of *Swertia* 11 between the Asian *S. macrosperma* and *S. papuana* occurring in Papuasia-New Guinea appears to be even more recent. Although the emerging of the massive Himalayan barrier may be expected to have caused important altitudinal vicariance, this was only observed in the *Swertia* s. str. and *Swertia* 8 lineages (see above). Divergence between high and lower altitude taxa in these lineages was estimated to have taken place at different dates, and whether it is directly attributable to different orogenic episodes is uncertain.

Four lineages clearly originated in Asia but diversified mostly elsewhere. This is the case for *Halenia* with few species in Asia but many in South America. Its relatives are all Asian (*Swertia* 3 in south-eastern temperate Asia and *Swertia* 4 in south-central China and north-eastern Eurasia), as well as its basal species *H. elliptica* and *H. corniculata*. The severance between Asian species of *Halenia* and their American relatives appears to have occurred at the most 2.5 mya, suggesting that the radiation of *Halenia* in the Andes may be of very recent age. Likewise, two lineages of *Swertia* (African species of *Swertia* s. str. and *Swertia* 10) have an Asian sister species but diversified in Africa. However, according to Fig. 4, dispersal to Africa in *Swertia* s. str. seems to have happened much later than in *Swertia* 10. Too few

species were included to elaborate a more precise biogeographic scenario, but it may be interesting to note that in both cases, divergence times (1.3 - 3.1 mya and 4.4 - 12.4 mya respectively) were estimated within a range of major faunal turnovers in Eastern Africa (Behrensmeyer *et al.*, 1997; Winkler, 2002). As regards the *Gentianella* s. str. lineage, all other lineages related to it are exclusively Asian, implying an Asian origin for its progenitor. Different biogeographic hypotheses were inferred for *Gentianella* s. str. from nuclear and chloroplast phylogenies (Hagen & Kadereit, 2001). Our results revealed the same discrepancy, but the analysis of combined data differed in that it favoured a North American origin of the fimbriate species and paraphyly for the efimbriate Eurasian and southern hemispheric species (compare Fig. 3 with Hagen & Kadereit, 2001).

Four species included in our sample are island endemics. *Swertia matsudae* (Taiwan) and *Swertia rosulata* (Madagascar) apparently diverged only recently (Fig. 4). *Swertia swertopsis* (Japan) and *S. tashiroi* (Taiwan and south-western Japan), on the other hand, represent two rather ancient lineages (Figs. 3 & 4), but it is not clear if their distribution reflects an initial insular isolated evolution, or if the present day distribution is secondary.

As regards genera of exclusively North America distribution, the 3 (or 4, depending on the viewpoints) species of *Bartonia* and the single species of *Obolaria* have an eastern North American distribution rather typical for Tertiary warm temperate relict floras comprising a number of disjunct eastern North American - East Asian sister-taxa (Milne & Abbott, 2002). However, this is not so for the species of *Frasera*, of which 13 out of 14 grow in western North America. In this lineage, speciation seems to have responded to changes in hydric conditions, as also supported by phylogenetic relationships. Whereas species of the *F. speciosa* - *F. fastigiata* grade are mesophilous and grow at a higher altitude/latitude, those of the *F. tubulosa* - *F. albicaulis* clade are mostly xerophilous plants distributed in the intermountain system. These two groups were estimated to have diverged 2.2 - 6.9 mya (Fig. 4), probably as a consequence of the xeric conditions in the Basin and Range Province that resulted from the rain shadow caused by the appreciably uplifted Cascade-Sierra Nevada and Coast Ranges at the Pliocene (Graham, 1993). Yet it appears that the progenitor of *Frasera* lived several million years before these events, but the origin and affinities of this lineage are far from clear.

Key to the lineages of *Swertia* (including *Frasera*)

1 Seeds ≥ 4mm in length, to 10 mm 2
– Seeds < 4mm in length 3

2 Flowers 4-merous; style distinct; leaves opposite or verticillate, conduplicate with a silvery white margin, or leaves verticillate, flat with a green margin; pollen striate to striate-reticulate (Plants from North America)..... *Frasera*
– Flowers 5-merous; style distinct; leaves opposite, flat with a green margin; inflorescence a fasciculate cyme or umbel; pollen spinose-punctate. (Plant from Japan)..... *Swertia* 2

3 Seeds 1-4, testa smooth; nectaries 2, oblong, fimbriate, not saccate; pollen verrucate-punctate with very short colpi ..... *Swertia* 11
– Seeds numerous, testa variable; nectaries 1 or 2, variable; pollen not verrucate-punctate (except in *S. yunnanensis*, see 18) 4

4 Plant perennial 5
– Plant annual or biennial 9

5 Taproot long and fleshy; flowers 4-merous, nectary 1,..... *Swertia* 8
– Flowers 4- or 5-merous, nectary 1 or 2, plant with rhizomes (long or short and thickened) or stolons 6

6 Seeds corrugate, corrugate-cristate, polyhedral-winged, or flattened with an annular wing..... *Swertia* s. str.
– Seeds sub-globose to ovoid, testa smooth to finely alveolate 7

7 Flowers 4-merous, nectary 1 (*S. handeliana* and *S. ramosa*) ..... *Swertia* 8 ?
– Flowers 5-merous, nectaries 1 or 2 8

8 Nectaries 2; style absent; pollen spinose-punctate ..... *Swertia* 10
– Nectary 1; style short, stout; pollen striate reticulate ..... *Swertia* 5

9 Filaments flattened, connate or free at the base, pubescent; style distinct; nectaries usually inverted (opening towards the receptacle), rarely naked 10
– Filaments filiform to subulate, glabrous; style present or absent; nectaries variable but not inverted 11

10 Nectary 1..... *Swertia* 9
– Nectaries 2 ..... *Swertia*? *hispidical*

11 Nectary naked 12
– Nectary fimbriate or dentate at the margin..... 14

12 Nectaries 2; seeds with smooth to finely alveolate testa ..... *Swertia* 3
– Nectary 1; seeds with conspicuously sculptured testa 13

Key to the lineages of *Swertia* (continued)

13 Basal leaves rosulate, present at anthesis, leaves somewhat fleshy; seeds echinate; style absent; pollen spinose (tuberculate)-punctate (Plant from Japan & Taiwan) ..... *Swertia* 1
– Basal leaves absent at anthesis, leaves not fleshy; seeds corrugate; style short; pollen striate-reticulate (Plant from temperate Himalaya, *S. cordata*) ..... *Swertia* s. str.

14 Seeds sub-globose to ovoid, testa smooth to finely alveolate 15
– Seeds corrugate, corrugate cristate, echinate, or polyhedral and warted 20

15 Nectary radially elongated or elliptic and protruding abaxially, with 2 shortly fimbriate scales attached to its longitudinal edges only, fimbriae compound, flowers 4-merous ..... *Swertia* 4
– Nectary not so, flowers 4- or 5-merous 16

16 Nectary 1, watch-pocket shaped, with long compound fimbriae; flowers 5-merous; ..... *Swertia*? *tenuis*
– Nectaries 2, not watch-pocket shaped, fimbriate or dentate; flowers 4 or 5-merous 17

17 Taproot thickened, fleshy; style short, stout; stigma lobes oblong to linear; flowers 4-merous; nectaries 2 ..... *Swertia* 8
Taproot, if present, not thickened and fleshy; style absent; stigma lobes sub-orbicular; flowers 4 or 5-merous; nectaries 2 18

18 Nectary oblong, saccate, opening laterally; scale of the nectary laciniate, attached to the inner longitudinal side of the secretory tissue; flowers 5-merous ..... *Swertia*? *yunnanensis*
– Nectary oblong or +- V-shaped, opening radially; scale of the nectary saccate-fimbriate, fimbriate or dentate, attached at least on both sides of the secretory tissue; flowers 4 or 5-merous 19

19 Pollen spinose-punctate; corolla lobes obtuse to acute. (Plant from Africa of SW India) ..... *Swertia* 10
– Pollen striate to striate-reticulate; corolla lobes acute to acuminate. (Plant from Asia, but not SW India) *Swertia* 12

20 Flowers 5-merous; style distinct, slender; stigma lobes orbicular, broad; nectaries 2, saccate, with compound fimbriae; seeds minute, polyhedral, warted ..... *Swertia* 7
– Flowers 4-merous; style short, stout; stigma lobes sub-orbicular, not broad; nectaries 1 or 2, variable; seeds variable 21

21 Nectaries 2, +- triangular; scale of the nectary fimbriate, attached to the base and to the inner longitudinal side of the secretory tissue ..... *Swertia* 6
– Nectary 1, sub-orbicular or watch-pocket shaped; scale fimbriate, not attached as above ..... *Swertia* s. str.

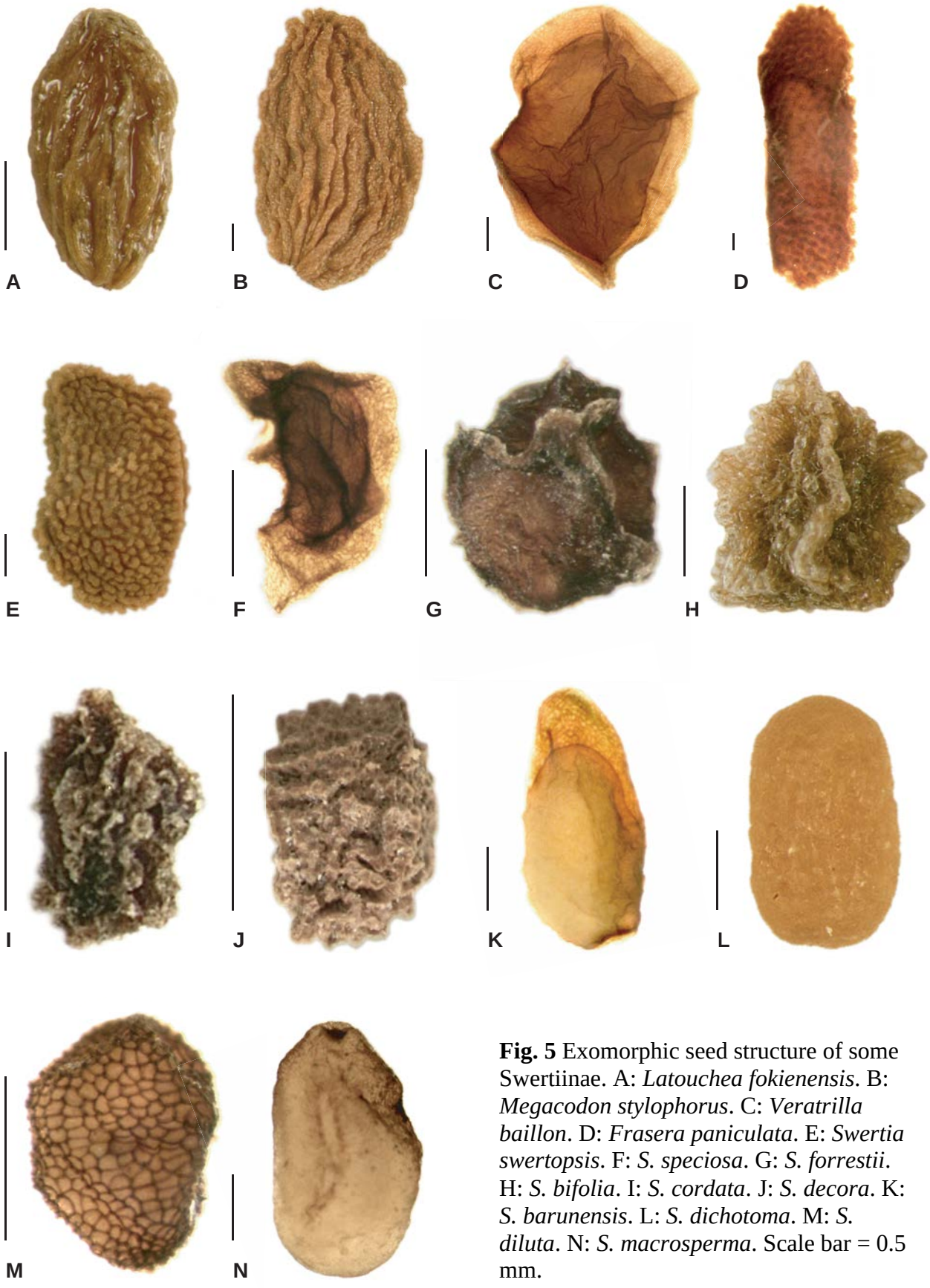


Fig. 5 Exomorphic seed structure of some Swertiinae. A: *Latouchea fokienensis*. B: *Megacodon stylophorus*. C: *Veratrilla baillon*. D: *Frasera paniculata*. E: *Swertia swertopsis*. F: *S. speciosa*. G: *S. forrestii*. H: *S. bifolia*. I: *S. cordata*. J: *S. decora*. K: *S. barunensis*. L: *S. dichotoma*. M: *S. diluta*. N: *S. macrosperma*. Scale bar = 0.5 mm.

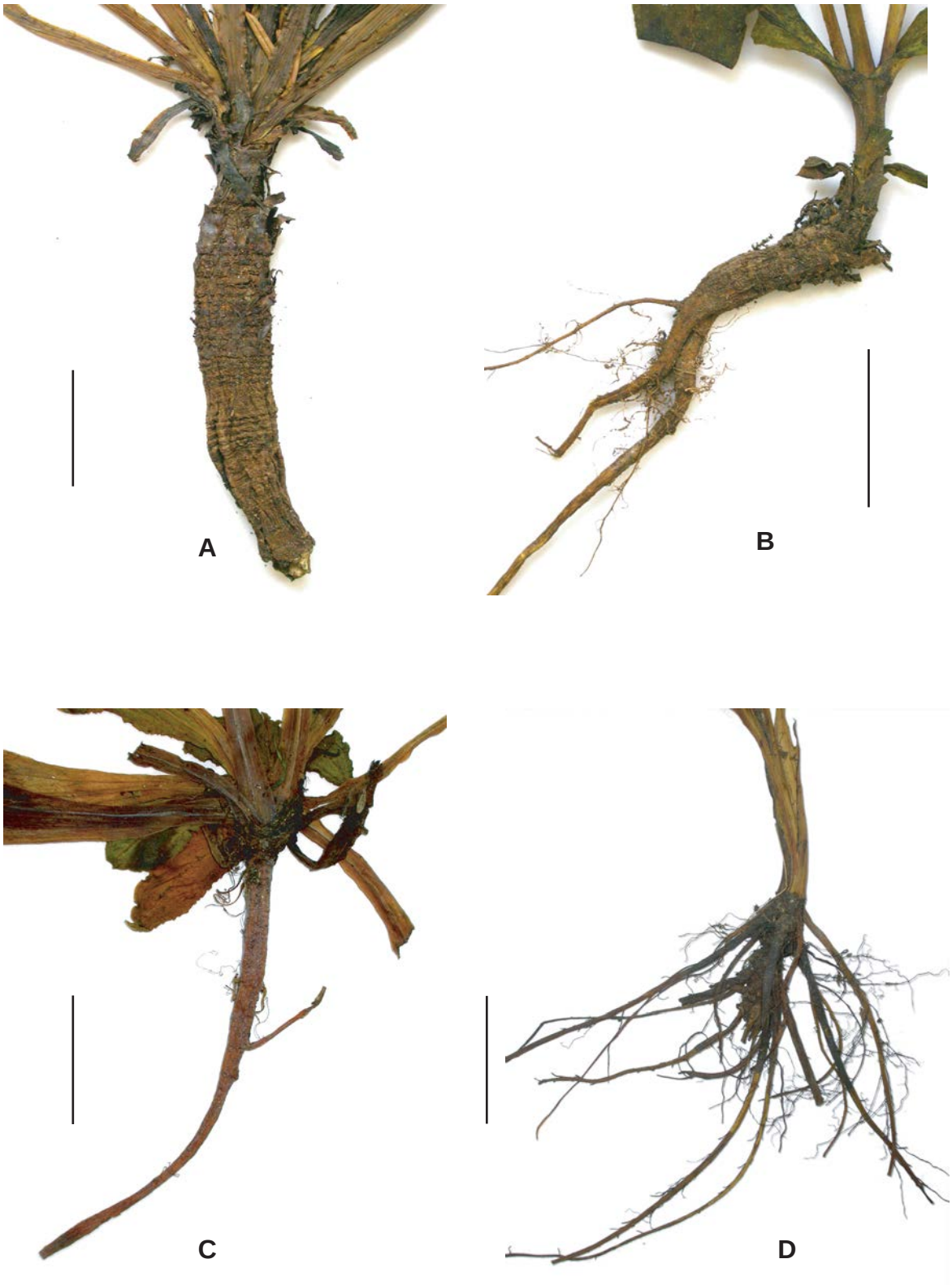


Fig. 6 Fleshy and long taproot of the perennial species of clade *Swertia* 8 (Fig. 2). A: *S. barunensis*, perennial. B: Similar but reduced taproot in the biennial *S. chirayita*, blooming plant. C: *S. membranifolia*, vegetative rosette. D: For comparison, the mostly lateral roots observed in annuals or biennials of other clades of *Swertia* (here a vegetative rosette of *S. bimaculata*).

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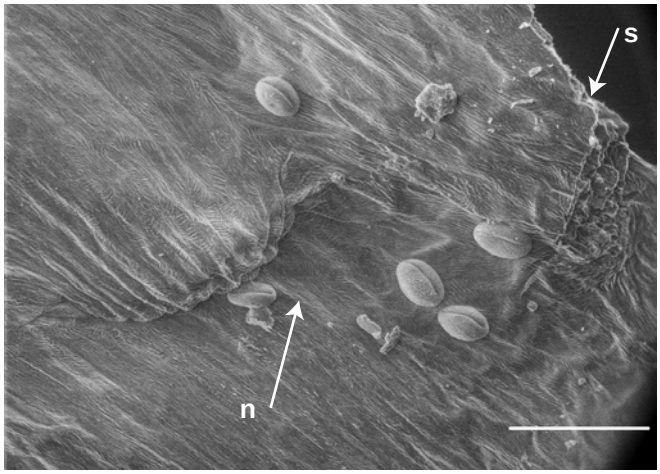

Fig. 7 Nectariferous fold (n) in *Gentianella pygmaea*, at the point of insertion of the stamen (s) (broken). Scale bar = 100 µm.

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V

A NEW SPECIES OF *SWERTIA* L. (GENTIANACEAE) FROM NEPAL

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A new species of *Swertia* L. (Gentianaceae) from Nepal

PHILIPPE CHASSOT

Université de Neuchâtel, Institut de Botanique, Laboratoire de Botanique évolutive, Case postale 2, 2007 Neuchâtel 7, Switzerland

Swertia barunensis P. Chassot **sp. nov.** from Nepal is described and illustrated. It was collected in 1997 in an alpine meadow in the Makalu Barun National Park at an elevation of 4200m. It belongs to *Swertia* section *Macranthos* T.-N. Ho & S.-W. Liu and resembles *S. pseudohookeri* H. Smith from which it differs mainly by the shape of the nectary and the exomorphic seed structure. A key to all the species of sect. *Macranthos* is provided. The affinities of *S. barunensis* with some other taxa in the subtribe *Swertiinae* (Griseb.) Rchb. are also briefly discussed.

ADDITIONAL KEYWORDS: Himalaya — *Macranthos* — systematics — taxonomy.

INTRODUCTION

The genus *Swertia* is a rather well documented taxon of about 150 species distributed mainly in the mountains of Asia and Africa. The important botanical explorations in the Himalaya during the last century, coupled with the thorough descriptions of herbarium collections realised in the past 30 years has cast a new light on the number and distribution of the species in this genus. Since Shah's (1984) monograph of the genus, only a few taxa have been subject to synonymic modifications, while new species are discovered only rarely, e. g. *S. luquanensis* S.-W. Liu from China and *S. chiangdaoensis* P. Suksathan from Thailand.

The new species described here belongs to section *Macranthos* T.-N. Ho & S.-W. Liu. It was collected in the Makalu Barun National Park in Nepal, in an alpine meadow at an altitude of 4200 m near the Shipton pass, on the path leading to the Barun valley from Tashi Gaon (Fig. 1). It is known only from the type locality. Including the species presently described, sect. *Macranthos* comprises 12 species that were all, until recently, considered as one, namely *S. hookeri* C. B. Clarke. H. Smith (1965, 1970) reviewed the specimens of *S. hookeri* s. l. collected during the British expeditions in the Himalaya and recognised 8 additional species: *S. assamensis*, *S. candelabrum*, *S. crossoloma*, *S. grandiflora*, *S. pseudohookeri*, *S. splendens*, *S. staintonii* and *S. virescens*. From the collections examined by Smith, one additional species, *S. taylorii*, was described by Shah (1984). One more taxon was discovered in Tibet in 1975 and subsequently described by T.-N. Ho & S.-W. Liu (1980) as *S. verticillifolia*.

SPECIES DESCRIPTION

SWERTIA BARUNENSIS P. CHASSOT, **SP. NOV.**
(SECT. *MACRANTHOS* T.-N. HO & S.-W. LIU)
(FIGS. 2, 3)

Diagnosis: Ad *Swertiae* species sectionis *Macranthi* T.-N. Ho & S.-W. Liu, gregi informali IX (Shah, 1984) congruentis, pertinens. *S. pseudohookeri* affinis sed, inter alia, nectario orbiculari et seminibus ovoideis in extremitate unica alatis distat.

Planta 60 cm alta, caulis teres. Radix robusta, 15 cm longa vel ultra, 2 cm diametro, numerosis cicatribus foliarum praecedentium annorum notata, basi modice ramificans, apice caulem floriferum singulum ferens. Folia rosularia

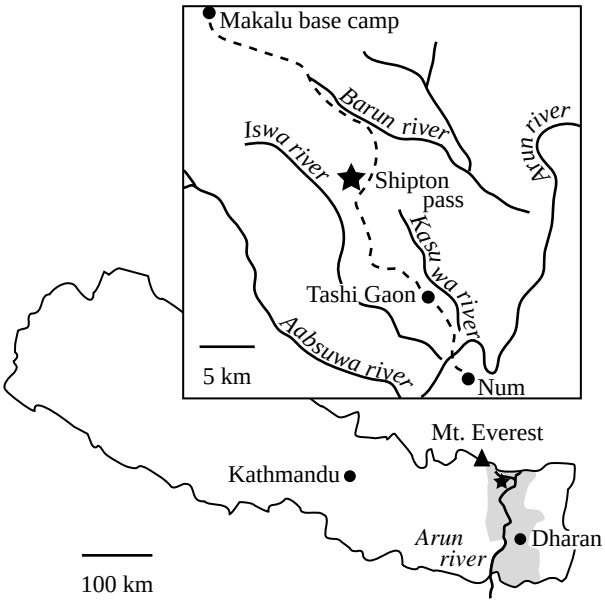

Figure 1. Map of Nepal showing the collection locality of ***Swertia barunensis* sp. nov.** (✶) in the Kosi district (shaded).

Figure 2. *Swertia barunensis* sp. nov. before pressing.

plantae florentis marcida vel delapsa, non florentis elliptica, ad 17 cm longa et ad 5 cm lata, apice subacuta, basin in petiolum latum sensim attenuata. Folia caulina ovata, sessilia, subacuta, 4-verticillata, infimo nodo 6cm longa et 3 cm lata, apicem versus decrescentia. Inflorescentia pyramidalis, e basi et nodis 2 rami 4, pluriflori, suberecti, florendi tempore 7-9 cm longi editi, flores in superioribus nodis numerosi, erecti. Flores 4-meri, virescentes, brunneo-striati; 1.5-6 cm longe pedicellati, pedicellis glabris, apice sepalis decurrentibus leviter alatis. Calycis tubus perbrevis, lobi subaequales ovati, obtusi, ad 1 cm longi et 0.6 cm lati, margine integri. Corollae tubus perbrevis, lobi obcuneati, apice truncati et parum erosi, ad 1.5 cm longi et 0.9 cm lati, nectario orbiculari circum longe fimbriato. Stamina corollae tubo adnata, filamentis liberis 6 mm longis et thecis 2.5 mm longis et 2 mm latis. Ovarium ovatum, stigmatibus parvis et rotundatis, stylo ad 1 mm longo. Capsula ad 1.8 cm longa, seminibus ovoideis in extremitate unica irregulatim alatis, ca. 1.2 mm longis et 1 mm latis.

Type: Nepal, Kosi: Makalu Barun National park, Shipton pass, alpine meadow, ca. 4200m, 16 ix 1997, Chassot 97-28 (holotypus: NEU).

Description: Robust perennial herb 60 cm high. ROOT fleshy to woody, yellow, 2 cm thick and 15 cm or more long, unbranched excepted at the base, bearing a single flowering stem, circularly marked by scars of leaves from preceding years. STEM robust, hollow, terete, to 1 cm in diameter. BASAL LEAVES represented by fibrous remains, rosulate, withered on the flowering plant, those of the vegetative rosettes elliptic, subacute, up to 17 cm long and 5 cm wide, gradually attenuated on the enlarged petiole, margin entire. CAULINE LEAVES ovate, sessile, subacute, verticillate by 4, the lowest 6 cm long and 3 cm wide, gradually diminishing in size towards the apex of the stem,

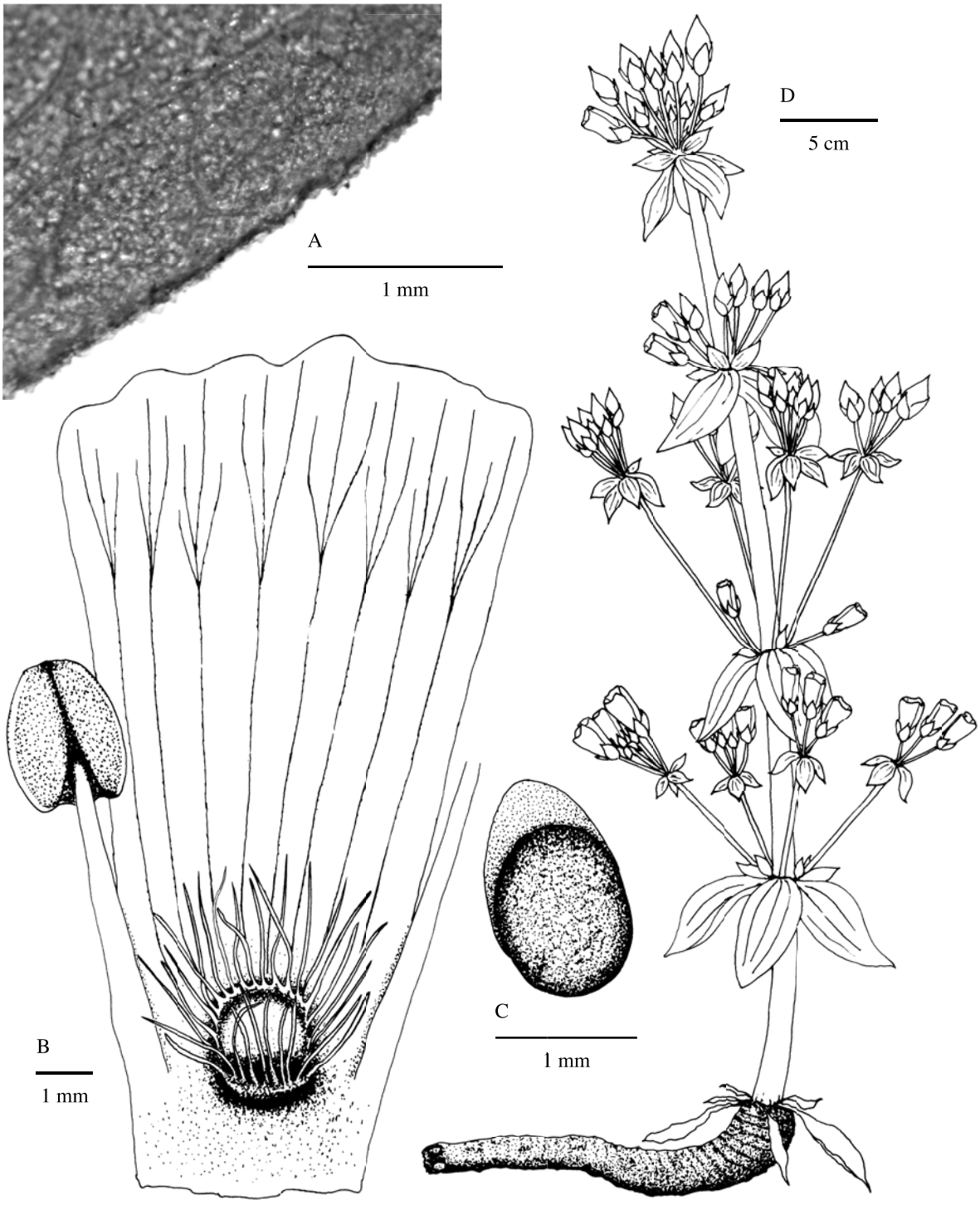

Figure 3. ***Swertia barunensis*** P. Chassot **sp. nov.** (A) Marginal portion of sepal showing entire margin. (B) Corolla lobe with fimbriate nectary and stamen. (C) Seed showing one winged extremity. (D) Whole plant in late flowering stage. Note that the few flowers still bearing petals are closed.

margin entire. INFLORESCENCE narrowly pyramidal, paniculate, branched from the lowermost node to the middle of the stem; upper half not branched but with whorls of axillary solitary flowers and 3- flowered cymes with a minute, swollen peduncle; lower nodes with solitary flowers or cymes and 4 branches 7 - 9 cm long terminated by 4 verticillate bracts bearing solitary flowers and cymes. FLOWERS tetramerous, greenish tinged with blue, and with brown striation, numerous. PEDICELS 1.5 to 6 cm long, slightly winged at the top by the decurrent sepals. CALYX tube very short, lobes ovate, obtuse, to 1 cm long and 0.6 cm wide,

with entire margin. COROLLA tube very short, lobes obcuneate, apex truncate and erose, to 1.5 cm long and 0.9 cm wide, with 1 orbicular fimbriate nectary. STAMENS adnate to the corolla tube; filaments free, 6 mm long; anthers 2.5 mm long and 2 mm wide. OVARY ovate; style indistinct on young ovaries but up to 1 mm on mature ones; stigmas rounded and small; capsule up to 1.8 cm long. SEEDS ovoid, irregularly winged at one end. (Figs. 2, 3)

DISCUSSION

Within the genus *Swertia*, section *Macranthos* is clearly distinguished from all other species by relatively large tetramerous flowers bearing a single nectary on each petal, verticillate leaves (usually opposite and rarely alternate in the genus), and a robust growth, in particular of the subterranean part of the plant. Indeed, the vegetative rosettes develop a thick and fleshy tap-root during several years before setting flowers. Shah (1984) states that the species of this section are perennial without specifying if they are monocarpic or polycarpic. Unfortunately, herbarium specimens rarely include adequate material to draw definite conclusions on the life habit of the species in this section. Nevertheless, the individuals of *S. barunensis* observed in the field seemed to be plietesial. They were always found either as single rosettes or as flowering plants, but then with withered rossular leaves and without any trace of future development.

The morphological diversity between the species of *Swertia* is high, although the basic floral structure remains constant. Shah (1984) was thus able to recognise 35 informal groups in the genus. Within each group, the species are obviously of close affinity, but their identification is made possible by a small number of relatively constant characters. The morphology of the seeds, as well as the shape of the nectaries and the structure of their appendages were shown to be highly reliable throughout the genus and are commonly used in determination keys (Ho & Pringle, 1996; Maiti & Banerji, 1976; Shah, 1984; Smith, 1970). Regarding sect. *Macranthos*, Smith (1970) noted that the leaves and sepals are invariably bordered by 0.3 - 0.5 mm long lacinules in some species, but entire in the others, and conferred an important taxonomic reliability to this particularity. The major criteria used in the taxonomy of this section are listed in Table 1.

The exomorphic seed structure of *Swertia barunensis*, i. e. ovoid and winged at one end, clearly differentiates it from most other species of sect. *Macranthos*, which have wingless seeds. Only *S. hookeri* and *S. verticillifolia* also have winged seeds, but the seeds of *S. hookeri* are flat with an annular wing, and those of *S. verticillifolia* are ovoid but winged at both extremities. Furthermore, *S. verticillifolia* is of a

Table 1. Main diagnostic characters in *Swertia* sect. *Macranthos*. Data compiled from Smith (1970) and Shah (1984).

Species	Leaf and sepal margin	Seed	Nectary Scale	Nectary Shape	Distribution
S. barunensis P. Chassot	Entire	Ovoid winged (1 end)	Fimbriate	Orbicular	Nepal
S. verticillifolia T.-N. He & S.-W. Liu	Entire	Ovoid winged (both ends)	Absent	Triangular	Tibet
S. pseudohookeri Harry Sm.	Entire	Ovoid wingless	Fimbriate	Rhomboid	Bhutan
S. grandiflora Harry Sm.	Entire	Ovoid wingless	Membranous dentate	Orbicular	Bhutan
S. taylorii J. Shah	Entire	Ovoid wingless	Absent	Orbicular	Tibet
S. virescens Harry Sm.	Entire	Ovoid wingless	Absent	Triangular	Bhutan, Tibet
S. hookeri C. B. Clarke	Entire	Flat, annular winged	Absent to membranous dentate	Orbicular	Nepal, Sikkim, Tibet
S. crossoloma Harry Sm.	Lacinulate	Unknown	Fimbriate	Orbicular	Bhutan
S. splendens Harry Sm.	Lacinulate	Unknown	Fimbriate	Orbicular	Tibet
S. assamensis Harry Sm.	Lacinulate	Unknown	Membranous dentate	Orbicular	Assam
S. candelabrum Harry Sm.	Lacinulate	Ovoid wingless	Membranous dentate	Orbicular	Nepal
S. staintonii Harry Sm.	Lacinulate	Ovoid wingless	Absent	Triangular	Bhutan, Nepal

much larger size than *S. barunensis* and has naked triangular nectaries. The seeds of three species (*S. assamensis*, *S. crossoloma*, *S. splendens*) are not documented to date, but, unlike *S. barunensis*, they have lacinulate leaf and sepal margins, and also differ by a number of other characters like flower colour and size, prolonged connective of anthers, winged pedicels and inflorescence structure. The presence of fimbriae around the nectary is also a feature that *S. barunensis* shares with few species of sect. *Macranthos*, i. e. *S. crossoloma*, *S. splendens* and *S. pseudohookeri*. As mentioned above, *S. crossoloma* and *S. splendens* can easily be distinguished from the new species. On the other hand, *S. pseudohookeri* from Bhutan seems to be closely related to *S. barunensis*. In addition to sharing fimbriate nectaries, both species have entire leaf and sepal margins. However, in *S. pseudohookeri* the nectary is rhomboid and the raised rim-like scale bearing the fimbriae and surrounding the nectary presents a small opening on its proximal side. In *S. barunensis* the nectary is orbicular and entirely surrounded by the scale. Furthermore, the seeds of *S. pseudohookeri* are wingless, and its leaves are smaller and narrower. In regard to its distribution, *S. barunensis* does not seem to be related to the species collected in the mountains neighbouring the Arun valley, close to where it was found (Fig. 1), i. e. *S. candelabrum* and *S. staintonii*. Both species have lacinulate leaf and sepal margins, nectaries without fimbriae and wingless seeds. *S. barunensis* is, therefore, recognised as a new species.

The habitat of *S. barunensis* perfectly reflects the altitudinal range of the other species in this section, which mostly occur above 4000 m in the rainy eastern Himalaya. It is interesting to note that in this paraphyletic genus (Chassot *et al.*, 2001), several lineages have adapted to high altitude. But among the Asian and African alpine species of *Swertia*, the morphological

KEY TO THE SPECIES OF SECT. *MACRANTHOS*

1. Leaf blade (not petiolar part) and sepal margin entire. 2
— Leaf blade and sepal margin lacinulate, lacinules chlorophyllaceous, 0.3-0.5 mm. long. 8

2. Seeds more or less winged. 3
— Seeds wingless. 5

3. Seeds flat, with an annular wing. ..... *S. hookeri*
— Seeds ovoid, winged at one or both ends. 4

4. Nectary orbicular, fimbriate. ..... *S. barunensis*
— Nectary more or less triangular, naked. ..... *S. verticillifolia*

5. Nectary fimbriate. ..... *S. pseudohookeri*
— Nectary naked or with dentate or entire peripheral scale. 6

6. Corolla lobes 2-3 cm; nectary with membranous dentate scale. ..... *S. grandiflora*
— Corolla smaller; nectary naked. 7

7. Nectary more or less triangular; anther 2.5-3 mm. ..... *S. taylorii*
— Nectary orbicular; anther 0.5-1 mm. ..... *S. virescens*

8. Nectary fimbriate. 9
— Nectary naked or with dentate scale. 10

9. Connective of anthers prolonged, claw-like; flowers pale creamy. ..... *S. crossoloma*
— Connective of anthers not prolonged; flowers brick red. ..... *S. splendens*

10. Stem pentangular; wings of stem and pedicels ciliate; nectary naked. ..... *S. staintonii*
— Stem terete or lineolate. 11

11. Inflorescence congested; pedicels 1-1.5 cm. ..... *S. assamensis*
— Inflorescence lax with few flowers on curvingly ascending branches; pedicels 2-6 cm. ..... *S. candelabrum*

consequences of this adaptation such as observed in sect. *Macranthos* are unique. Perennial robust growth, long, thick and fleshy roots, whorled leaves and tetramery remind more some species of the North American genus *Frasera,* i. e. *F. caroliniensis, F. fastigiata, F. speciosa, F. umpquaensis,* than any other species of *Swertia*. Furthermore, some *Frasera* species (e. g. *F. caroliniensis, F. speciosa*) also have a plietesal life habit, like it seems to be the case for *S. barunensis*. But despite the overall resemblance, the aforementioned species of *Frasera,* a still debated genus closely allied to *Swertia*, differ from *Swertia* sect. *Macranthos* by their intrastaminal corona and larger seeds that are flat with an annular wing. From a molecular phylogenetic point of view (Chassot *et al.* 2001), the distant relationship found between *S. barunensis* (as *S. aff. pseudohookeri*) and *Frasera* shows that the similarities in the gross morphology are probably due to parallel evolution. Conversely, in the same study, *S. barunensis* was shown to be closely related to *S. chirayita*, a species that has tetramerous flowers but with 2 nectaries on each lobe, opposite leaves, and which is a rather typical representative of the annual species of the genus growing at lower elevations. This example illustrates how phylogenetic affinities such as suggested by DNA sequence data in Chassot *et al*. (2001) have been masked by the morphological similarities brought about during the course of evolution and adaptation of the species of *Swertia* and closely related genera.

Considering the extensive and detailed taxonomic work achieved in *Swertia* sect. *Macranthos*, it is very unlikely that more species will be described from past collections. Moreover, given the robustness of these plants and the resulting reticence of collectors to put them in press, the paucity of herbarium material is not surprising, and more species are probably still to be discovered in the vast Himalayan domain.

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APPENDIX

SPECIMENS COLLECTED FOR THIS STUDY AND DESPOSITED AT THE HERBARIUM OF THE UNIVERSITY OF NEUCHÂTEL (NEU)

COLLECTED BY PHILIPPE CHASSOT & YONG-MING YUAN

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Canscora andrographioides Griff. ex C. B. Clarke	Ph. Chassot	99-234	26 octobre 1999	Thailand	Top of hill with transmission tower, km 55 on the road to Chiangdao from Chiangmai	900
Canscora diffusa (Vahl) R. Br. ex Roem. & Schult.	Ph. Chassot	99-235	26 octobre 1999	Thailand	roadside at the top of the pass between MaeRim and Samoeng	
Canscora sp.	Ph. Chassot	99-231	23 octobre 1999	Thailand	en route to Doi Inthanon summit from Chomtong, Chomtong	1250
Canscora sp.	Ph. Chassot	99-232	23 octobre 1999	Thailand	en route to Doi Inthanon summit from Chomtong, Vachiratharn falls, Chomtong	750
Centaurium erythraea Rafin.	Ph. Chassot	00-13	2 juillet 2000	Ecuador	old road Loja-Zamora, at top of pass	3000
Centaurium sp.	Ph. Chassot	00-32	16 juillet 2000	USA	1. 5km S of Sunnyside, on ditch next to lake, Nye Co. , NE	1500
Centaurium sp.	Ph. Chassot	00-35	19 juillet 2000	USA	Santa Rosa ecological reserve, CA	
Comastoma chayuense Liu & Ho	Ph. Chassot & Y.-M. Yuan	99-97	25 septembre 1999	China	White Horse Mountain, Deqin	4300
Comastoma cyananthiflorum (Franch.) Holub	Ph. Chassot & Y.-M. Yuan	99-141	1 octobre 1999	China	yak farm west of Da Xueshan, Wongshui, Zhongdian	4200
Comastoma pedunculatum (Royle ex G. Don) Holub	Ph. Chassot	97-9	1 septembre 1997	Nepal	Langtang to Giangjin Gompa, Langtang	
Comastoma pulmonarium (Turcz.) Toyokuni	Ph. Chassot & Y.-M. Yuan	99-111	27 septembre 1999	China	White Horse Mt. , west pass, Deqin	3800
Comastoma stellariifolium (Franch.) Holub	Ph. Chassot	97-25	2 septembre 1997	Nepal	KongmaLa, Langtang	ca 5000
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-49	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-50	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-55	22 septembre 1999	China	vicinity of Zhongdian	3200
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-73	23 septembre 1999	China	Bita lake, Zhongdian	3500
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-112	28 septembre 1999	China	30 km north of Zhongdian	3300
Comastoma traillianum (Forrest) Holub	Ph. Chassot & Y.-M. Yuan	99-222	11 octobre 1999	China	Behind spruce meadow ropeway, Baishui (Yun Sha Ping), Lijiang	3100
Comastoma sp.	Ph. Chassot & Y.-M. Yuan	99-109	27 septembre 1999	China	White Horse Mt. , west pass, Deqin	3800
Crawfurdia speciosa Wall.	Ph. Chassot	97-26	15 septembre 1997	Nepal	Tashi Gaon to TutuLa pass, Makalu Barun National Park	3000
Crawfurdia sp.	Ph. Chassot & Y.-M. Yuan	99-188	7 octobre 1999	China	Zhong He peak, valley north of top, Dali	3700
Crawfurdia sp.	Ph. Chassot & Y.-M. Yuan	99-189	7 octobre 1999	China	Zhong He peak, valley north of top, Dali	3500-3800

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Exacum sutaepense Hosseus	Ph. Chassot	99-230	23 octobre 1999	Thailand	Under km. 41. 5, en route to Doi Inthanon summit, Chomtong	1900
Frasera albicaulis Griseb. in Hook.	Ph. Chassot	00-38	23 juillet 2000	USA	Foothills, Pine Hill, El Dorado Co. , CA	500
Frasera albomarginata S. Watson	Ph. Chassot	00-23	9 juillet 2000	USA	Mojave, Cedar canyon road, Pinto Mt. , Ca	1700
Frasera albomarginata S. Watson	Ph. Chassot	00-24	10 juillet 2000	USA	Grand canyon, west rim, Mohave point	
Frasera albomarginata S. Watson	Ph. Chassot	00-30	15 juillet 2000	USA	Buckskin Mt. , ca 15 mi off Hy 89 on rd 1026, near Fredona AZ	1500
Frasera albomarginata S. Watson	Ph. Chassot	00-33	19 juillet 2000	USA	around Sunnyside, Nye Co. , NE	
Frasera coloradensis Rogers	Ph. Chassot	00-28	13 juillet 2000	USA	ESE of Kim, S of Carizo Mt. , rd 24. 4, Las Animas Co. , CO	
Frasera fastigiata (Pursh) Heller	Ph. Chassot	00-41	27 juillet 2000	USA	Winchester park campground, Idaho Co. , ID	1200
Frasera gypsicola (Barneby) Post	Ph. Chassot	00-31	16 juillet 2000	USA	300m S of Sunnyside, Nye Co. , NE	1580
Frasera montana Mulf.	Ph. Chassot	00-39	25 juillet 2000	USA	McCall, Ponderosa park entrance, Valley Co. , ID	1500
Frasera montana Mulf.	Ph. Chassot	00-40	26 juillet 2000	USA	11mi up Grimes Creek road from Hy 21, Boise Co. , ID	1200
Frasera neglecta Hall	Ph. Chassot	00-22	8 juillet 2000	USA	Chilao campground, silver mocassin trail, CA	1770
Frasera paniculata Torr.	Ph. Chassot	00-29	14 juillet 2000	USA	bridge ove Piute creek on rd 98, Navajo Co. , AZ	1500
Frasera parryi Torr.	Ph. Chassot	00-34	19 juillet 2000	USA	Blue jay campground, Cleveland Natl forest, San Diego Co. , CA	
Frasera puberulenta Davidson	Ph. Chassot	00-37	21 juillet 2000	USA	Onion valley, 500m after trailhead, Inyo Co. , CA	2900
Frasera speciosa Griseb. in Hook.	Ph. Chassot	00-25	11 juillet 2000	USA	roadside south of Flagstaff, Coconino Co. , AZ	
Frasera speciosa Griseb. in Hook.	Ph. Chassot	00-27	12 juillet 2000	USA	west side of pass from Taos to Raton on road 64	2800
Frasera tubulosa Coville	Ph. Chassot	00-36	20 juillet 2000	USA	Olancha pass, summit meadow, Inyo Co. , CA	2830
Frasera umpquaensis M. Peck & Applegate	Ph. Chassot	00-42	11 août 2000	USA	Elk meadow, near London, Douglas Co. , OR	1260
Gentiana ampla Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-43	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Gentiana arethusae Burkill	Ph. Chassot & Y.-M. Yuan	99-45	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Gentiana arethusae Burkill	Ph. Chassot & Y.-M. Yuan	99-79	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Gentiana arethusae Burkill	Ph. Chassot & Y.-M. Yuan	99-104	27 septembre 1999	China	White Horse Mt. , east pass, Deqin	3800
Gentiana atuntsiensis W. W. Smith	Ph. Chassot & Y.-M. Yuan	99-56	22 septembre 1999	China	Zhongdian, hotspring	3500
Gentiana atuntsiensis W. W. Smith	Ph. Chassot & Y.-M. Yuan	99-140	1 octobre 1999	China	Da Xueshan, Wongshui, Zhongdian	4200
Gentiana cephalantha Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-59	22 septembre 1999	China	Dayangchang, Zhongdian	3500

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Gentiana cephalantha Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-70	23 septembre 1999	China	Jidiba, Zhongdian	2900
Gentiana cephalantha Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-183	6 octobre 1999	China	Wu Wei Si, Dali	2300-2500
Gentiana crassicaulis Gilg	Ph. Chassot & Y.-M. Yuan	99-51	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Gentiana crassuloides Franch.	Ph. Chassot & Y.-M. Yuan	99-108	27 septembre 1999	China	White Horse Mt. , west pass, Deqin	3800
Gentiana delavayi Franch.	Ph. Chassot & Y.-M. Yuan	99-38	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3800
Gentiana delavayi Franch.	Ph. Chassot & Y.-M. Yuan	99-161	4 octobre 1999	China	en route from Dali to Binchuan, Shang Dang pass	2200
Gentiana expansa Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-203	9 octobre 1999	China	Yu Hu, Lijiang	2700
Gentiana helophylla I. B. Balf. & Forrest	Ph. Chassot & Y.-M. Yuan	99-58	22 septembre 1999	China	Jidiba, Zhongdian	3600
Gentiana hynaldii Kanitz.	Ph. Chassot & Y.-M. Yuan	99-42	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Gentiana hynaldii Kanitz.	Ph. Chassot & Y.-M. Yuan	99-53	22 septembre 1999	China	vicinity of Zhongdian	3200
Gentiana hynaldii Kanitz.	Ph. Chassot & Y.-M. Yuan	99-76	23 septembre 1999	China	Hotspring, Zhongdian	3300
Gentiana haynaldii Kanitz.	Ph. Chassot & Y.-M. Yuan	99-116	28 septembre 1999	China	old radar station (30 km north of Zhongdian), Zhongdian	3300
Gentiana leptoclada I. B. Balf. & Forrest	Ph. Chassot	99-239	29 octobre 1999	Thailand	Doi Chiangdao summit, Chiangdao	2000
Gentiana lineolata Franch.	Ph. Chassot & Y.-M. Yuan	99-52	21 septembre 1999	China	on the way from Zhongdian to Napa lake	3000
Gentiana lineolata Franch.	Ph. Chassot & Y.-M. Yuan	99-164	4 octobre 1999	China	Dian Nan, Jian Chuan	2500
Gentiana lineolata Franch.	Ph. Chassot & Y.-M. Yuan	99-210	9 octobre 1999	China	Ming Yin, Lijiang	2800
Gentiana melandrifolia Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-185	6 octobre 1999	China	Wu Wei Si, Dali	2700
Gentiana melandrifolia Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-191	7 octobre 1999	China	Zhong He peak, valley north of top, Dali	3200-3600
Gentiana nanobella C. Marquand	Ph. Chassot & Y.-M. Yuan	99-80	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Gentiana nanobella C. Marquand	Ph. Chassot & Y.-M. Yuan	99-103	27 septembre 1999	China	White Horse Mt. , east pass, Deqin	3800
Gentiana panthaica Burkill	Ph. Chassot & Y.-M. Yuan	99-184	6 octobre 1999	China	Wu Wei Si, Dali	2700

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Gentiana pantheica Burkill	Ph. Chassot & Y.-M. Yuan	99-190	7 octobre 1999	China	Zhong He peak, valley north of top, Dali	3000
Gentiana phylocalyx C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-94	25 septembre 1999	China	White Horse Mountain, Deqin	4600
Gentiana picta Franch. ex Hemsl.	Ph. Chassot & Y.-M. Yuan	99-136	30 septembre 1999	China	Mailang, yak farm, Dongwang, Zhongdian	3140
Gentiana praticola Gilg	Ph. Chassot & Y.-M. Yuan	99-35	19 septembre 1999	China	bambu temple, Kunming	2200
Gentiana primuliflora (?) Franch.	Ph. Chassot & Y.-M. Yuan	99-163	4 octobre 1999	China	Dian Nan, Jian Chuan	2500
Gentiana regescens Franch.	Ph. Chassot & Y.-M. Yuan	99-34	19 septembre 1999	China	bambu temple, Kunming	2200
Gentiana regescens Franch.	Ph. Chassot & Y.-M. Yuan	99-182	6 octobre 1999	China	Wu Wei Si, Dali	2400
Gentiana rhodantha Franch. ex Hemsl	Ph. Chassot & Y.-M. Yuan	99-178	6 octobre 1999	China	pass behind Hai Dong, Dali	2200
Gentiana rhodantha Franch. ex Hemsl	Ph. Chassot & Y.-M. Yuan	99-194	8 octobre 1999	China	Deng Chuan, Eryuan	2300
Gentiana rhondantha Franch. ex Hemsl	Ph. Chassot & Y.-M. Yuan	199-58	3 octobre 1999	China	Hai Xi, Lijiang	2000
Gentiana sedifolia Kunth	Ph. Chassot	00-4	18 juin 2000	Ecuador	Ilinizas	4300-4700
Gentiana sedifolia Kunth	Ph. Chassot	00-11	28 juin 2000	Ecuador	paso del Inca	4100
Gentiana souliei Franch.	Ph. Chassot & Y.-M. Yuan	99-217	10 octobre 1999	China	pass behind FungKe, Lijiang	3450
Gentiana souliei Franch.	Ph. Chassot & Y.-M. Yuan	99-221	11 octobre 1999	China	Behind spruce meadow ropeway, Baishui (Yun Sha Ping), Lijiang	3100
Gentiana stellulata (?) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-77	23 septembre 1999	China	Bita lake, Zhongdian	3500
Gentiana stragulata I. B. Balf. & Forrest	Ph. Chassot & Y.-M. Yuan	99-93	25 septembre 1999	China	White Horse Mountain, Deqin	4300
Gentiana suborbisepala C. Marquand	Ph. Chassot & Y.-M. Yuan	99-95	25 septembre 1999	China	White Horse Mountain, Deqin	4300
Gentiana suborbisepala C. Marquand	Ph. Chassot & Y.-M. Yuan	99-105	27 septembre 1999	China	White Horse Mt. , east pass, Deqin	3800
Gentiana suborbisepala C. Marquand	Ph. Chassot & Y.-M. Yuan	99-123	29 septembre 1999	China	Xiao Xueshan pass, Zhongdian	3800
Gentiana tongolensis Franch.	Ph. Chassot & Y.-M. Yuan	99-124	29 septembre 1999	China	Xiao Xueshan pass, Zhongdian	3800
Gentiana tongolensis Franch.	Ph. Chassot & Y.-M. Yuan	99-146	1 octobre 1999	China	5 km after crossroad Dongwang-Sechuan, Zhongdian	4700

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Gentiana veitchiorum Hemsl.	Ph. Chassot & Y.-M. Yuan	99-150	3 octobre 1999	China	Yisong, Zhongdian	3300
Gentiana veitchiorum Hemsl.	Ph. Chassot & Y.-M. Yuan	99-107	27 septembre 1999	China	White Horse Mt. , west pass, Deqin	3800
Gentiana veitchiorum Hemsl.	Ph. Chassot & Y.-M. Yuan	99-142	1 octobre 1999	China	Da Xueshan, Wongshui, Zhongdian	3800
Gentiana veitchiorum Hemsl.	Ph. Chassot & Y.-M. Yuan	99-145	1 octobre 1999	China	5 km after crossroad Dongwang-Sechuan, Zhongdian	4700
Gentiana wardii W. W. Smith	Ph. Chassot & Y.-M. Yuan	99-91	25 septembre 1999	China	White Horse Mountain, Deqin	4600
Gentiana yunnanensis Franch.	Ph. Chassot & Y.-M. Yuan	99-41	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Gentiana yunnanensis Franch.	Ph. Chassot & Y.-M. Yuan	99-75	23 septembre 1999	China	Hotspring, Zhongdian	3300
Gentiana af yunnanensis Franch.	Ph. Chassot & Y.-M. Yuan	99-89	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	2900
Gentiana af. yunnanensis Franch.	Ph. Chassot & Y.-M. Yuan	99-57	22 septembre 1999	China	Zhongdian, hotspring	3500
Gentiana aff. yunnanensis Franch.	Ph. Chassot & Y.-M. Yuan	99-44	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Gentiana sp.	Ph. Chassot	99-10	2 septembre 1999	Taiwan	en route from Dayuling to Wushe, parking in front of park office building	2800
Gentiana sp.	Ph. Chassot	99-9	2 septembre 1999	Taiwan	en route from Dayuling to Wushe, parking in front of park office building	2800
Gentiana sp.	Ph. Chassot	99-15	8 septembre 1999	Japan	Hiking trail from Mt. Aso to Aso youth hostel, Kumamoto pref.	
Gentiana sp.	Ph. Chassot	99-16	8 septembre 1999	Japan	Hiking trail from Mt. Aso to Aso youth hostel, Kumamoto pref.	
Gentiana sp.	Ph. Chassot	99-24	12 septembre 1999	Japan	top of Mt. Sobo, Kumamoto pref.	1700
Gentiana sp.	Ph. Chassot	99-25	15 septembre 1999	Japan	Nishikodokuryo, Kamickochi, Takayama	2700
Gentiana sp.	Ph. Chassot	99-26	15 septembre 1999	Japan	Nishikosanso, Kamikochi, Takayama	2100
Gentiana sp.	Ph. Chassot	99-27	15 septembre 1999	Japan	Nishikosanso, Kamikochi, Takayama	2100
Gentiana sp.	Ph. Chassot	99-28	15 septembre 1999	Japan	Nishikodokuryo, Kamikochi, Takayama	2500
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-92	25 septembre 1999	China	White Horse Mountain, Deqin	4600
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-125	29 septembre 1999	China	Xiao Xueshan pass, Zhongdian	3800
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-137	30 septembre 1999	China	Mailang, yak farm, Dongwang, Zhongdian	3900-4000
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-143	1 octobre 1999	China	yak farm west of Da Xueshan, Wongshui, Zhongdian	3800

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-170	5 octobre 1999	China	pass near Shazhi, Jizushan, Binchuan	2195
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-187	7 octobre 1999	China	Zhong He peak, Dali	4000
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-224	16 octobre 1999	China	Wanniansi to Huayan Peak, valley east of main path (grave yard), Emei Shan	1280
Gentiana sp.	Ph. Chassot	00-43	11 août 2000	USA	Paradise meadow, Mt Rainer Natl Park, WA	
Gentiana sp.	Ph. Chassot & Y.-M. Yuan	99-179	6 octobre 1999	China	pass behind Hai Dong, Dali	2200
Gentiana sp.(aff. primuliflora)	Ph. Chassot & Y.-M. Yuan	99-193	8 octobre 1999	China	Deng Chuan, Eryuan	2300
Gentiana sp.(aff. primuliflora)	Ph. Chassot & Y.-M. Yuan	99-199	8 octobre 1999	China	Song Gui, Huo Qin	2300
Gentiana sp.(angusta?)	Ph. Chassot	99-13	2 septembre 1999	Taiwan	Taroko national park, after the pass on the road Dayuling-Wushe	2800
Gentiana sp.(atkinsoni?)	Ph. Chassot	99-6	30 août 1999	Taiwan	Shei-Pa national park, bellow Kuanwu	1900
Gentianella amarella (L.) Börner	Ph. Chassot	00-26	12 juillet 2000	USA	west side of pass from Taos to Raton on road 64	2800
Gentianella anomala (C. Marquand) T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-144	1 octobre 1999	China	yak farm west of Da Xueshan, Wongshui, Zhongdian	3800
Gentianella azurea (Bunge) Holub	Ph. Chassot & Y.-M. Yuan	99-82	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Gentianella azurea (Bunge) Holub	Ph. Chassot & Y.-M. Yuan	99-110	27 septembre 1999	China	White Horse Mt. , west pass, Deqin	3800
Gentianella cerastioides (Kunth) Fabris	Ph. Chassot	00-1	18 juin 2000	Ecuador	Ilinizas	4000-4700
Gentianella cerastioides (Kunth) Fabris	Ph. Chassot	00-2	18 juin 2000	Ecuador	Ilinizas	4000-4700
Gentianella foliosa (Kunth) Fabris	Ph. Chassot	00-3	18 juin 2000	Ecuador	Ilinizas	4500-4700
Gentianella gentianoides (Franch.) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-201	9 octobre 1999	China	Yu Hu, Lijiang	2700
Gentianella gentianoides (Franch.) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-223	11 octobre 1999	China	Behind spruce meadow ropeway, Baishui (Yun Sha Ping), Lijiang	above 3300
Gentianella giioides (Gilg) Fabris	Ph. Chassot	00-16	2 juillet 2000	Ecuador	Nudo de Cajanuma, Zamora	3000
Gentianella giioides (Gilg) Fabris	Ph. Chassot	00-20	3 juillet 2000	Ecuador	Station San Francisco, transect 2, Zamora	2400-3000
Gentianella moorcroftiana (Wall. ex Griseb.) AiryShaw	Ph. Chassot	97-66	12 octobre 1997	Nepal	Jumla to Chauta, Karnali	3000
Gentianella moorcroftiana (Wall. ex Griseb.) AiryShaw	Ph. Chassot	97-70	14 octobre 1997	Nepal	Chauta to Rara Lake, Karnali	3800

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Gentianella rapunculoides (Willd. ex Schult.) J. S. Pringle	Ph. Chassot	00-10	27 juin 2000	Ecuador	Laguna de Cuicocha, Imbabura	3380
Gentianella rapunculoides (Willd. ex Schult.) J. S. Pringle	Ph. Chassot	00-12	28 juin 2000	Ecuador	paso del Inca	4100
Gentianopsis barbata (Froel.) Ma	Ph. Chassot & Y.-M. Yuan	99-87	24 septembre 1999	China	Nixi, Zhongdian	2900
Gentianopsis barbata var. stenocalyx H. W. Li ex T. N. Ho	Ph. Chassot & Y.-M. Yuan	99-115	28 septembre 1999	China	30 km north of Zhongdian	3300
Gentianopsis barbata var. stenocalyx H. W. Li ex T. N. Ho	Ph. Chassot & Y.-M. Yuan	99-74	23 septembre 1999	China	Hotspring, Zhongdian	3300
Gentianopsis contorta (Roye) Ma	Ph. Chassot	97-69	12 octobre 1997	Nepal	Bhargaon to Chauta, Karnali	2300
Gentianopsis contorta (Roye) Ma	Ph. Chassot & Y.-M. Yuan	99-88	24 septembre 1999	China	Nixi, Zhongdian	2900
Gentianopsis contorta (Roye) Ma	Ph. Chassot & Y.-M. Yuan	99-121	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Gentianopsis contorta (Roye) Ma	Ph. Chassot & Y.-M. Yuan	99-101	26 septembre 1999	China	Meili glacier, left side of valley, Deqin	2500
Gentianopsis contorta (Roye) Ma	Ph. Chassot & Y.-M. Yuan	99-216	10 octobre 1999	China	pass behind FungKe, Lijiang	3450
Gentianopsis grandis Ma	Ph. Chassot & Y.-M. Yuan	99-157	3 octobre 1999	China	Tu guan Cun, Zhongdian	2900
Gentianopsis grandis Ma	Ph. Chassot & Y.-M. Yuan	99-195	8 octobre 1999	China	Deng Chuan, Eryuan	2300
Gentianopsis grandis Ma	Ph. Chassot & Y.-M. Yuan	99-215	10 octobre 1999	China	pass behind FungKe, Lijiang	3450
Gentianopsis paludosa (Hook. f.) Ma	Ph. Chassot & Y.-M. Yuan	99-106	27 septembre 1999	China	White Horse Mt. , east pass, Deqin	3800
Gentianopsis paludosa (Hook. f.) Ma	Ph. Chassot & Y.-M. Yuan	99-122	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Gentianopsis sp.	Ph. Chassot & Y.-M. Yuan	99-41'bis	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Halenia brevicornis	Ph. Chassot	00-8	27 juin 2000	Ecuador	Laguna de Cuicocha, Imbabura	3380
Halenia elliptica (Kunth) G. Don	Ph. Chassot & Y.-M. Yuan	99-47	21 septembre 1999	China	on the way from Zhongdian to Napa lake	3400
Halenia gracilis Griseb.	Ph. Chassot	00-7	27 juin 2000	Ecuador	Laguna de Cuicocha, Imbabura	3380
Halenia weddelliana	Ph. Chassot	00-5	18 juin 2000	Ecuador	Ilinizas	4000-4700
Halenia weddelliana Gilg	Ph. Chassot	00-9	27 juin 2000	Ecuador	Laguna de Cuicocha, Imbabura	3380
Halenia elliptica D. Don	Ph. Chassot	97-3	30 août 1997	Nepal	Shyabru to Lama Hotel, Langtang	2200
Halenia elliptica D. Don	Ph. Chassot	97-15	4 septembre 1997	Nepal	Giangjin Gompa to Lama Hotel, Langtang	3000

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Lomatogonium brachyantherum (C. B. Clarke) Fern.	Ph. Chassot	97-41	19 septembre 1997	Nepal	Makalu Barun National Park, West of Seto Pokhari	5000
Lomatogonium brachyantherum (C. B. Clarke) Fern.	Ph. Chassot & Y.-M. Yuan	99-84	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Lomatogonium carinthiacum (Wulf.) Reichb.	Ph. Chassot	97-10	1 septembre 1997	Nepal	Langtang to Giangjin Gompa, Langtang	3600
Lomatogonium carinthiacum (Wulf.) Reichb.	Ph. Chassot	97-E12	2 septembre 1997	Nepal	Langtang	
Lomatogonium chumbicum (Burkill) Harry Sm.	Ph. Chassot	97-42	19 septembre 1997	Nepal	Makalu Barun National Park, West of Seto Pokhari	5000
Lomatogonium chumbicum (Burkill) Harry Sm.	Ph. Chassot	97-43	19 septembre 1997	Nepal	Makalu Barun National Park, Sherson	4800
Lomatogonium forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-114	28 septembre 1999	China	30 km north of Zhongdian	3300
Lomatogonium forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-119	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Lomatogonium forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-130	29 septembre 1999	China	Wung Shui, Zhongdian	3000
Lomatogonium forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-138	30 septembre 1999	China	crossroad Sechuan-Dongwang, Zhongdian	3600
Lomatogonium af. forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-71	23 septembre 1999	China	Hotspring, Zhongdian	3300
Lomatogonium af. forresti (Balf. f.) Fern.	Ph. Chassot & Y.-M. Yuan	99-196	8 octobre 1999	China	Song Gui, Huo Qin	2300
Lomatogonium gamosepalum (Burkill) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-134	30 septembre 1999	China	Mailang, Dongwang, Zhongdian	3800-4000
Lomatogonium gamosepalum (Burkill) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-135	30 septembre 1999	China	Mailang, Dongwang, Zhongdian	4100
Lomatogonium graciliflorum Harry Sm.	Ph. Chassot	97-73	17 octobre 1997	Nepal	Rara to Guru Singha, Chuchemara	3700
Lomatogonium lijiangense T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-156	3 octobre 1999	China	Tu guan Cun, Zhongdian	2900
Lomatogonium lijiangense T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-205	9 octobre 1999	China	Yu Hu, Lijiang	2700
Lomatogonium lijiangense T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-207	9 octobre 1999	China	after crossroad Daju-FungKe, Lijiang	3000
Lomatogonium lijiangense T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-209	9 octobre 1999	China	Ming Yin, Lijiang	2800
Lomatogonium longifolium Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-78	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Lomatogonium longifolium Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-81	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Lomatogonium longifolium Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-98	25 septembre 1999	China	White Horse Mountain, Deqin	4300

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Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot	97-37	18 septembre 1997	Nepal	Makalu Barun National Park	
Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot	97-40	19 septembre 1997	Nepal	Makalu Barun National Park, West of Seto Pokhari	4500
Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot	97-44	19 septembre 1997	Nepal	Makalu Barun National Park, Sherson	4800
Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot & Y.-M. Yuan	99-83	24 septembre 1999	China	White Horse Mountain, between the two passes, Deqin	4200
Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot & Y.-M. Yuan	99-96	25 septembre 1999	China	White Horse Mountain, Deqin	4300
Lomatogonium macranthum (Diels & Gilg) Fern.	Ph. Chassot & Y.-M. Yuan	99-214	10 octobre 1999	China	Top of Mountain behind FungKe, Lijiang	3900
Lomatogonium micranthum Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-127	29 septembre 1999	China	5 km north of Xiao Xueshan, Zhongdian	3400
Lomatogonium micranthum Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-151	3 octobre 1999	China	Yisong, Zhongdian	3300
Lomatogonium oreocharis (Diels) C. Marquand	Ph. Chassot & Y.-M. Yuan	99-219	11 octobre 1999	China	Behind spruce meadow ropeway, Baishui (Yun Sha Ping), Lijiang	3500
Lomatogonium rotatum (L.) Fries ex Nyman	Ph. Chassot & Y.-M. Yuan	99-61	22 septembre 1999	China	Zhongdian	3500
Lomatogonium sikkimense (Burkill) Harry Sm.	Ph. Chassot	97-13	2 septembre 1997	Nepal	Giangjin Gompa to Tserko Ri, Langtang	4800
Lomatogonium sikkimense (Burkill) Harry Sm.	Ph. Chassot	97-36	18 septembre 1997	Nepal	Barun valley, after Yak Karka, Makalu Barun National Park	4500
Lomatogonium sikkimense (Burkill) Harry Sm.	Ph. Chassot	97-45	19 septembre 1997	Nepal	Sherson, Makalu Barun National Park	4500
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-46	21 septembre 1999	China	on the way from Zhongdian to Napa lake	3400
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-54	22 septembre 1999	China	vicinity of Zhongdian	3200
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-72	23 septembre 1999	China	Hotspring, Zhongdian	3300
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-113	28 septembre 1999	China	30 km north of Zhongdian	3300
Lomatogonium zhongdianense S.-W. Liu & T.-N. Ho	Ph. Chassot & Y.-M. Yuan	99-120	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Lomatogonium sp.	Ph. Chassot & Y.-M. Yuan	99-48	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Macrocarpea ovalis (Ruiz & Pav.) Ewan	Ph. Chassot	00-15	2 juillet 2000	Ecuador	Nudo de Cajanuma, Zamora	3000
Megacodon stylophorus (C. B. Clarke) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-65	22 septembre 1999	China	Zhongdian, hotspring	3400
Megacodon stylophorus (C. B. Clarke) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-147	1 octobre 1999	China	N of Da Xue Shan pass	3800-4100

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Megacodon stylophorus (C. B. Clarke) Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-36	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Microrphium pubescens C. B. Clarke	Ph. Chassot	99-242	29 octobre 1999	Thailand	Khao Chang Lot, PhangNga	20
Microrphium pubescens C. B. Clarke	Ph. Chassot	99-243	29 octobre 1999	Thailand	Somdech Phra Srinagadindra park, PhangNga	20
Pterygocalyx volubilis Maxim.	Ph. Chassot & Y.-M. Yuan	99-100	26 septembre 1999	China	Meili glacier, left side of valley, Deqin	2500
Swertia alata (G. Don) C. B. Clarke	Ph. Chassot	97-61	10 octobre 1997	Nepal	vicinity of Jumla	2500
Swertia alata (G. Don) C. B. Clarke	Ph. Chassot	97-100		Nepal	Shivalaya, Jiri region	1820
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot	97-18	5 septembre 1997	Nepal	Lama Hotel to Shyabru bensi, Langtang	2400
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot	97-20	5 septembre 1997	Nepal	Lama Hotel to Shyabru bensi, Langtang	2400
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot & Y.-M. Yuan	99-172	5 octobre 1999	China	Jizushan, 5 km after entrance gate.	2135
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot & Y.-M. Yuan	99-180	6 octobre 1999	China	Wu Wei Si, Dali	2200
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot	99-229	22 octobre 1999	Thailand	Sun Ku shrine, en route to Doi Pui, Chiang Mai	1600
Swertia angustifolia Buch.-Ham. ex D. Don	Ph. Chassot	99-233	23 octobre 1999	Thailand	Under km. 41. 5, en route to Doi Inthanon summit, Chomtong	1900
Swertia atroviolacea Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-131	30 septembre 1999	China	Mailang yak farm, Dongwang, Zhongdian	3600-4000
Swertia atroviolacea Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-149	2 octobre 1999	China	Birong valley (Shangri-La), Zhongdian	3000
Swertia barunensis P. Chassot	Ph. Chassot	97-28	16 septembre 1997	Nepal	Shipton Pass, Makalu Barun National Park	4000-4500
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot	97-22	12 septembre 1997	Nepal	Chichila to Num, Makalu Barun National Park	1500
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot	99-18	9 septembre 1999	Japan	Fukabayama (kikuchy sky-line), Kumamoto pref.	
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot	99-19	9 septembre 1999	Japan	Fukabayama (kikuchy sky-line), Kumamoto pref.	
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot	99-22	12 septembre 1999	Japan	Mt. Sobo, Kumamoto pref.	1000
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot	99-23	12 septembre 1999	Japan	Mt. Sobo, Kumamoto pref.	1100
Swertia bimaculata (Sieb. & Zuc.) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-225	16 octobre 1999	China	Wanniansi to Huayan Peak, valley east of main path (grave yard), Emei Shan	1280
Swertia binchuanensis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-160	4 octobre 1999	China	en route from Dali to Binchuan, Shang Dang pass	2200
Swertia binchuanensis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-177	6 octobre 1999	China	pass behind Hai Dong, Dali	2200
Swertia binchuanensis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-197	8 octobre 1999	China	Song Gui, Huo Qin	2300
Swertia calcicola Kerr	Ph. Chassot	99-241	29 octobre 1999	Thailand	Doi Chiangdao summit, Chiangdao	2000
Swertia chirayta Karsten	Ph. Chassot	97-2	30 août 1997	Nepal	Shyabru to Lama Hotel, Langtang	2200

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Swertia chirayta Karsten	Ph. Chassot	97-4	30 août 1997	Nepal	Shyabru to Lama Hotel, Langtang	2200
Swertia chirayta Karsten	Ph. Chassot	97-5	30 août 1997	Nepal	Shyabru to Lama Hotel, Langtang	2200
Swertia ciliata (G. Don) B. L. Burtt	Ph. Chassot	97-6	31 août 1997	Nepal	Lama Hotel to Langtang, Langtang	2200
Swertia ciliata (G. Don) B. L. Burtt	Ph. Chassot	97-7	1 septembre 1997	Nepal	Langtang to Giangjin Gompa, Langtang	3000
Swertia ciliata (G. Don) B. L. Burtt	Ph. Chassot	97-8	1 septembre 1997	Nepal	Langtang to Giangjin Gompa, Langtang	3000
Swertia ciliata (G. Don) B. L. Burtt	Ph. Chassot	97-64	10 octobre 1997	Nepal	vicinity of Jumla	2500
Swertia cincta Burkill	Ph. Chassot & Y.-M. Yuan	99-33	19 septembre 1999	China	bambu temple, Kunming	2200
Swertia cincta Burkill	Ph. Chassot & Y.-M. Yuan	99-64	22 septembre 1999	China	Dayangchang, Zhongdian	3500
Swertia cordata (G. Don) C. B. Clarke	Ph. Chassot	97-17	5 septembre 1997	Nepal	Lama Hotel to Shyabru bensi, Langtang	2800
Swertia cordata (G. Don) C. B. Clarke	Ph. Chassot	97-60	10 octobre 1997	Nepal	vicinity of Jumla	2500
Swertia cordata (G. Don) C. B. Clarke	Ph. Chassot	97-65	10 octobre 1997	Nepal	vicinity of Jumla	2500
Swertia cuneata Wall.	Ph. Chassot	97-14	3 septembre 1997	Nepal	Giangjin Gompa to Ganja La, Langtang	4800
Swertia cuneata Wall.	Ph. Chassot	97-35	18 septembre 1997	Nepal	Barun valley, before base camp, Makalu Barun National Park	4500
Swertia cuneata Wall.	Ph. Chassot	97-71	16 octobre 1997	Nepal	Murma top, Rara Lake, Karnali	3900
Swertia cuneata Wall.	Ph. Chassot	97-71b	16 octobre 1997	Nepal	Murma top, Rara Lake, Karnali	3900
Swertia decora Franch.	Ph. Chassot & Y.-M. Yuan	99-169	5 octobre 1999	China	pass near Shazhi, Jizushan, Binchuan	2195
Swertia decora Franch.	Ph. Chassot & Y.-M. Yuan	99-176	6 octobre 1999	China	pass behind Hai Dong, Dali	2200
Swertia decora Franch.	Ph. Chassot & Y.-M. Yuan	99-192	8 octobre 1999	China	Deng Chuan, Eryuan	2300
Swertia decora Franch.	Ph. Chassot & Y.-M. Yuan	99-204	9 octobre 1999	China	Yu Hu, Lijiang	2700
Swertia delavayi Franch.	Ph. Chassot & Y.-M. Yuan	99-66	22 septembre 1999	China	Baishuitai, Zhongdian	2550
Swertia delavayi Franch.	Ph. Chassot & Y.-M. Yuan	99-213	10 octobre 1999	China	Hill behind Shan Man, FungKe, Lijiang	2200
Swertia forrestii Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-139	1 octobre 1999	China	yak farm west of Da Xueshan, Wongshui, Zhongdian	4125
Swertia japonica (Schultes) Makino	Ph. Chassot	99-20	9 septembre 1999	Japan	under grasses in a field left of Kikuchy sky-line, Aso, Kumamoto pref.	
Swertia kingii Hook. f.	Ph. Chassot	97-46	22 septembre 1997	Nepal	Tutu-La pass, Makalu Barun National Park	4800
Swertia kouitchensis Franch.	Ph. Chassot & Y.-M. Yuan	99-226	16 octobre 1999	China	Wanniansi to Huayan Peak, valley east of main path (grave yard), Emei Shan	1280
Swertia laticalyx J. Shah	Ph. Chassot & Y.-M. Yuan	99-220	11 octobre 1999	China	Behind spruce meadow ropeway, Baishui (Yun Sha Ping), Lijiang	3100-3500

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Swertia macrosperma (C. B. Clarke) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-31	19 septembre 1999	China	bambu temple, Kunming	2200
Swertia macrosperma (C. B. Clarke) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-32	19 septembre 1999	China	bambu temple, Kunming	2200
Swertia macrosperma (C. B. Clarke) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-60	22 septembre 1999	China	before Jidiba, Zhongdian	3600
Swertia macrosperma (C. B. Clarke) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-181	6 octobre 1999	China	Wu Wei Si, Dali	2200
Swertia membranifolia Franch.	Ph. Chassot & Y.-M. Yuan	99-186	7 octobre 1999	China	Zhong He peak, Dali	3300
Swertia multicaulis D. Don	Ph. Chassot	97-12	2 septembre 1997	Nepal	Giangjin Gompa to Tserko Ri, Langtang	4300
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot	98-2a	19 décembre 1997	Nepal	Jhinu, Anapurna sanctuary trekk	1500
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-30	19 septembre 1999	China	Bambu temple, Kunming	2200
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-67	22 septembre 1999	China	Baishuitai, Zhongdian	2550
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-152	3 octobre 1999	China	Song Yuan, Zhongdian	1880
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-171	5 octobre 1999	China	Jizushan, 5 km after entrance gate.	2135
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-198	8 octobre 1999	China	Song Gui, Huo Qin	2300
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-212	10 octobre 1999	China	Hill behind Shan Man, FungKe, Lijiang	2200
Swertia nervosa (G. Don) C. B. Clarke	Ph. Chassot & Y.-M. Yuan	99-227	16 octobre 1999	China	Wanniansi to Huayan Peak, valley east of main path (grave yard), Emei Shan	1280
Swertia paniculata Wall.	Ph. Chassot	97-27	15 septembre 1997	Nepal	Tashi Gaon to TutuLa pass, Makalu Barun National Park	3000
Swertia paniculata Wall.	Ph. Chassot	97-63	10 octobre 1997	Nepal	vicinity of Jumla	2500
Swertia paniculata Wall.	Ph. Chassot	98-3a	19 décembre 1997	Nepal	Jhinu, Anapurna sanctuary trekk	1500
Swertia paniculata Wall.	Ph. Chassot	97-101		Nepal	Shivalaya, Jiri region	1820
Swertia paniculata Wall.	Ph. Chassot & Y.-M. Yuan	99-126	29 septembre 1999	China	5 km north of Xiao Xueshan, Zhongdian	3400
Swertia paniculata Wall.	Ph. Chassot & Y.-M. Yuan	99-155	3 octobre 1999	China	Tu guan Cun, Zhongdian	2900
Swertia patula Harry Sm.	Ph. Chassot & Y.-M. Yuan	99-211	10 octobre 1999	China	Hill behind Shan Man, FungKe, Lijiang	2200
Swertia petiolata D. Don	Ph. Chassot	97-32	17 septembre 1997	Nepal	Barun valley, after Yak Kharka, Makalu Barun National Park	4200
Swertia pinetorum Kerr	Ph. Chassot	99-236	27 octobre 1999	Thailand	gew mae pan trail, Doi Inthanon, Chomtong	2120

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Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-39	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-63	22 septembre 1999	China	Dayangchang, Zhongdian	3500
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-68	22 septembre 1999	China	Baishuitai, Zhongdian	2550
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-86	24 septembre 1999	China	Nixi, Zhongdian	2900
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-102	26 septembre 1999	China	Meili glacier, left side of valley, Deqin	2500
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-117	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-129	29 septembre 1999	China	Wung Shui, Zhongdian	3000
Swertia punicea Hemsl.	Ph. Chassot & Y.-M. Yuan	99-132	30 septembre 1999	China	Mailang, Dongwang, Zhongdian	3100
Swertia racemosa Wall.	Ph. Chassot	97-11	1 septembre 1997	Nepal	Langtang to Giangjin Gompa, Langtang	3600
Swertia racemosa Wall.	Ph. Chassot	97-72	16 octobre 1997	Nepal	Murma top, Rara Lake, Karnali	3900
Swertia randaiensis Hayata	Ph. Chassot	99-2	29 août 1999	Taiwan	Shei-Pa National park, Kuanwu, road to army site	2450
Swertia randaiensis Hayata	Ph. Chassot	99-11	2 septembre 1999	Taiwan	Mt. Hehuan, Taroko national park	3000
Swertia randaiensis Hayata	Ph. Chassot	99-12	2 septembre 1999	Taiwan	Mt. Hehuan, Taroko national park	3000
Swertia randaiensis Hayata	Ph. Chassot	99-3	29 août 1999	Taiwan	Shei-Pa National park, Kuanwu, road to army site	2450
Swertia randaiensis Hayata	Ph. Chassot	99-8	2 septembre 1999	Taiwan	road from Dayuling to Wushe	2600
Swertia rosularis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-228	17 octobre 1999	China	Age old trees terrace to fairy peak, Emei Shan	1400
Swertia souliaei Burkill	Ph. Chassot & Y.-M. Yuan	99-218	10 octobre 1999	China	Top of Mountain behind FungKe, Lijiang	3900
Swertia speciosa D. Don	Ph. Chassot	97-68	12 octobre 1997	Nepal	Jumla to Chauta, first pass, Karnali	3200
Swertia striata Collett & Hemsl.	Ph. Chassot	99-240	29 octobre 1999	Thailand	Doi Chiangdao summit, Chiangdao	2000
Swertia tashiroi Makino	Ph. Chassot	99-7bis	31 août 1999	Taiwan	Shei-Pa national park, Tuchang to Kuanwu	1250
Swertia tashiroi Makino	Ph. Chassot	99-14	4 septembre 1999	Taiwan	Taipei, shiting Hsiang, Huangtitien mountain hiking loop, east end of the loop	600
Swertia tashiroi Makino	Ph. Chassot	99-1	29 août 1999	Taiwan	Shei-Pa National park, Kuanwu, on the road to the 5 huge trees	2000
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-40	21 septembre 1999	China	On the way to Napa lake from Zhongdian	3400
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-85	24 septembre 1999	China	Nixi, Zhongdian	2900

Taxon	Collector	Coll. N°	Date	Country	Locality	Alt. [m]
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-118	28 septembre 1999	China	Wung Shang, Zhongdian	2700
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-128	29 septembre 1999	China	Wung Shui, Zhongdian	3000
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-133	30 septembre 1999	China	Mailang, Dongwang, Zhongdian	2800-3500
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-159	3 octobre 1999	China	Hai Xi, Lijiang	2000
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-175	6 octobre 1999	China	pass behind Hai Dong, Dali	2200
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-202	9 octobre 1999	China	Yu Hu, Lijiang	2700
Swertia tenuis T.-N. Ho & S.-W Liu	Ph. Chassot & Y.-M. Yuan	99-206	9 octobre 1999	China	after crossroad Daju-FungKe, Lijiang	3000
Swertia tozanensis Hayata	Ph. Chassot	99-7	2 septembre 1999	Taiwan	east of Dayuling on the highway from Hualien	2600
Swertia yunnanensis Burkill	Ph. Chassot & Y.-M. Yuan	99-62	22 septembre 1999	China	Dayangchang, Zhongdian	3500
Swertia yunnanensis Burkill	Ph. Chassot & Y.-M. Yuan	99-69	23 septembre 1999	China	Dayangchang, Zhongdian	3200
Swertia yunnanensis Burkill	Ph. Chassot & Y.-M. Yuan	99-200	9 octobre 1999	China	Yu Hu, Lijiang	2700
Swertia yunnanensis Burkill	Ph. Chassot & Y.-M. Yuan	99-208	9 octobre 1999	China	Ming Yin, Lijiang	2800
Swertia yunnanensis Burkill	Ph. Chassot & Y.-M. Yuan	99-162	4 octobre 1999	China	Dian Nan, Jian Chuan	2500
Symbolanthus calygonus Griseb.	Ph. Chassot	00-14	2 juillet 2000	Ecuador	Nudo de Cajanuma, Zamora	3000
Tapeinostemon zamoranum Steyerm.	Ph. Chassot	00-17	3 juillet 2000	Ecuador	Station San Francisco, transect 2, Zamora	1500
Tapeinostemon zamoranum Steyerm.	Ph. Chassot	00-18	3 juillet 2000	Ecuador	Station San Francisco, transect 2, Zamora	3000
Tripterospermum sp.	Ph. Chassot	99-5	29 août 1999	Taiwan	Shei-Pa national park, Kuanwu, on the road to the military site	2500
Tripterospermum sp.	Ph. Chassot	99-17	9 septembre 1999	Japan	bottom of Hiking path Kikuchy sky-line to Uchinomae, Kumamoto pref.	
Tripterospermum sp.	Ph. Chassot	99-21	9 septembre 1999	Japan	Fukabayama (kikuchy sky-line), Kumamoto pref.	
Veratrilla bailloni Franch.	Ph. Chassot & Y.-M. Yuan	99-37	21 septembre 1999	China	Hill behind Napa lake, Zhongdian	3900
Veratrilla bailloni Franch.	Ph. Chassot & Y.-M. Yuan	99-90	24 septembre 1999	China	White Horse Mountain, just before east pass, deqin	3950
Veratrilla bailloni Franch.	Ph. Chassot & Y.-M. Yuan	99-148	1 octobre 1999	China	South of Da Xue Shan pass, wongshui, Zhongdian	3900-4200