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PATTERNS OF HOST-PLANT CHOICE IN BEES OF THE GENUS *CHELOSTOMA*: THE CONSTRAINT HYPOTHESIS OF HOST-RANGE EVOLUTION IN BEES

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To trace the evolution of host-plant choice in bees of the genus *Chelostoma* (Megachilidae), we assessed the host plants of 35 Palearctic, North American and Indomalayan species by microscopically analyzing the pollen loads of 634 females and reconstructed their phylogenetic history based on four genes and a morphological dataset, applying both parsimony and Bayesian methods. All species except two were found to be strict pollen specialists at the level of plant family or genus. These oligolectic species together exploit the flowers of eight different plant orders that are distributed among all major angiosperm lineages. Based on ancestral state reconstruction, we found that oligolecty is the ancestral state in *Chelostoma* and that the two pollen generalists evolved from oligolectic ancestors. The distinct pattern of host broadening in these two polylectic species, the highly conserved floral specializations within the different clades, the exploitation of unrelated hosts with a striking floral similarity as well as a recent report on larval performance on nonhost pollen in two *Chelostoma* species clearly suggest that floral host choice is physiologically or neurologically constrained in bees of the genus *Chelostoma*. Based on this finding, we propose a new hypothesis on the evolution of host range in bees.

KEY WORDS: **Ancestral state reconstruction, evolutionary constraint, oligolecty, phylogeny, pollination, supermatrix.**

Bees are the major pollinators of angiosperms in most ecosystems (Michener 2007). They provision their brood cells with large amounts of pollen and nectar, which makes the bees indispensable mutualists of flowering plants on the one hand and very effective herbivores on the other (Westerkamp 1996; Müller et al. 2006). In their natural habitats, bees are often confronted with a dazzling array of different flowers from which they have to make the most rewarding choice. In fact, while some bee species exploit a wide range of different flowers, others restrict their flower visits to closely related plant taxa. Robertson (1925) was the first to recognize that this floral specificity is limited to the collection of pollen but not to the uptake of nectar. He introduced the terms oligolectic for pollen specialists and polylectic for pollen generalists. Oligolectic bees are characterized by consistently collecting pollen from flowers of a single genus, subfamily, or family (Linsley and MacSwain 1958; Westrich 1989; Cane and Sipes 2006; Müller and Kuhlmann in press). In contrast, polylectic bees exploit flowers of more than one plant family.

Polylectic and oligolectic species coexist in all investigated bee faunas. Therefore, both polylecty and oligolecty obviously represent successful evolutionary strategies. Whereas polylecty is considered advantageous in reducing dependence upon a limited number of pollen hosts (Moldenke 1975; Eickwort and Ginsberg 1980), the ecological and evolutionary factors that select for oligolecty or act to maintain it remain subject of several, mostly untested hypotheses. One traditional assumption is that oligolecty has evolved to reduce interspecific competition for pollen (Robertson 1899, 1925; Michener 1954; Linsley 1958; Thorp 1969; Michener 1979). This hypothesis is based on the observation that pollen specialists are especially abundant in species-rich bee faunas, with up to 60% oligoleges in Californian deserts (Michener 1979; Minckley and Roulston 2006). If competition is the most important factor, closely related bee species are expected to harvest pollen on different plant taxa. However, several studies that combined bee phylogenies with pollen preferences suggest that close relatives are generally specialized on the same pollen hosts (Müller 1996; Wcislo and Cane 1996; Michez et al. 2004; Sipes and Tepedino 2005; Minckley and Roulston 2006; Patiny et al. 2007; Larkin et al. 2008; Michez et al. 2008). Another assumption addresses the possibly higher foraging efficiency of specialist bees compared to generalists that selects for host specificity (Lovell 1913, 1914). Indeed, some studies demonstrated that specialist bees are actually more efficient in pollen harvesting than generalists (Strickler 1979; Cane and Payne 1988; Laverty and Plowright 1988). However, a comparison of foraging rates of oligolectic and polylectic bees on *Medicago sativa* (Fabaceae) indicates that specialists are not always faster than generalists at using shared hosts (Pesenko and Radchenko 1993; Minckley and Roulston 2006). Similarly, no differences were observed in the flower handling techniques of oligolectic versus polylectic anthidiine bees (Müller 1996).

Traditionally, it has been a widely accepted assumption that oligolectic bees have evolved from polylectic ancestors (Michener 1954; Linsley 1958; MacSwain et al. 1973; Iwata 1976; Moldenke 1979; Hurd et al. 1980). Indeed, there do exist some clear examples of transitions from polylecty to oligolecty, e.g., in the genus *Lasioglossum* where oligolectic species have evolved twice within clades of polyleges (Danforth et al. 2003). However, growing evidence suggests that many generalist bee species have evolved from oligolectic ancestors. The basal clades of most bee families include a high proportion of pollen specialists (Westrich 1989; Wcislo and Cane 1996; Patiny et al. 2007). The Dasypodaidae and Melittidae, which are probably the most basal bee families, are predominantly composed of oligoleges, suggesting that oligolecty might be the ancestral state in bees (Danforth et al. 2006b; Michez et al. 2008). Oligolecty is also assumed to be the plesiomorphic condition in the genus *Andrena*, with polylecty having independently evolved several times (Larkin et al. 2008). Furthermore, polylecty appears to be a derived trait in several anthidiine bees as well as in a pollen-collecting masarine wasp (Müller 1996; Mauss et al. 2006). Given the huge pollen quantities needed to rear a single bee larva (Schlindwein et al. 2005; Müller et al. 2006), strong selection should act on oligolectic bees to reduce their heavy dependence upon a limited number of host plants. However, pollen specialists are widespread and outnumber the generalists in numerous bee clades as well as in some habitats (Westrich 1989; Minckley and Roulston 2006; Michener 2007). Therefore, oligolecty in bees is possibly best considered as an evolutionary constraint that has been repeatedly overcome in many polylectic bee lineages (Müller 1996; Larkin et al. 2008).

Recently, two possible constraints have been identified that might prevent oligolectic bee species from becoming polylectic or from switching hosts, that is, constraints linked to pollen digestion and neurological (including cognitive) constraints. First, the failure of several specialized bee species to develop on nonhost pollen clearly indicates that the pollen of some plant taxa possesses unfavorable or protective properties that render its digestion difficult (Praz et al. 2008). Similarly, the pattern of use of Asteroideae pollen by bees of the genus *Colletes* suggests that this pollen has chemical properties that interfere with its digestion by generalists (Müller and Kuhlmann in press). Therefore, physiological adaptations might be needed to overcome the protective properties of some pollen types. This in turn may constrain the bees' capability to use other pollen types similar to herbivorous insects, where adaptations to the secondary chemistry of their hosts may result in a lower capability to exploit alternative hosts (Strauss and Zangerl 2002; Singer 2008). Second, constraints in recognizing or handling nonhost flowers are likely to prevent the pollen-specialist bee *Heriades truncorum* from becoming polylectic (Praz et al. in press). This species, which exclusively collects pollen on Asteraceae in nature, was found to be able to develop on several types of nonhost pollen. However, the females refused to collect nonhost pollen despite its suitability for larval development even in the absence of the normal host. This finding suggests that neurological limitations are more important than nutritional constraints in shaping the host range of this species.

Phylogenetic inference is a powerful method to uncover patterns of host-plant choice and to test hypotheses on the evolution of host-plant associations (Harvey 1996). So far, only a few studies applied phylogenetic inference to analyze bee-flower relationships at species level (Müller 1996; Michez et al. 2004; Sipes and Tepedino 2005; Larkin et al. 2008). Most of these studies, however, were restricted to one biogeographical region and did not include the whole diversity of the bee taxon under investigation. In the present study, we used phylogenetic inference to analyze patterns of host plant choice in bees of the genus *Chelostoma* (Megachilidae, Osmiini). Although this genus is assumed to consist mainly of oligoleges (Michener 2007), solely the pollen preferences of the few central European and North American species are known so far (Hurd and Michener 1955; Moldenke and Neff 1974; Krombein et al. 1979; Parker 1988; Westrich 1989, 1993; Gogala 1999; Amiet et al. 2004; Michener 2007). By including a substantial proportion of species from all three biogeographical regions in which the genus is known to occur, we provide the first study of bee-flower relationships on a worldwide scale. Specifically, we addressed the following questions: (1) What are the flower preferences of the different *Chelostoma* species? (2) Is oligolecty the ancestral state in the genus *Chelostoma* and have polylectic species evolved from oligolectic ancestors or vice versa? (3) Is host-plant choice a conserved trait with members of the same clade having the same host preferences? (4) Are the observed patterns of host-plant choice consistent with the hypothesis that the oligolectic habit is constrained by physiological or neurological limitations? Based on our findings, we outline a new hypothesis on the evolution of host range in bees.

Material and Methods

BEE SPECIES

The genus *Chelostoma*, which is divided into six subgenera by Michener (2007), is represented by 42 described species in the Palearctic region, nine species in North America, and a single species in subtropical and tropical southeast Asia (Michener 2007; Ungricht et al. in press). The center of diversity is situated in the eastern Mediterranean area of Europe and western Asia. There is strong evidence that the genus *Chelostoma* is monophyletic and sister to all other species of the tribe Osmiini (Praz et al., in press). For the present study, we selected a total of 35 species and subspecies (26 taxa from the Palearctic, eight from the Nearctic and one from Indomalaya; Table 1) for which enough pollen samples were available for assessing floral host range. These species represent all six subgenera currently recognized and encompass most of the morphological variability within *Chelostoma*. Four species are new to science, they are referred to as *species 2, 3, 23,* and *24,* respectively. Their description is in preparation (A. Müller unpubl. ms.).

Voucher specimens of all bee taxa selected for the present study are deposited in the Entomological Collection at the ETH Zurich. The nomenclature follows Krombein et al. (1979) and Ungricht et al. (in press) for the genus *Chelostoma*, Ungricht et al. (in press) for the Osmiini and Schwarz et al. (1996) for the other megachilid species.

HOST PLANTS

To assess the pollen hosts of the 35 *Chelostoma* taxa selected for this study, we analyzed the scopal contents of 634 females from museum or private collections by light microscopy using the method outlined by Westrich and Schmidt (1986). For each species, we sampled specimens from as many localities as possible to account for potential differences in pollen-host use of different populations. Before removing pollen from the abdominal scopae, we estimated the degree to which they were filled. The amount of pollen was assigned to five classes ranging from 5/5 (full load) to 1/5 (filled only to one-fifth). The pollen grains were stripped off the scopae with a fine needle onto a slide and embedded in glycerine gelatine. We estimated the percentages of different pollen types by counting the grains along four lines chosen randomly across the cover slip at a magnification of 400×. Pollen types represented by less than 10% of the counted grains were excluded to prevent potential bias caused by contamination. In loads consisting of two or more different pollen types, we corrected the percentages of the number of pollen grains by their volume. After assigning different weights to scopae according to how filled they were (full loads were five times more strongly weighted than scopae filled to only one-fifth), we summed up the estimated percentages over all pollen samples of each species. The pollen grains were identified at a magnification of 400× or 1000× with the aid of the literature cited in Westrich and Schmidt (1986) and an extensive reference collection. Identification of the pollen samples from the North American species was facilitated by Constance and Chuang (1982) who give a survey on the pollen morphology of the Hydrophyllaceae. In general, we identified the pollen grains down to family or, if possible, to genus level, those of the Asteraceae down to the subfamilies Asteroideae and Cichorioideae, respectively.

To characterize different degrees of host-plant association among the *Chelostoma* species investigated, we used the two categories oligolecty and polylecty (sensu Westrich 1989; Müller and Kuhlmann in press). We did not differentiate between the subcategories of oligolecty and polylecty as defined by Cane and Sipes (2006) and Müller and Kuhlmann (in press), respectively. To classify a *Chelostoma* species as oligolectic, we applied two different approaches introduced by Müller (1996) and Sipes and Tepedino (2005), respectively. A species was designated as oligolectic if (1) 95% or more of the pollen grain volume belonged to the same plant family or genus, or (2) if 90% or more of the females collected pure loads of one plant family or genus. Both approaches yielded exactly the same categorizations for all *Chelostoma* species analyzed. To infer the host range of those species for which only a small number of pollen loads was available, both the literature and unpublished field data were also considered (Appendix 1).

Table 1. The nine outgroup species and the 35 *Chelostoma* species included in this study, with locality information, and GenBank accession numbers.

Species	Locality	Collector	GenBank accession numbers			
			EF	Opsin	CAD	COI
Outgroup						
Lithurgus chrysurus	Italy, Abruzzen, Massa	AM	EU851523	EU851629	EU851418	EU863054
Anthidium punctatum	Switzerland, Weiach	AM	EU851525	EU851631	EU851420	EU863055
Megachile pilidens	Switzerland, Weiach	AM	EU851531	EU851637	EU851426	EU863056
Ochreriades fasciatus	Jordan, Wadi Shu'ayb	CP, CS & AM	EU851590	EU851696	EU851485	EU863057
Hofferia schmiedeknechti	Greece, Chimara	CP & CS	EU851556	EU851662	EU851451	EU863058
Hoplitis adunca	Italy, Aosta	AM	EU851572	EU851678	EU851467	EU863059
Osmia cornuta	Switzerland, Zürich	AM	EU851609	EU851714	EU851504	EU863060
Protosmia minutula	Switzerland, Embd	CP	EU851620	EU851725	EU851515	EU863061
Heriades truncorum	Switzerland, Winterthur	AM	EU851553	EU851659	EU851448	EU863062
Subgenus *Ceraheriades*						
Chelostoma lamellum	China, Yunan Province	CS	EU851545	EU851651	EU851440	EU863063
Subgenus *Chelostoma*						
Chelostoma carinulum	–	–	–	–	–	–
Chelostoma diodon	Greece, Lesbos	A. Grace	EU863111	EU863132	EU863090	EU863064
Chelostoma edentulum	Morocco, Souss	AM	EU863112	EU863133	EU863091	EU863065
Chelostoma emarginatum	Greece, Platania	K. Standfuss	EU863113	EU863134	EU863092	–
Chelostoma florisomne	Switzerland, Chur	E. Steinmann	EU851546	EU851652	EU851441	EU863066
Chelostoma grande	Switzerland, Erschmatt	CS	EU863114	EU863135	EU863093	EU863067
Chelostoma mocsaryi	Greece, Platania	CS & CP	EU863115	EU863136	EU863094	EU863068
Chelostoma nasutum	Greece, Andhritsena	CS & CP	EU863116	EU863137	EU863095	EU863069
Chelostoma species 2	Greece, Cyprus	C. Schmid-Egger	EU863117	EU863138	EU863096	EU863070
Chelostoma species 3	Jordan, Dead Sea	CS, CP & AM	EU863118	EU863139	EU863097	EU863071
Chelostoma transversum	Greece, Zachlorou	CS & CP	EU863119	EU863140	EU863098	EU863072
Subgenus *Eochelostoma*						
Chelostoma aureocinctum	Thailand, Chiang Mai	CS	EU851547	EU851653	EU851442	EU863073
Subgenus *Foveosmia*						
Chelostoma bytinskii	Jordan, Wadi Mujib	CS, CP & AM	EU863120	EU863141	EU863099	EU863074
Chelostoma californicum	USA, CA, Mariposa Co	T. Griswold	EU851548	EU851654	EU851443	EU863075
Chelostoma campanularum	Switzerland, Winterthur	AM	EU851549	EU851655	EU851444	EU863076
Chelostoma cockerelli	USA, CA, Tuolomne Co.	J. Gibbs	EU863121	EU863142	EU863100	EU863077
Chelostoma distinctum	Switzerland, Emdt	AM	EU863122	EU863143	EU863101	EU863078
Chelostoma foveolatum	Italy, Toscana	AM	EU863123	EU863144	EU863102	EU863079
Chelostoma garrulum	–	–	–	–	–	–
Chelostoma hellenicum	Greece, Tygetos Mts	CS & CP	EU863124	EU863145	EU863103	EU863080
Chelostoma incisulum	USA, CA, Tuolomne Co.	L. Fuerst	EU863125	EU863146	EU863104	EU863081
Chelostoma isabellinum	–	–	–	–	–	–
Chelostoma laticaudum	Greece, Andhritsena	CS & CP	EU863126	EU863147	EU863105	EU863082
Chelostoma m. marginatum	–	–	–	–	–	–
Chelostoma m. incisuloides	USA, CA, Tuolomne Co.	H. W. Ikerd	EU863127	EU863148	EU863106	EU863083
Chelostoma minutum	USA, CA, Tuolomne Co.	T. Griswold	EU863128	EU863149	EU863107	EU863084
Chelostoma phaceliae	USA, CA, Tuolomne Co.	L. Fuerst	EU863129	EU863150	EU863108	EU863085
Chelostoma species 23	–	–	–	–	–	–
Chelostoma species 24	–	–	–	–	–	–
Chelostoma styriacum	Greece, Michas-Lakomata	CS & CP	EU863130	EU863151	EU863109	EU863086
Chelostoma tetramerum	–	–	–	–	–	–
Chelostoma ventrale	Turkey, Ankara	E. Scheuchl	EU863131	EU863152	EU863110	EU863087
Subgenus *Gyrodromella*						
Chelostoma rapunculi	Switzerland, Fully	CP	EU851550	EU851656	EU851445	EU863088
Subgenus *Prochelostoma*						
Chelostoma philadelphi	USA, MD, Pr. George's co.	S. Droege	EU851551	EU851657	EU851446	EU863089

Names of the authors of the present study are abbreviated. For species lacking locality data and GenBank accession numbers, only morphological data were available.

Table 2. **Primers used for the four genes Elongation factor-1α, LW-rhodopsin, Conserved ATPase Domain (CAD), and Cytochrome Oxidase I (COI).**

Primer	Sequence 3–5′	Reference
Elongation factor-1α		
HaF2For1	GGG YAA AGG WTC CAA RTA TGC	Danforth et al. 2004
F2Rev1-Meg	AAT CAG CAG CAC CCT TGG GTG G	Praz et al., in press
For4a	AGC TTT GCA AGA GGC TGT TC	Praz et al., in press
Cho10	ACR GCV ACK GTY TGH CKC ATG TC	Danforth et al. 2004
F2-Intron-Rev	AAA AAT CCT CCG GTG GAA AC	Praz et al., in press
Exon2For	CCG ACT AGA CCT ACA GAC AAA GCT C	Praz et al., in press
Exon3Rev	GAG CAA ATG TGA CAA CCA TAC C	Praz et al., in press
PCR conditions:	HaF2For1/F2Rev1-Meg: 30″ 94°C, 30″ 58°C, 40″ 72°C For4a/Cho10: 30″ 94°C, 30″ 58°C, 30″ 72°C Exon2For/F2Rev1-Meg: 30″ 94°C, 30″ 58°C, 30″ 72°C	
LW-Rhodopsin		
OpsFor-Osm	AAT TGY TAY TWY GAG ACA TGG GT	Praz et al., in press
OpsinRev3	GCC AAT TTA CAC TCG GCA CT	Praz et al., in press
OpsinFor5c	GCG TGT GGC ACC GAT TAT TTC	this study
OpsinRev6	GCC ARY GAY GGG AAT TTC T	this study
PCR conditions:	OpsinFor-Osm/OpsinRev3: 30″ 94°C, 30″ 55°C, 30″ 72°C OpsinFor5c/OpsinRev6: 30″ 94°C, 30″ 58°C, 30″ 72°C	
CAD		
CADFor4	TGG AAR GAR GTB GAR TAC GAR GTG GTY CG	Danforth et al. 2006a
CADRev1-Meg	GCC ATC ACT TCY CCT AYG CTC TTC AT	Praz et al., in press
PCR conditions:	CADFor4/CADRev1-Meg: 30″ 94°C, 30″ 55°C, 30″ 72°C	
COI		
UEA3	TAT AGC ATT CCC ACG AAT AAA TAA	Lunt et al. 1996
UEA6	TTA ATW CCW GTW GGN CAN GCA ATR ATT AT	Lunt et al. 1996
UEA10	CAA TGC ACT TAT TCT GCC ATA TT	Lunt et al. 1996
COI-Chel-Rev	GTW GGW ACN GCA ATR ATT ATR GTT G	this study
COI-Chel-For	GGA ATT GGA TTT TTA GGA TTT ATT G	this study
PCR conditions:	UEA3/UEA6: 30″ 94°C, 45″ 51°C, 45″ 72°C UEA3/UEA10: 30″ 94°C, 45″ 51°C, 45″ 72°C UEA3/COI-Chel-Rev: 30″ 94°C, 30″ 51°C, 30″ 72°C COI-Chel-For /UEA10: 30″ 94°C, 30″ 51°C, 30″ 72°C	

MOLECULAR PHYLOGENY

DNA sequences

Freshly collected material allowing for DNA extraction was available for 28 of the 35 *Chelostoma* taxa included in the present study (Table 1). As outgroup species, we selected five representatives of the tribe Osmiini (*Heriades truncorum, Hofferia schmiedeknechti, Hoplitis adunca, Osmia cornuta*, and *Protosmia minutula*), one species each of the tribes Lithurgini (*Lithurgus chrysurus*), Anthidiini (*Anthidium punctatum*) and Megachilini (*Megachile pilidens*), and *Ochreriades fasciatus*, a megachilid bee originally assigned to the Osmiini (but see Praz et al., in press). We generated a DNA matrix composed of 3018 aligned sequences from four genes: the three nuclear genes Elongation factor-1α (F2-copy; hereafter EF), Long-wave rhodopsin (opsin), Conserved ATPase domain (CAD), and the mitochondrial gene Cytochrome oxidase subunit 1 (COI). Preliminary phylogenetic analyses indicated that the coding sequence of EF was too conserved for the phylogenetic level to considered here: there was almost no variation in codon positions 1 and 2 in the ingroup and only little, mostly silent, variation in position 3, which was AT-biased and had a biased base composition across species. We therefore sequenced only the two introns (approximately 200 and 240 bp, respectively) included in the 1600 bp fragment often used to infer bee phylogeny (Danforth et al. 2004). For opsin, we included both the coding sequence (600 bp) and three introns (approximately 80–100 bp each). The fragment used for CAD (448 bp) had no introns and corresponds to exon 6 in the fragment described by Danforth et al. (2006a). For these three nuclear markers, we used primers designed for bees in general (Danforth et al. 2004, 2006a), for the Osmiini (Praz et al., in press) as well as new primers specific to *Chelostoma* (Table 2). The fragment of COI consisting of 1219 bp was amplified with universal primers for insects (Zhang and Hewitt 1996;

Table 2). For five *Chelostoma* species, chromatograms of COI sequences revealed several double peaks indicating the presence of pseudogenes (Bensasson et al. 2001). As these double peaks concerned less than 1% of the complete sequence for two species and less than 5% for two others, and as no indels were found, we did not use cloning techniques to separate the different copies but coded all base pairs with double peaks as "N." The sequence of *C. emarginatum* contained too many double peaks and was therefore excluded from the analyses.

DNA was extracted from bees preserved in 100% ethanol and from a few pinned specimens up to 5-year old following the extraction protocol of Danforth (1999). PCR-amplification products were purified using GFX DNA purification kit (GE Healthcare Europe GmbH, Freiburg, Germany). Automated sequencing of the PCR products was performed on an ABI Prism (Applied Biosystems, Foster City, CA) 3130×1 sequencer using BigDye technology (Applied Biosystem). GenBank accession numbers for the DNA sequences and the locality data for the specimens used for DNA extraction are listed in Table 1.

Alignments for all genes were performed manually using MacClade version 4.08 for OSX (Maddison and Maddison 2005). Some regions in the ingroup as well as all outgroup intron sequences could not be aligned unambiguously and were excluded. We initially coded five indels that could be unambiguously aligned as additional characters, but as they did not influence the phylogenetic results, we excluded them from all subsequent analyses. To ensure that the correct reading frame of each gene was found, the coding sequences were converted into amino acid sequences. No stop codons were found in any of the exons of the genes. The complete alignment is deposited in TreeBASE (www.treebase.org/treebase/index.html) under the study accession number S2125.

Phylogenetic analyses

We performed parsimony analyses of each gene separately in Paup version 4.0b10 for Macintosh (Swofford 2002) with the following parameter settings: unweighted analysis, heuristic search, TBR branch swapping, 100 bootstrap replicates, 10 random sequence additions, four trees held at each step, maximum of 500 trees retained . As very little incongruence was observed between the four genes and as no conflicting topology was supported by bootstrap values above 60%, we combined the four genes into a single matrix and analyzed the combined dataset with the same parameter as above performing 1000 bootstrap replicates. We performed analyses with and without the third nucleotide position of COI, which was strongly AT-biased and hence prone to high level of homoplasy (Danforth et al. 2003).

For the Bayesian analyses, the four genes were analyzed collectively under four different partitioning regimes. First, we partitioned the dataset by gene, yielding four partitions. Second, we partitioned opsin into two partitions, the coding sequence and the introns, resulting in a total of five partitions. Third, we partitioned COI into three partitions for the first, second, and third nucleotide position, which yielded seven partitions. Lastly, we performed a fully partitioned analysis with 11 partitions, with a separate GTR model applied to each gene and codon position (GTR + SSR). Analyses by MrModeltest (Nylander 2004) identified the following best models of sequence evolution for each partition: EF intron, HKY + G; opsin, GTR + I + G; opsin coding sequence, HKY + I + G; opsin intron, GTR + I; CAD, K80 + G; COI, GTR + I; COI nt1, GTR + I + G; COI nt2, GTR + I + G; COI nt3, GTR + G. A posteriori examination of parameter plots with Tracer version 1.4 (Rambaut and Drummond 2003) indicated that the proportion of invariant sites (I) and the shape (G) parameters could not be properly estimated for the three site-specific partitions of COI, and hence we applied the GTR model to these three partitions. To select between these partitioning regimes, we calculated Bayes factors (Nylander et al. 2004) and Akaike Information Criteria (AIC) using the formula of McGuire et al. (2007).

Markov Chain Monte Carlo analyses were performed using MrBayes 2.1.1 (Huelsenbeck and Ronquist 2001). We performed two simultaneous runs with one cold and three heated chains each (temperature parameter fixed to 0.05) for two million generations, sampling trees and parameters every 100 generations. The onset of stationarity was determined by an examination of plots for log-likelihood values and for all parameters using Tracer. All trees sampled before stationarity (usually 10%) were discarded and the remaining trees from both runs were combined into a single majority rule consensus tree in Paup.

MORPHOLOGICAL PHYLOGENY AND SUPERMATRIX ANALYSIS

For seven of the 35 *Chelostoma* species included in this study, only morphological data were available. To include these seven taxa into the phylogeny, we combined the molecular and the morphological dataset into a total-evidence "supermatrix" (Queiroz and Gatesy 2006). To collect morphological characters, both males and females of all 35 *Chelostoma* species were examined externally using a dissecting microscope. In addition, we dismembered the abdomen of the males to get appropriate views of the otherwise hidden sterna and the genitalia, and embedded the scopal hairs of the females in glycerine gelatine for microscopical study. The search for morphological characters was facilitated by the publications of Michener (1938, 1942, 2007), Hurd and Michener (1955), Warncke (1991), and Schwarz and Gusenleitner (2000). The morphological analysis yielded 48 characters (see Appendices 2 and 3). We did not code morphological characters for the outgroup species, as homology proved impossible to ensure. We selected *C. aureocinctum* as an outgroup for analyzing the morphological dataset alone, as this species unambiguously appeared as sister to all other *Chelostoma* species in the molecular phylogenetic analyses. We first performed parsimony analysis of the morphological dataset alone in Paup 4.0b10 with the following parameter settings: all characters unweighted and treated as unordered, heuristic search, TBR branch swapping, 1000 bootstrap replicates, 10 random sequence additions, four trees held at each step, maximum of 500 trees retained. Then, we combined the morphological and the molecular datasets and performed parsimony and Bayesian analyses with only those 28 species for which both datasets were available. For the morphological partition, we applied a simple character model with all transition rates equal and a fixed proportion of character states. A gamma shape distribution of rates was not fitted to the morphological partition, as preliminary analyses failed to correctly estimate this parameter. Lastly, we ran a Bayesian analysis with all 35 species included, coding both the lacking molecular data for the seven additional species and the lacking morphological data for the outgroup as "missing data." This "supermatrix" was analyzed with MrBayes under the favored partitioning regime for molecular data and an additional partition composed of the morphological dataset, applying the same model as above. We ran 5 million generations and constrained the ingroup (*Chelostoma*) to be monophyletic to reach stationarity within a reasonable period of time.

EVOLUTION OF HOST-PLANT CHOICE

To reconstruct the evolution of host-plant choice within the genus *Chelostoma*, we first applied parsimony mapping in MacClade, using the topology of the majority rule consensus of trees saved in the Bayesian analysis of the "supermatrix." As parsimony reconstruction of ancestral state does not take branch length into account, we used maximum likelihood inference of ancestral character states as implemented in BayesTraits (Pagel et al. 2004; Pagel and Meade 2006). Transition rates between all states (i.e., pollen hosts) were assumed to be equal using the "restrictall" command in BayesTraits. We used two samples of trees saved during Bayesian analyses of the "supermatrix": first, the analysis including only those 28 species for which both molecular and morphological data were available, and second, the analysis with all 35 species. In the second "supermatrix" analysis, the length of branches leading to the seven species without molecular data could not satisfactorily be estimated due to the missing DNA data. However, as these species were all closely related to other species and well nested within the clades for which ancestral state reconstruction was performed, we postulate that the biased branch lengths did not substantially affect the results. The Bayesian analyses were allowed to run for 2 million generations after convergence, saving trees every 4000 generations in both runs resulting in a total of 1002 trees. We excluded the outgroup taxa in Mesquite for OSX (Maddison and Maddison 2007).

We reconstructed the ancestral pollen host for five important and well-supported nodes with posterior probabilities of 100% with the "AddNode" command in BayesTraits. In addition, we used the "most recent common ancestor approach" implemented in BayesTraits (command "AddMRCA") to specifically test whether specialization to one pollen host had occurred only once. This approach enabled ancestral host inference for each of the 1002 trees in the most recent common ancestor of all species specialized to a given pollen host. If this ancestor was found to be specialized on this host, we concluded that specialization to this host had happened only once. Lastly, to assess the robustness of some ancestral state reconstructions that had important implications for the understanding of host associations in the genus *Chelostoma*, we used the "Fossil" command in BayesTraits enabling the comparison of the likelihoods associated with alternative states. In all three approaches, we used samples of trees with and without the seven additional species lacking molecular data.

Results

HOST PLANTS

We classified 26 of the 35 *Chelostoma* taxa selected for this study as oligolectic based on the microscopical analysis of pollen loads (Table 3) as well as on the literature and unpublished field data (Appendix 1). These specialized species restrict pollen harvesting to Campanulaceae (10 species), Hydrophyllaceae (6), Ranunculaceae (3), Asteraceae (2), Dipsacaceae (2), Brassicaceae (1), *Ornithogalum* (Hyacinthaceae) (1), and *Philadelphus* (Hydrangeaceae) (1). In six additional species, the pollen loads exclusively consisted of pollen of Campanulaceae (3 species), Ranunculaceae (1), *Allium* (Alliaceae) (1), and *Schima* (Theaceae) (1). The small number of pollen loads available for study and the lack of literature or field data did not allow unambiguous classification of these six species as pollen specialists (Table 3). However, because the closest relatives of the putative Campanulaceae specialists *C. isabellinum*, *C. garrulum*, and *C. species 24* are strictly oligolectic on Campanulaceae, and as the presumed Ranunculaceae specialist *C. species 2* is a member of a clade that contains several Ranunculaceae oligoleges, these four species are most probably pollen specialists as well. Similarly, *C. tetramerum* and *C. aureocinctum* are treated as oligolectic on *Allium* and *Schima*, respectively. Four of the five pollen loads of *C. tetramerum* originated from different localities and the 10 females of *C. aureocinctum* available for study were collected at eight different localities in India, Nepal, Thailand, and China, which clearly points to a pollen specialization. Two *Chelostoma* species turned out to be polylectic harvesting pollen on at least five (*C. species 3*) and three plant families (*C. minutum*), respectively (Table 3). The host range of *C. lamellum*, which is known only from Sichuan, Yunnan and Gansu province in China, is still

Table 3. Host-plant preferences and inferred category of host range in 35 *Chelostoma* species and subspecies.

Subgenus and species	*n*	N	Results of microscopical analysis of pollen loads		Host range
			% pollen grain volume	% pure loads of preferred host	
Subgenus *Ceraheriades*					
Chelostoma lamellum	2	2	HYDRA (cf. *Philadelphus*)	100	unknown, possibly oligolectic on *Philadelphus* (HYDRA), see text
Subgenus *Chelostoma*					
Chelostoma carinulum	10	6	RAN 100	100	oligolectic on RAN
Chelostoma diodon	36	17	AST (Asteroideae) 89.4, AST (Cichorioideae) 10.2, BRA 0.4	97.2	oligolectic on AST
Chelostoma edentulum	28	20	BRA 95.3, RES 3.3, RAN 1.3, (Asteroideae) 0.2	92.9	oligolectic on BRA
*Chelostoma emarginatum**	32	27	RAN 100	100	oligolectic on RAN
*Chelostoma florisomne**	46	42	RAN 100	100	oligolectic on RAN
*Chelostoma grande**	20	16	DIP 100	100	oligolectic on DIP
*Chelostoma mocsaryi**	32	22	HYA (cf. *Ornithogalum*) 99.8, BRA 0.1, API 0.1	93.8	oligolectic on *Ornithogalum*
*Chelostoma nasutum**	24	11	CAM 100	100	oligolectic on CAM
Chelostoma species 2	4	4	RAN 100	100	probably oligolectic on RAN
Chelostoma species 3	13	9	CIS 40.7, RAN 31.2, BRA 24.0, RES 3.4, ZYG 0.7	15.4	polylectic
*Chelostoma transversum**	21	10	DIP 100	100	oligolectic on DIP
Subgenus *Eochelostoma*					
Chelostoma aureocinctum	10	8	THE (*Schima*) 100	100	probably oligolectic on *Schima*
Subgenus *Foveosmia*					
*Chelostoma bytinskii**	4	3	CAM 100	100	oligolectic on CAM
*Chelostoma californicum**	26	22	HYD (*Phacelia*) 100	100	oligolectic on *Phacelia*
*Chelostoma campanularum**	34	24	CAM 100	100	oligolectic on CAM
*Chelostoma cockerelli**	14	11	HYD 100	100	oligolectic on HYD
*Chelostoma distinctum**	18	16	CAM 100	100	oligolectic on CAM
*Chelostoma foveolatum**	15	10	CAM 100	100	oligolectic on CAM
Chelostoma garrulum	9	6	CAM 100	100	oligolectic on CAM
*Chelostoma hellenicum**	11	8	CAM 100	100	oligolectic on CAM
*Chelostoma incisulum**	29	19	HYD (*Phacelia*) 95.2, ALL (cf. *Allium*) 4.8	96.6	oligolectic on *Phacelia*
Chelostoma isabellinum	2	2	CAM 100	100	probably oligolectic on CAM
*Chelostoma laticaudum**	15	11	CAM 100	100	oligolectic on CAM
*Chelostoma m. marginatum**	5	2	HYD (*Phacelia*) 100	100	oligolectic on HYD
*Chelostoma m. incisuloides**	-	-	-	-	oligolectic on HYD
*Chelostoma minutum**	18	12	HYD 69.9, ALL (cf. *Allium*) 23.6, unknown 6.5	70.6	polylectic
*Chelostoma phaceliae**	46	17	HYD (*Phacelia*) 100	100	oligolectic on *Phacelia*
*Chelostoma species 23**	1	1	CAM 100	100	oligolectic on CAM
Chelostoma species 24	2	1	CAM 100	100	probably oligolectic on CAM
*Chelostoma styriacum**	11	5	CAM 100	100	oligolectic on CAM
Chelostoma tetramerum	5	4	ALL (cf. *Allium*) 100	100	probably oligolectic on *Allium*
Chelostoma ventrale	34	25	AST (Asteroideae) 100	100	oligolectic on AST
Subgenus *Gyrodromella*					
*Chelostoma rapunculi**	36	31	CAM 100	100	oligolectic on CAM
Subgenus *Prochelostoma*					
*Chelostoma philadelphi**	21	11	HYDRA (cf. *Philadelphus*) 100	100	oligolectic on *Philadelphus*

n, total number of pollen loads; *N*, number of pollen loads from different localities. Abbreviations of plant taxa: ALL, Alliaceae; API, Apiaceae; AST, Asteraceae; BRA, Brassicaceae; CAM, Campanulaceae; CIS, Cistaceae; DIP, Dipsacaceae; HYA, Hyacinthaceae; HYD, Hydrophyllaceae; HYDRA, Hydrangeaceae; RAN, Ranunculaceae; RES, Resedaceae; THE, Theaceae; ZYG, Zygophyllaceae. The nomenclature of *Chelostoma* follows Ungricht et al. (in press) for the Palearctic and Indomalayan species and Krombein et al. (1979) for the Neartic species. Subgeneric classification of *Chelostoma* according to Michener (2007). Species for which literature and unpublished field data were used to infer host range in addition to the results of the microscopical pollen analysis are marked with an asterisk (see Appendix 1 for a compilation of published and unpublished flower records).

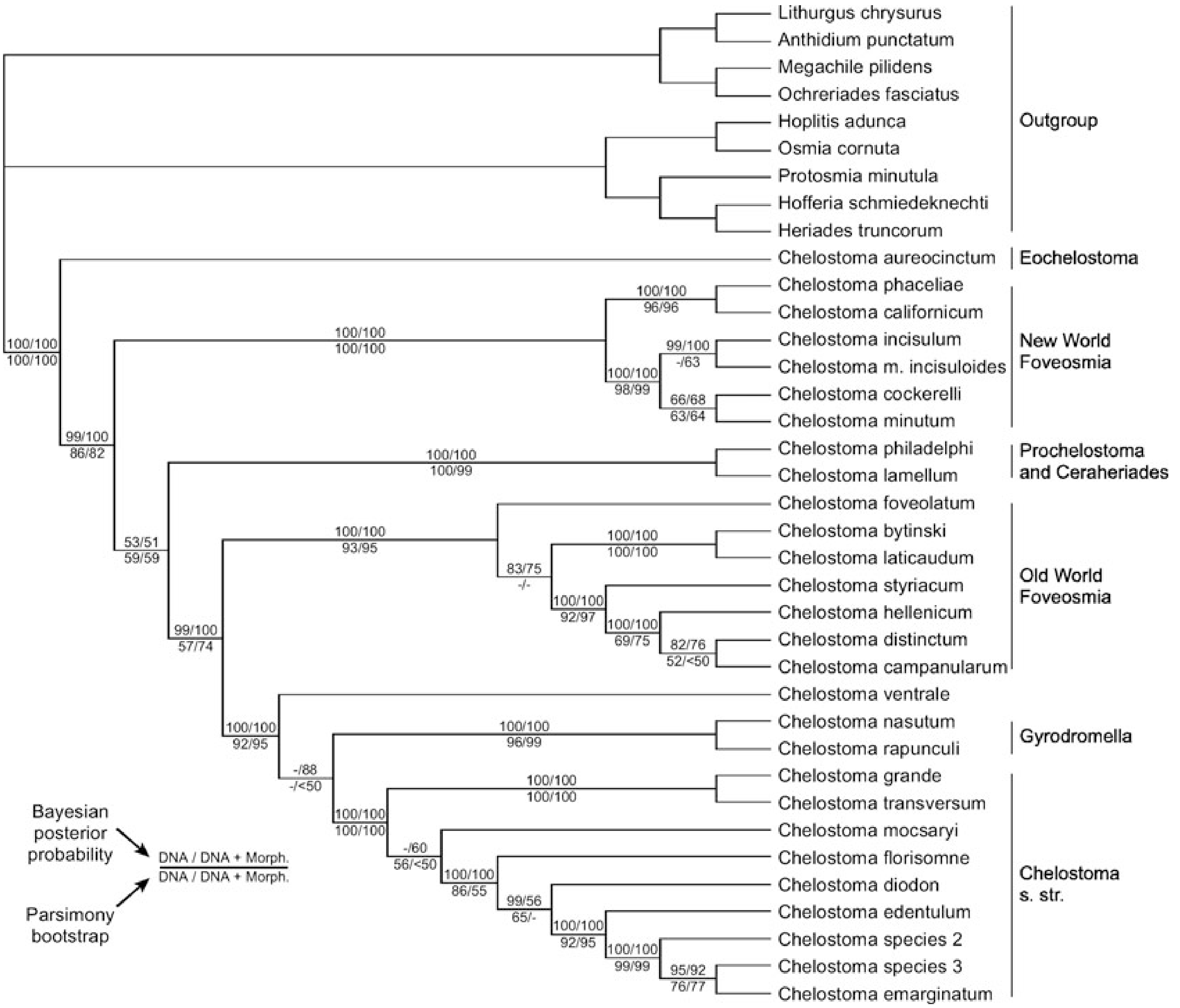

Figure 1. Phylogenetic relationships within the genus *Chelostoma*. The tree shown is the 50%-majority rule tree of trees 5000–50,000 in the favored Bayesian analysis of the combined dataset (four genes divided into five partitions plus morphology; COI nt3 included). Values above branches give the posterior probabilities for the Bayesian analyses (left: without morphology; right: with morphology). Values below branches give the parsimony bootstrap values without COI nt3 (left: without morphology; right: with morphology). Missing values ("-") indicate clades not recovered in the analysis.

unknown. The only two pollen loads available for study contained pollen grains that could not be differentiated microscopically from pollen samples of *Philadelphus* (Hydrangeaceae). Small pollen packages removed from the labrum and clypeus of two females, which have been collected at two different localities in Yunnan province in 1992 and 1993, respectively, were also composed of *Philadelphus* grains. In addition, the only two individuals of *C. lamellum* we collected in China in July 2006 were observed flying near flowering *Philadelphus* shrubs. Therefore, we hypothesize that *Philadelphus* is an important or even the exclusive pollen host of *C. lamellum*. This assumption is supported by the finding that one pollen load of the closely related Chinese species *C. sublamellum*, which could not be included in our phylogeny due to the lack of specimens for study, entirely consisted of *Philadelphus* pollen.

PHYLOGENY

Maximum parsimony bootstrap analysis of the combined molecular dataset with the exclusion of the third codon position of COI yielded an almost completely resolved tree (26 of the 27 nodes with bootstrap support above 50%, see values in Fig. 1). The inclusion of the strongly AT-biased third codon position of COI had only minor influences on the tree topology, however the tree was slightly less well supported (22 of the 27 nodes with bootstrap support above 50%). Parsimony analysis of the morphological dataset alone yielded 385 most parsimonious trees. The topology of the strict consensus tree (Appendix 4) was largely congruent with the molecular trees based on parsimony. Parsimony analysis of the combined molecular and morphological dataset produced a well-resolved consensus tree (values in Fig. 1) highly similar to that of the molecular data alone but with slightly better support.

In the Bayesian analyses, log-likelihood values and AIC scores favored the partitioning regime with five partitions (harmonic means of likelihood values: −23072, −23033, −24364, −24256, and −23574, for the analyses under four, five, six, seven partitions, and the GTR + SSR analysis, respectively). All five analyses yielded consensus trees that were almost fully resolved and virtually identical in their topology. The majority-rule consensus tree in the favored analysis had only two polytomies, and 21 of the 25 other nodes had posterior probabilities of above 95% (values in Fig. 1). Adding the morphological dataset as a supplementary partition to this analysis resulted in similar or slightly higher supports for all nodes apart from one with substantially lower support (Fig. 1). Overall, only five nodes were not recovered by all four analyses (parsimony and Bayesian analyses each with and without morphology; Fig. 1): *C. marginatum incisuloides* was sister to *C. cockerelli* and *C. minutum* in one parsimony analysis; *C. bytinskii* and *C. laticaudum* were sister group of *C. foveolatum* in both parsimony analyses but not in the Bayesian analyses; there was a polytomy with *C. ventrale*, *Gyrodromella*, and *Chelostoma* s. str. in one Bayesian analysis, and *C. ventrale* was sister to *Gyrodromella* in one parsimony analysis; lastly, *C. diodon* and *C. mocsaryi* had a different position in one parsimony analysis.

The total-evidence Bayesian analysis of the "supermatrix" resulted in a tree (Fig. 2) with no conflict in topology with the combined molecular phylogeny (Fig. 1). All seven species, for which only morphological data were available, clustered with the same sister species in both the total-evidence phylogeny and the strict consensus tree based on the morphological data alone (Appendix 4).

EVOLUTION OF HOST-PLANT CHOICE

Based on the total-evidence phylogeny and the assumption of parsimony, oligolecty is the ancestral state in the genus *Chelostoma*. Parsimony mapping of pollen hosts (Fig. 2) reveals a derived position of the two polylectic species *C. minutum* and *C. species 3*, which provides solid evidence that polylecty independently arose twice within *Chelostoma*. The two polylectic species broadened their diet under maintenance of the ancestral host within the clade from which they are derived (Table 3): *C. minutum* added pollen of *Allium* to the Hydrophyllaceae diet and *C. species 3* broadened the Ranunculaceae diet with pollen of mainly Cistaceae and Brassicaceae. Classifying the six species as polylectic for which only few pollen samples were available (*C. aureocinctum, C. tetramerum, C. isabellinum, C. garrulum, C. species 24, C. species 2*) would still result in two independent transitions from oligolecty to polylecty. However, due to the basal position of *C. aureocinctum*, the ancestral state of host range (oligolecty vs. polylecty) in the genus *Chelostoma* would be equivocal.

Ancestral reconstruction of host-plant choice at the five selected nodes (Fig. 2) confirmed the results based on parsimony. Likelihood values of inferred hosts did not substantially differ between analyses with and without the seven taxa lacking molecular data (values in Fig. 2). The ancestor of the American *Foveosmia* species (node A) was most likely a specialist of Hydrophyllaceae and the common ancestor of the Palearctic *Foveosmia* species, *Gyrodromella*, and *Chelostoma* s. str. (node B) a specialist of Campanulaceae. The ancestral hosts for nodes C and D were less clear (Fig. 2). The probable ancestral host at node E was Ranunculaceae with likelihood values of 83.3% and 87.5% for the analyses with 28 and 35 species, respectively (Fig. 2). These relatively low values are likely due to the unstable position of the Asteraceae specialist *C. diodon* within this clade (in 49% of the trees sister to all members of clade E, in 51% sister to all members of clade E except *C. florisomne*). To circumvent this problem, we applied the "most common recent ancestor approach." The common ancestor of the four Ranunculaceae specialists (with or without *C. diodon*, depending on the tree sampled) was most likely a Ranunculaceae specialist (likelihood values 91.8% and 95.4% in analyses with 28 and 35 species, respectively). Similarly, the "most common recent ancestor approach" revealed that the specializations to each of the other pollen hosts had occurred only once except for the Asteraceae (Hydrophyllaceae: 99.5% and 99.5% in analyses with 28 and 35 species, respectively; Campanulaceae: 97.1% and 97.6%; Dipsacaceae: 99.5% and 99.6%). The most common ancestor of *C. ventrale* and *C. diodon* was unlikely to be an Asteraceae specialist (20.0% and 15.5%), but rather a Campanulaceae specialist (44.4% and 46.5%) indicating two independent switches away from the Campanulaceae. These two independent switches are further confirmed by likelihood comparisons of analyses with the ancestor of node C successively constrained to be specialized on either Campanulaceae, Asteraceae or Ranunculaceae (average likelihood values over 1002 trees −28.35, −32.24, and −32.39, respectively). As a difference of two log-units is conventionally taken as strong evidence (Pagel 1999), there is substantial support that the ancestor at node C was a Campanulaceae specialist. We did not infer the ancestral host of the ancestor of *C. philadelphi* and *C. lamellum* as the host-plant spectrum of the latter species is not definitely known. However, *Philadelphus*, the exclusive pollen host of *C. philadelphi* (Fig. 2, Table 3), is also a pollen host of *C. lamellum*, which strongly suggests that a specialization to *Philadelphus* had occurred only once.

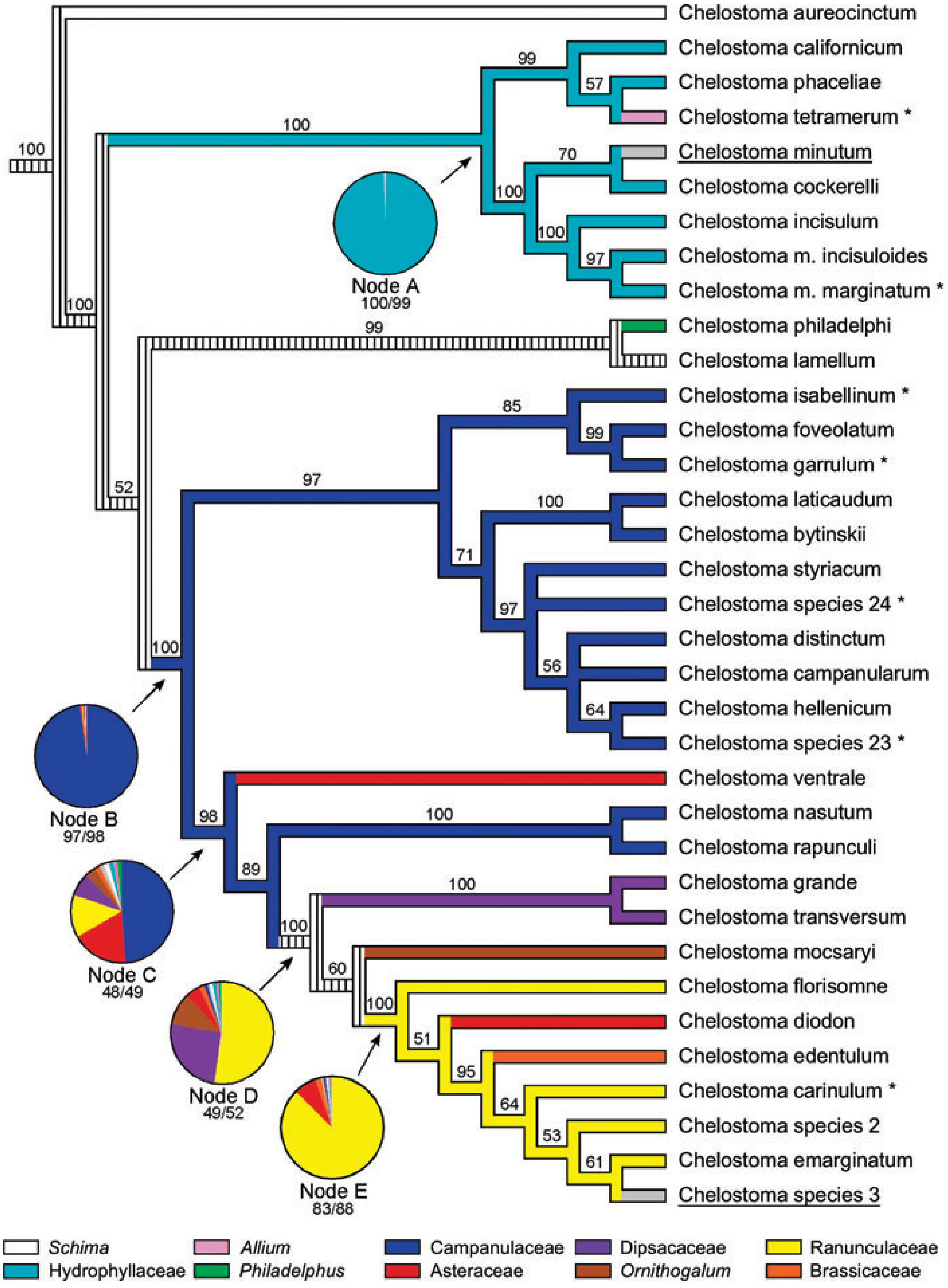

Figure 2. Majority-rule consensus tree of trees 5000–50,000 in the Bayesian analysis of the "supermatrix" including those seven *Chelostoma* species (indicated by asterisks) for which only the morphological dataset was available. Outgroup species are omitted from the figure. The floral hosts of the 33 oligolectic species are mapped onto the tree using the criterion of maximum parsimony. Both polylectic species (gray branches and underlined) as well as *Chelostoma lamellum*, whose pollen preferences are not definitely known, were coded as "missing data." The values at the five selected nodes A–E give the average probabilities of having the most-likely state at this node in maximum likelihood ancestral state reconstruction (left value: reconstruction with those 28 species for which both molecular and morphological data were available; right value: reconstruction with all 35 species included). The pie diagrams represent the ancestral state reconstructions for all 10 pollen hosts for each of the five nodes.

Discussion

PHYLOGENY

The present study provides a well-supported phylogenetic hypothesis for 35 *Chelostoma* species enabling the evolutionary reconstruction of host-plant associations within this bee genus on a worldwide scale. Our phylogeny differs from the current subgeneric classification of the genus *Chelostoma* (Michener 2007; see Table 3) in three respects. Most interestingly, these three divergences are strongly corroborated by floral host choice. First, the subgenus *Foveosmia* is not monophyletic as previously assumed but was found to consist of three distinct clades: a North American clade closely associated with flowers of the family Hydrophyllaceae, a Palearctic clade comprising all *Foveosmia* species specialized on Campanulaceae, and *C. ventrale*, an oligolege of the Asteraceae. Second, the North American *C. philadelphi* and the eastern Palearctic *C. lamellum* are closely related and visit the flowers of *Philadelphus* to collect pollen. Thus, their inclusion in the two different subgenera *Prochelostoma* and *Ceraheriades* is no longer justified. Third, *C. nasutum*, classified as a member of the subgenus *Chelostoma* s. str., is a member of the subgenus *Gyrodromella* and is a specialist of Campanulaceae as is its close relative *C. rapunculi*.

EVOLUTION OF HOST-PLANT CHOICE

The genus *Chelostoma* mainly consists of oligolectic species. Only two of the 35 taxa investigated were found to be pollen generalists. These two polylectic species evolved from oligolectic ancestors. In both species, the evolution of polylecty followed a distinct pattern. First, both species maintained the exclusive pollen host of their closest relatives in their polylectic diet (Hydrophyllaceae in *C. minutum*, Ranunculaceae in *C. species 3*). The fact that three of four transitions from oligolecty to polylecty in the western Palearctic anthidiine bees and seven of eight cases of host broadening in North American *Diadasia* species occurred under maintenance of the original pollen hosts (Müller 1996; Sipes and Tepedino 2005) indicates that this pattern of increase in diet breadth might be widespread in bees. Second, some of the additional pollen hosts incorporated into the diets of the two polylectic *Chelostoma* species are already used by closely related species. In *C. minutum*, the additional host *Allium* is the pollen source of *C. tetramerum*, whereas flowers of the Brassicaceae, one of the new hosts of *C. species 3*, are the exclusive pollen source of *C. edentulum*. Similarly, alternative host use in bees of the genus *Diadasia* (Emphorini) is strongly biased toward host families that are already exploited by other *Diadasia* or Emphorini species as primary hosts (Sipes and Tepedino 2005). Host switches constrained to plants that are used by related species were also found in phytophagous insects, e.g., in the beetle genus *Ophraella* (Futuyma et al. 1993, 1994, 1995) and in the butterfly tribe Nymphalini (Janz et al. 2001).

It has been repeatedly shown that floral specializations in bees are highly conserved, with sister species generally exploiting the same host (Müller 1996; Wcislo and Cane 1996; Michez et al. 2004; Sipes and Tepedino 2005; Minckley and Roulston 2006; Patiny et al. 2007; Larkin et al. 2008; Michez et al. 2008). Phylogenetically conserved host associations were also found in the genus *Chelostoma*. Except for two independent specializations to the Asteraceae, switches to all other host plant taxa happened only once. Most remarkable in this respect is the utilization of the same host, that is, *Philadelphus*, by both the North American *C. philadelphi* and the Chinese *C. lamellum*, indicating a floral host choice that might have been conserved for several million years after a dispersal event probably from the eastern to the western hemisphere had occurred (Hines 2008; Praz et al. in press). Thus, floral host choice in the genus *Chelostoma* does not appear to be a labile trait easily shaped by selective forces, as for example by flower supply or by interspecific competition. In fact, in southeastern Europe, up to five different *Chelostoma* species can be observed to simultaneously exploit the same *Campanula* patch together with several *Campanula* oligoleges of the genera *Andrena* and *Hoplitis* (C. Sedivy, C. Praz, A. Müller, unpubl. field data). This supports the view championed by Minckley and Roulston (2006) that, rather than restricting foraging to plants not exploited by other specialist bees, oligoleges are often specialized on widely used host plants where competition for pollen appears to be especially severe (Hurd and Linsley 1975; Hurd et al. 1980; Sipes and Wolf 2001; Minckley et al. 2000). Hence, oligolecty in bees seems to be maintained or selected for by specific plant traits rather than by the avoidance of interspecific competition alone.

The oligolectic *Chelostoma* species exploit the flowers of 10 different plant families, which belong to eight orders. These eight orders are distributed among all major angiosperm lineages from the more basal ones to the most derived ones (Soltis et al. 2005), that is, the monocots (Asparagales) and the eudicots, the latter including the Ranunculales, the rosids (Brassicales), the Cornales, the Ericales as well as both the euasterids I (Boraginales), and the euasterids II (Dipsacales, Asterales). Similarly, the host-plant taxa newly added by the two polylectic *Chelostoma* species are not related to their ancestral hosts (Asparagales in addition to Boraginales in *C. minutum*; Malvales and Brassicales in addition to Ranunculales in *C. species 3*). Host switches to distantly related hosts were also observed in bees of the genus *Diadasia* (Sipes and Tepedino 2005). These findings show that host shifts in bees do not necessarily involve switches to closely related plants and indicate that other factors than host-plant phylogeny might underlie floral host specialization.

Indeed, visual appearance is strikingly similar across flowers of several plant taxa exploited by bees of the genus *Chelostoma*. The flowers of many species among those host-plant taxa that have been newly incorporated by *C. species 3* into its polylectic diet (Cistaceae, Brassicaceae) are as brightly yellow as the flowers of *Ranunculus*, its presumed ancestral host. The multistaminate androecium of the flowers of both the Ranunculaceae and the Cistaceae additionally contributes to their highly similar visual appearance. Furthermore, the flowers of both *Schima* (Theaceae) and *Philadelphus* (Hydrangeaceae) are of similar size, have a conspicuous white corolla, and possess many yellow stamens. In addition, both taxa are shrubs or trees. Several genera among the Hydrophyllaceae (e.g., *Eriodictyon, Nama, Phacelia*) contain species characterized by distinctly bell-shaped and often blue- or purple-colored flowers that are surprisingly similar to those of the Campanulaceae, although the mechanism of pollen presentation is completely different in these two plant families. Our phylogeny does not reveal direct switches between *Schima* and *Philadelphus* and between Hydrophyllaceae and Campanulaceae. However, the support for the phylogenetic position of *C. philadelphi* and *C. lamellum* is weak (Figs. 1 and 2). Based on our morphological data alone (Appendix 4), these two species are more basal forming the sister group of all other *Chelostoma* species except *C. aureocinctum*, which is also supported by the plesiomorphic morphology of the labial palpus they have in common with *C. aureocinctum* (Michener 2007). A more basal position of *C. philadelphi* and *C. lamellum* would result in direct switches between *Schima* and *Philadelphus* and between Hydrophyllaceae and Campanulaceae. Vision is a key sensory modality in host-plant recognition in hymenopteran species including bees (Fischer et al. 2001; Giurfa and Lehrer 2001). Thus, the presented cases of a striking floral similarity in otherwise nonrelated hosts might point to an important role of floral shape, morphology, or color in directing the selection of new hosts in bees in general. This hypothesis is supported by the visually very similar but unrelated hosts of two closely related sister species of the anthidiine bees (Müller 1996). *Trachusa dumerlei* is a strict specialist of knapweeds and thistles (Asteraceae), whereas *Trachusa interrupta* exclusively collects pollen on Dipsacaceae. Both plant taxa have mostly red- to blue-colored flowers that are concentrated into compact inflorescences resulting in a very similar visual appearance. The use of visually similar but unrelated floral hosts is also found in pollen specialist bees of the genera *Macrotera* and *Diadasia* (Danforth 1996; Sipes and Tepedino 2005). In both genera, several species exploit the similarly shaped and colored flowers of some Cactaceae (e.g., *Opuntia*) or Malvaceae (e.g., *Sphaeralcea*), respectively.

EVOLUTIONARY CONSTRAINTS

Several findings detailed above indicate that evolutionary constraints have strongly influenced host-plant choice in bees of the genus *Chelostoma*. First, the increase in diet breadth in the two polylectic *Chelostoma* species appears to have been far from an accidental process. Its distinct pattern suggests that the newly added hosts might necessitate similar physiological or neurological (including cognitive) capabilities to cope with as the ancestral host, or that these capabilities were inherited from a common ancestor. As a result, only a limited set of flowers may fulfill the bees' requirements, which may explain why the two polylectic *Chelostoma* species included hosts into their diets already used by closely related species. Second, the highly conserved floral specializations found in the genus *Chelostoma* as well as in many other bee lineages indicate difficulties in escaping from the oligolectic habit. Third, the selection of unrelated hosts with a striking floral similarity suggests that bees of the genus *Chelostoma* might be neurologically limited to exploit or detect flowers of significantly differing shapes, morphologies, or colors.

The constraints acting on host range in bees of the genus *Chelostoma* may be classified into two types: physiological constraints related to pollen digestion and neurological constraints related to the recognition or handling of flowers. Evidence for both types of constraints comes from rearing experiments conducted with *C. rapunculi* and *C. florisomne* (Praz et al. 2008). *Chelostoma rapunculi*, which exclusively collects pollen on Campanulaceae, failed to develop on four different diets of non-host pollen, namely *Buphthalmum* (Asteraceae), *Ranunculus* (Ranunculaceae), *Sinapis* (Brassicaceae), and *Echium* (Boraginaceae), suggesting a strong limitation in host range associated with pollen digestion. The first three of these four pollen hosts are exploited by members of the subgenus *Chelostoma* s. str., which indicates that these species have evolved physiological adaptations to successfully use them. Our data suggest that this physiological constraint has been overcome two times independently, once in *C. ventrale* and once in the ancestor of *Chelostoma* s. str (node D in Fig. 2). In the latter case, the path was open to specialize on several other hosts, such as Brassicaceae, Dipsacaceae, *Ornithogalum*, and *Ranunculus*. In contrast, *C. florisomne*, which is strictly specialized on Ranunculaceae, was found to be able to develop on two nonhost pollen diets, namely *Campanula* and *Brassica* (Praz et al. 2008). Our study shows that none of the members of the subgenus *Chelostoma* s. str, including *C. florisomne*, exploit Campanulaceae for pollen, although they evolved from ancestors that were oligolectic on this plant family (nodes B and C in Fig. 2). This finding clearly points to constraints that are not related to nutrition but rather to host recognition or flower handling. Such information-processing constraints are actually assumed to be the reason why the solitary bee *Heriades truncorum* refused to harvest pollen on *Campanula* and *Echium* in the absence of its specific host, the Asteraceae, although both types of nonhost pollen support larval development (Praz et al. in press). Neurological constraints might explain why related species in the genus *Chelostoma* as well as in other groups of bees tend to specialize on flowers that are similar in shape, morphology, or color. In fact, there is evidence that adult bees have limited memory

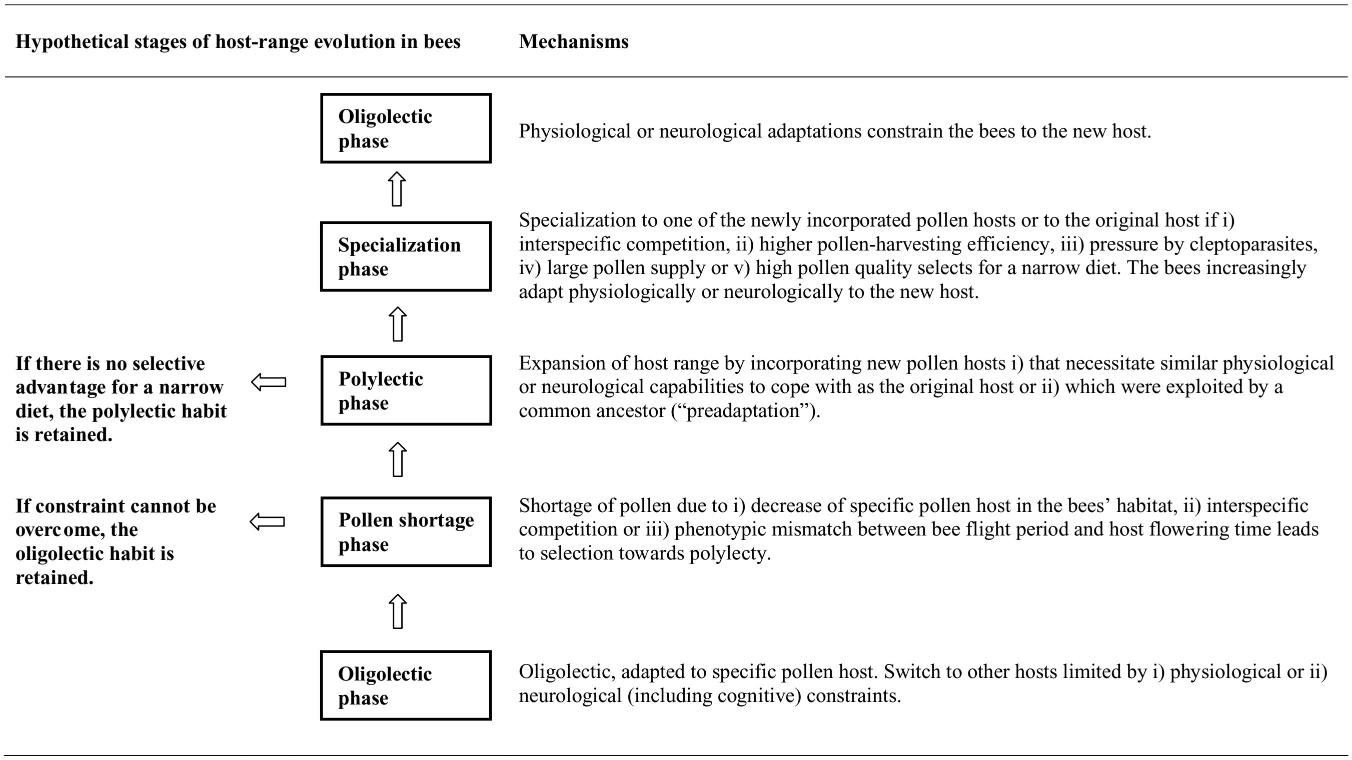

Figure 3. The constraint hypothesis of host-range evolution in bees.

and learning capacities for shapes and colors (Betrays and Wcislo 1994; Chittka et al. 2001; Giurfa and Lehrer 2001).

THE CONSTRAINT HYPOTHESIS

The results of our study strongly support the hypothesis that oligolecty in the genus *Chelostoma* is evolutionarily constrained. Based on this finding, we propose a new hypothesis on the evolution of host range in bees (Fig. 3). This constraint hypothesis is related to the oscillation hypothesis of host-plant range recently postulated for herbivorous insects (Janz and Nylin 2008). Indeed, patterns of host use in *Chelostoma* as well as in other bee lineages display striking similarities to those of phytophagous insects (Sipes and Tepedino 2005; Müller and Kuhlmann in press).

Our constraint hypothesis distinguishes five consecutive stages of host-range evolution (Fig. 3). *Oligolectic phase*: Numerous oligolectic bee species appear to be highly adapted to their specific hosts and, as shown above, are probably constrained by physiological or neurological limitations (including vision or possibly also olfaction as cognitive sensory modalities) rendering switches to or incorporations of other hosts difficult. In fact, many host-specific herbivorous insects were found to be physiologically adapted to the secondary chemistry of their host plants, but less adapted to utilizing other hosts (Slansky 1993; Strauss and Zangerl 2002; Cornell and Hawkins 2003; Singer 2008). In other phytophagous insects, limited information-processing abilities, that is, neurological or cognitive constraints, are assumed to underlie host-plant specialization (Bernays 1998, 2001; Egan and Funk 2006). *Pollen shortage phase*: The quantitative pollen requirements of bees are enormous (Schlindwein et al. 2005; Müller et al. 2006). Therefore, as soon as pollen becomes limiting, strong selection pressure should act on oligolectic bees to reduce their heavy dependence upon a limited number of pollen hosts. *Polylectic phase*: If the constraint cannot be overcome in a period of pollen shortage, the oligolectic habit will be retained. If, however, an alternative pollen host is available that demands similar physiological or neurological abilities to cope with as the original host or that is suited because the bees inherited the machinery to successfully use it from a common ancestor, it may become incorporated into the diet under maintenance of the original host. Evidence for this process comes from butterflies of the tribe Nymphalini, for which *Urtica* is probably the ancestral host (Janz et al. 2001). Most of the tested species still showed the capacity to feed on *Urtica*, regardless of their actual host plant. *Specialization phase*: If there is no selective advantage for a narrow diet, bees will retain or even broaden the polylectic habit. If, however, a narrow diet is selected for, bees are expected to either respecialize on the original host if pollen shortage is no longer prevailing or to increasingly adapt to the new host. Respecialization was indeed found to occur in herbivorous insects. In butterflies of the tribe Nymphalini, clear tendencies were observed to respecialize on either the original host or on one of the newly incorporated hosts after a phase of expanded host range (Janz et al.

2001; Weingartner et al. 2006). *Oligolectic phase*: Physiological or neurological adaptations again constrain the bees to the new host.

The selective forces acting during the postulated phase of expanded host-range (Box "polylectic phase" in Fig. 3) decide whether a bee species will keep or further broaden its polylectic habit, or whether it will respecialize. Indications exist that many bee species currently are in this transitional stage. Numerous polylectic bee species show a striking preference for a distinct plant clade (Müller 1996; Müller and Kuhlmann in press), which suggests that they may, in future, either consolidate their polylectic habit or become strictly specialized. Similarly, the flexibility of several specialized bee species to switch hosts in the absence of their usual hosts (e.g., Michener and Rettenmeyer 1956; Westrich 2008) may point to their capability to become either fully polylectic or strictly oligolectic, depending on the direction of selection. Most important, the phylogenetic traces of a host-range expansion can be lost with time, that is, a period of expanded host range followed by specialization on one of the new hosts will in retrospect appear as a clean host switch, once all traces of the additional hosts are lost (Janz and Nylin 2008). This probably also applies to the 10 seemingly clean host switches in the genus *Chelostoma*, which are strongly expected to have proceeded over a shorter or longer transitional phase of expanded host range.

In conclusion, host-plant choice in bees appears to be a dynamic process enabling transitions both from a narrower to a broader diet and vice versa (Waser et al. 1996; Sipes and Tepedino 2005). However, floral host choice does not appear to be a highly flexible trait that can be easily changed by selective forces. Instead, it appears to be evolutionarily much more constrained than hitherto thought.

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Appendix 1: Host-plant preferences of bees of the genus *Chelostoma* based on the literature and on unpublished field data.

Subgenus and species	Literature and unpublished field data
Subgenus *Chelostoma*	
Chelostoma emarginatum	oligolectic on *Ranunculus* (Amiet et al. 2004)
Chelostoma florisomne	oligolectic on *Ranunculus* (Westrich 1989; Amiet et al. 2004)
Chelostoma grande	oligolectic on Dipsacaceae (Westrich 1993; Amiet et al. 2004)
Chelostoma mocsaryi	oligolectic on *Ornithogalum* (Gogala 1999; C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Italy, France and Greece)
Chelostoma transversum	oligolectic on Dipsacaceae (C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Greece)
Subgenus *Foveosmia*	
Chelostoma bytinskii	oligolectic on *Campanula* (C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Jordan)
Chelostoma californicum	oligolectic on *Phacelia* (Moldenke and Neff 1974; Krombein et al. 1979, T. Griswold in litt.)
Chelostoma campanularum	oligolectic on *Campanula* (Westrich 1989; Amiet et al. 2004)
Chelostoma cockerelli	oligolectic on *Eriodictyon* (Moldenke and Neff 1974; Krombein et al. 1979, T. Griswold in litt.)
Chelostoma distinctum	oligolectic on *Campanula* (Westrich 1989; Amiet et al. 2004)
Chelostoma foveolatum	oligolectic on *Campanula* (Amiet et al. 2004)
Chelostoma hellenicum	oligolectic on *Campanula* (K. Standfuss in litt., C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Greece)
Chelostoma incisulum	oligolectic on *Phacelia* (Moldenke and Neff 1974; Krombein et al. 1979, T. Griswold in litt.)
Chelostoma laticaudum	oligolectic on *Campanula* (K. Standfuss in litt., C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Greece)
Chelostoma m. marginatum	oligolectic on Hydrophyllaceae (Krombein et al. 1979; Moldenke and Neff 1974)
Chelostoma m. incisuloides	oligolectic on Hydrophyllaceae (Krombein et al. 1979; Moldenke and Neff 1974)
Chelostoma minutum	oligolectic on *Phacelia* (Moldenke and Neff 1974), polylectic on *Phacelia*, *Allium* and *Sedum* (Parker 1988)
Chelostoma nasutum	oligolectic on *Campanula* (K. Standfuss in litt., C. Sedivy, C. J. Praz and A. Müller unpubl. field data from Greece)
Chelostoma phaceliae	oligolectic on *Phacelia* (Moldenke and Neff 1974; Krombein et al. 1979, T. Griswold in litt.)
Chelostoma species 23	oligolectic on *Campanula* (A. Müller unpubl. field data from Rhodos)
Chelostoma styriacum	oligolectic on *Campanula* (Standfuss in litt., C. Sedivy and C. J. Praz unpubl. field data from Greece)
Subgenus *Gyrodromella*	
Chelostoma rapunculi	oligolectic on *Campanula* (Westrich 1989; Amiet et al. 2004)
Subgenus *Prochelostoma*	
Chelostoma philadelphi	oligolectic on *Philadelphus* (Michener 2007)

Appendix 2: Morphological character and character states used in the cladistic analysis of the genus *Chelostoma*. If not otherwise stated, the characters refer to both sexes. The terminology of bee morphology follows Michener (2007).

(1) Vertex with a distinct preoccipital ridge developed at least medially (1); rounded, without a distinct preoccipital ridge (0).
(2) Antenna of male reddish-colored (1); uniformly dark-colored (0).
(3) Antennal segment 3 of male with long and erect bristle-like hairs (1); only microscopically haired (0).
(4) Antennal segment 3 of male 1.5× to 2× as long as pedicel (1); shorter to slightly longer than pedicel (0).
(5) Base of labrum of female strongly bent (1); more or less straight (0).
(6) Apex of labrum of female tripartite, with a distinct projection on both sides of the median projection (1); rounded, truncated or emarginate (0).
(7) Hypostomal carina of male forming a distinct tooth (1); straight to evenly rounded (0).
(8) Lower side of mandible of male with a distinct pilosity, which is as long and nearly as dense as that on the adjacent genal area (1); with a less distinct pilosity composed of rather short and scattered hairs (0).
(9) Inner margin of mandible of female more or less straight without a prominent tooth behind the apical two teeth (1); with a prominent, triangular tooth (0).
(10) Segment 3 of labial palpus flattened, its axis a continuation of that of segment 2 (1); not flattened, its axis directed laterally (0).

Continued

Appendix 2: Continued

(11) Number of segments of maxillary palpi: 4 (1); 3 (0).

(12) Parapsidal line short, less than 40% of length of tegula (1); long, more than 50% of length of tegula (0).

(13) Pronotal lobe of female rounded all around (1); slightly keeled or bulging at its base (0).

(14) Tegula of female punctured only apically and along inner margin (1); densely punctured across its whole surface (0).

(15) Jugal lobe connate to the hindwing for less than two-thirds of its length (1); connate to the hindwing for two-thirds of its length or more (0).

(16) Hind coxa with a carina along inner ventral margin (1); not carinate (0).

(17) Posterior margin of basal area of propodeum keeled or thickened along its whole width (1); rounded or only laterally keeled (0).

(18) Propodeum densely chagreened, dull (1); polished (0).

(19) Apical hair fringes on terga of female strongly developed (2); weakly developed (1); absent (0).

(20) Metasomal tergum 7 of male with dorsal pit (1); without dorsal pit (0).

(21) Metasomal tergum 7 of male evenly rounded (0); of different shape (1).

(22) Metasomal tergum 7 of male a single rounded projection (1); of different shape (0).

(23) Metasomal tergum 7 of male with two broad and truncated teeth (1); of different shape (0).

(24) Metasomal tergum 7 of male with two pointed teeth, incision about as broad as one tooth (1); of different shape or incision much broader or much narrower than one tooth (0).

(25) Metasomal tergum 7 of male three-toothed (1); of different shape (0).

(26) Metasomal tergum 7 of male four-toothed (1); of different shape (0).

(27) Median teeth of the four-toothed metasomal tergum 7 of male fused (1); not fused or absent (0).

(28) Lateral tooth of metasomal tergum 7 of male distinctly curved inwards (1); not curved inwards or absent (0).

(29) Scopal hairs of female distinctly tapered toward the apex (1); apically blunt (0).

(30) Scopal hairs of female with spiral swellings clearly visible at a magnification of 400× (1); with smooth surface (0).

(31) Scopal hairs of female with short side-branches (1); unbranched (0).

(32) Metasomal sternum 2 of male with a distinct median elevation or a hump (1); without projection or hump (0).

(33) Median elevation on metasomal sternum 2 of male half-elliptically shaped (1); of another shape (0).

(34) Median elevation on metasomal sternum 2 of male distinctly concave (1); not distinctly concave (0).

(35) Base of metasomal sternum 3 of male densely covered with plumose hair (1); without a dense cover of plumose hair (0).

(36) Metasomal sternum 3 of male with two spots of black bristles developed near the center of the sternum (3); between the center and the apical margin (2); at the apical margin (1); black bristles lacking (0).

(37) Membraneous flaps at apical margin of metasomal sternum 4 of male nearly as long as disc of sternum 4 or longer (1); half as long as disc of sternum 4 or less (0).

(38) Plumose hair on metasomal sternum 4 of male densely covering the whole sternal surface (3); loosely covering the sternal surface (2); developed only lateroapically (1); absent (0).

(39) Apical margin of metasomal sternum 4 of male with a dense and uninterrupted fringe of hairs, which are bent at right angles to the sternal surface (2); with a dense but medially interrupted fringe of such hairs (1); without a fringe of such hairs (0).

(40) Apical margin of metasomal sternum 5 of male apically fringed (1); not fringed (0).

(41) Lateral margin of metasomal sternum 5 of male lifted and distinctly keeled, at least in the apical half (1); flat and normally rounded (0).

(42) Hair comb at the apical margin of metasomal sternum 5 of male dense, short and developed along the whole sternal width (slightly interrupted medially in *Chelostoma hellenicum*) (1); of other shape or absent (0).

(43) Apical margin of metasomal sternum 5 of male with a bowl-shaped comb of hairs (1); different (0).

(44) Comb hairs at apex of metasomal sternum 5 of male shaped like a pearl necklet (3); wavy (2); zigzagged (1); of other shape or absent (0).

(45) Apex of metasomal sternum 6 of male carinate laterally, resulting in a triangular to rounded projection (1); of different shape (0).

(46) Apical margin of sternum 8 of male truncated to slightly emarginate, with a tuft of hairs medially (1); of other shape and without a median tuft of hairs (0).

(47) Gonostylus apically clubbed and beset with long hairs on inner and outer side (2); apically clubbed and hairless or only microscopically haired (1); apically slender (0).

(48) Inner margins of penis valves distinctly divergent (1); more or less parallel, lying close together (0).

Appendix 3: **Morphological data matrix used in the cladistic analysis of the genus *Chelostoma*. Unknown states are coded as "?" and polymorphic states as "P".**

Chelostoma philadelphi	000001000000001001000100000000000100000000000000000
Chelostoma phaceliae	0000000001P0010100211000100100010000000100000000
Chelostoma californicum	000000000100010100211000100100010000000100000000
Chelostoma cockerelli	0000000001000101000110000101000100000000100000?00
Chelostoma minutum	00000000010001010001100001010001000000001000000100
Chelostoma m. marginatum	0000000011000101000110000101000100000000100020000
Chelostoma m. incisuloides	000000001100010100011000011100010000000010002 0?00
Chelostoma tetramerum	0000000011100101001110001001000100000000100000000
Chelostoma incisulum	010000001100010100011000011100010000000010000 0?00
Chelostoma aureocinctum	000000000010000100200000000000000000000000100030000
Chelostoma ventrale	10000000010001011021100100001101000000000100000001
Chelostoma diodon	10001001110101110021100000001101000202011 0000120
Chelostoma edentulum	011100011101011100211010000011011113031110101120
Chelostoma emarginatum	011100011101011100211010000011011113031110101120
Chelostoma species 3	011100010101011100211010000011011113031110101120
Chelostoma florisomne	011100011101011111211010000010111103032110131120
Chelostoma carinulum	011100011101011101211010000011011113031110101120
Chelostoma species 2	011100011101011110211010000011011110031110101110
Chelostoma transversum	010110111111011100211010000011010011110110130120
Chelostoma grande	010110111111011100211010000011010011110110131120
Chelostoma mocsaryi	111100010101011110211000000011010112031110101120
Chelostoma rapunculi	1000000001000101002110000000110100010001 10110011
Chelostoma nasutum	1000000001000111002110100000110100010001 10110111
Chelostoma foveolatum	00000000110001010001110000001101000000000000000000
Chelostoma laticaudum	0000000001001101001111000000110100000000100000001
Chelostoma garrulum	00000000110001010001110000001101000000000000000000
Chelostoma bytinskii	0000000001001101001011000000110100000000000000001
Chelostoma isabellinum	00000000110001010001100000001101000000000100000000
Chelostoma species 24	00000000010001011011100100001101000000000100000000
Chelostoma campanularum	00000000010001011001100100001111000000000101000000
Chelostoma distinctum	00000000010001011001100100001101000000000100000000
Chelostoma hellenicum	00000000010001011001100100001101000000000101000000
Chelostoma styriacum	00000000010001011001100100001101000000000100000000
Chelostoma species 23	00000000010001011001100100001101000000000101000000
Chelostoma lamellum	00000100101001010021100000000101000000000000000000

Appendix 4: Strict consensus tree of 385 equally parsimonious tree in equal weights parsimony analysis of the morphological dataset.