

Pack hunting by minute soil testate amoebae: nematode hell is a naturalist's paradise

Edward A. D. Mitchell*

Laboratory of Soil Biology, University of Neuchâtel,
Neuchâtel CH-2000, Switzerland.

We may be experiencing a new golden era in protistology in which classical microscopy methods are combined with the latest developments in high throughput sequencing. This however will only be possible if strong expertise in the two fields is combined. This is the case in a new study by Geisen and colleagues (2015b) who report a little-documented and quite unlikely behaviour of soil protozoa: pack hunting. Geisen and colleagues (2015b) show that the organisms responsible for this hitherto unknown behaviour are very common in soils – and thus so is this function.

Pack hunting is well known in large animals such as wolves and lions and has fascinated generations of biologists as well as the general public. This behaviour involves complex cognitive abilities and often reveals the existence of a complex social structure. This is of course made possible by the evolution of a complex brain and associated sensory organs used to detect the prey and communicate among members of the predator community.

Protozoa are a well-known group of soil organisms and their roles as microbial grazers has long been established (Sandon, 1927). Yet since the early works of pioneers of protistology (Leidy, 1879; Penard, 1902) and despite numerous studies documenting their incredible phylogenetic (Lara *et al.*, 2007), taxonomic – albeit intense debate – (Foissner, 1997; 1999; Finlay *et al.*, 2001; Bass *et al.*, 2007) and functional diversity (Bonnet, 1964; Foissner, 1987; Ekelund and Rønn, 1994; Coûteaux and Darbyshire, 1998; Adl, 2003), including the increasing recognition of the importance of parasitism (De Vargas *et al.*, 2015; Geisen *et al.*, 2015a), this diversity is still ignored by most biologists including soil microbiologists. Indeed, many perhaps most scientists in these

fields recognize the existence of different broad categories, as flagellates, ciliates and amoebae, but then still lump them mostly into one functional group: bacterial feeders.

Research efforts on the major groups of soil organisms identified as 'bacteria', 'fungi', 'protozoa' and 'invertebrates' are strongly imbalanced as attested by the results of a search in the ISI Web of Science database (11.07.2015: soil AND bacteria = 53 782 hits, soil AND fungi = 33 485, soil AND protozoa = 1797, and soil AND invertebrate = 3290). Although these numbers could be refined, they clearly show that research efforts on bacteria and fungi are more than one order of magnitude higher than that on protozoa. Soil protozoologists often feel this gap, including when trying to get their share of attention at meetings and when trying to publish their work in high-ranking journal. Yet there are also clear advantages of working in a comparatively under-studied research field, and among these is the relatively high likelihood of making important discoveries.

Cryptodiffugia is a genus of minute (mostly 15–20 µm) testate amoebae described 125 years ago by Eugène Penard (1890), a pioneer protistologist who in addition to describing numerous species reported extensively on their behaviour. The biology of the genus has been studied using clonal cultures, but these used bacteria as a food source (Page, 1966; Hedley *et al.*, 1977), and thus until now the ability of this species to engage in coordinated group hunting of micro-metazoans had gone unnoticed.

Towards the end of his life, Penard published a series of papers on 'protozoa and psychology' in which he reflected that 'protozoa know what they are doing' (Penard, 1940a,b,c,d). He did not go as far as implying that they had the same capacity as animals possessing a complex brain, but over his very long career he had seen enough examples of behaviour ('instinct', capacity to respond rapidly to positive and negative stimuli, etc.) to realize that they possessed at least some basic sensory capacity. The full nature and mechanisms involved however remained largely unknown and this still represents a fascinating and wide-open field of research.

*For correspondence. E-mail edward.mitchell@unine.ch; Tel. +41 (0)32 718 23 45; Fax +41 (0)32 718 30 01.

I started this comment with the suggestion that we may be experiencing a new golden era in protistology and this has been a growing feeling in the last decade (Corliss, 2004; Clarholm, 2005). The study of Geisen and colleagues (2015b) illustrates perfectly the kind of major finding that can be done if one spends time looking at these organisms under the microscope to better characterize how they interact with other microorganisms and thus to better understand their true ecological function. Such careful observation of living organisms combined with other classical (light and electron microscopy, cultures) and newly developed (e.g. high throughput sequencing, whole genome analyses) tools will certainly force biologists to reconsider the common assumption that soil protozoa are simple organisms that can all be lumped into a single functional category.

With the exponential increase in molecular data showing the true extent of protist diversity in soil as well as in other habitat (Pawlowski *et al.*, 2012; Heger *et al.*, 2013; Geisen *et al.*, 2014; Lentendu *et al.*, 2014; De Vargas *et al.*, 2015; Singer *et al.*, 2015), we urgently need to substantially increase the number of trained protistologists. Expert protistologists are indeed essential to correctly interpret these data (I insist on 'correctly', as many gross errors are being published due to uncritical assignments of high throughput sequencing data using existing databases that suffer from huge gaps in phylogenetic coverage and contain many errors that only specialists can detect). Only experts with an intimate knowledge of the organisms will be able to conduct the kind of basic biological and ecological observations that have allowed Geisen and colleagues to make a quite stunning discovery. At the institutional level, the challenge is now also for research institutes and funding agencies to provide the opportunities for these enthusiastic and excellent young researchers to develop the ambitious research programs needed to make this golden era of protistology a reality.

References

- Adl, S.M. (2003) *The Ecology of Soil Decomposition*. Oxon, UK: CABI Publishing.
- Bass, D., Richards, T.A., Matthai, L., Marsh, V., and Cavalier-Smith, T. (2007) DNA evidence for global dispersal and probable endemicity of protozoa. *BMC Evol Biol* **7**: 13.
- Bonnet, L. (1964) Le peuplement thécamobiens des sols. *Revue d'Écologie et de Biologie du Sol* **1**: 123–408.
- Clarholm, M. (2005) Soil protozoa: an under-researched microbial group gaining momentum. *Soil Biol Biochem* **37**: 811–817.
- Corliss, J.O. (2004) Why the world needs protists! *J Eukaryot Microbiol* **51**: 8–22.
- Coûteaux, M.M., and Darbyshire, J.F. (1998) Functional diversity amongst soil protozoa. *Appl Soil Ecol* **10**: 229–237.
- De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., *et al.* (2015) Eukaryotic plankton diversity in the sunlit global ocean. *Science* **348**: 1261605–1–1261605–11.
- Ekelund, F., and Rønne, R. (1994) Notes on protozoa in agricultural soil with special emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiol Rev* **15**: 321–353.
- Finlay, B.J., Esteban, G.F., Clarke, K.J., and Olmo, J.L. (2001) Biodiversity of terrestrial protozoa appears homogeneous across local and global spatial scales. *Protist* **152**: 355–366.
- Foissner, W. (1987) Soil protozoa: fundamental problems, ecological significance, adaptation in ciliates and testaceans, bioindicators, and guide to the literature. *Prog Protozool* **2**: 69–212.
- Foissner, W. (1997) Global soil ciliate (Protozoa, Ciliophora) diversity: a probability-based approach using large sample collections from Africa, Australia and Antarctica. *Biodivers Conserv* **6**: 1627–1638.
- Foissner, W. (1999) Protist diversity: estimates of the near-imponderable. *Protist* **150**: 363–368.
- Geisen, S., Fiore-Donno, A.M., Walochnik, J., and Bonkowski, M. (2014) Acanthamoeba everywhere: high diversity of *Acanthamoeba* in soils. *Parasitol Res* **113**: 3151–3158.
- Geisen, S., Laros, I., Vizcaíno, A.N., Bonkowski, M., and de Groot, G.A. (2015a) Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. *Mol Ecol* **24**: 4556–4569.
- Geisen, S., Rosengarten, J., Koller, R., Mulder, C., Ulrich, T., and Bonkowski, M. (2015b) Pack hunting by the widespread soil amoeba *Cryptodiffugia operculata* on nematodes. *Environ Microbiol* [Epub ahead of print]. DOI: 10.1111/1462-2920.12949.
- Hedley, R.H., Ogden, C.G., and Mordan, N.J. (1977) Biology and fine structure of *Cryptodiffugia oviformis* (Rhizopodea: Protozoa). *Bull Br Mus (Natural History) Zool* **30**: 311–328.
- Heger, T.J., Mitchell, E.A.D., and Leander, B.S. (2013) Holarctic phylogeography of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida) reveals extensive genetic diversity explained more by environment than dispersal limitation. *Mol Ecol* **22**: 5172–5184.
- Lara, E., Berney, C., Ekelund, F., Harms, H., and Chatzinotas, A. (2007) Molecular comparison of cultivable protozoa from a pristine and a polycyclic aromatic hydrocarbon polluted site. *Soil Biol Biochem* **39**: 139–148.
- Leidy, J. (1879) *Freshwater Rhizopods of North America*. Washington, DC, USA: United States Geological Survey of the Territories.
- Lentendu, G., Wubet, T., Chatzinotas, A., Wilhelm, C., Buscot, F., and Schlegel, M. (2014) Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. *Mol Ecol* **23**: 3341–3355.
- Page, F.C. (1966) *Cryptodiffugia operculata* n. sp. (Rhizopodea – Arcellinida, Cryptodiffugiidae) and status of genus *Cryptodiffugia*. *Trans Am Microsc Soc* **85**: 506–515.
- Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., *et al.* (2012) CBOL Protist Working Group:

- barcoding eukaryotic richness beyond the animal, plant and fungal kingdom. *PLoS Biol* **10**: e1001419.
- Penard, E. (1890) Études sur les Rhizopodes d'eau douce. *Mémoires de la Société de Physique et d'Histoire Naturelle de Genève* **31**: 1–230.
- Penard, E. (1902) *Faune Rhizopodique du Bassin du Léman*. Genève, Switzerland: Kündig.
- Penard, E. (1940a) Protozoaires et psychologie. *Archives des Sciences Physiques et Naturelles de Genève* **22**: 179–200.
- Penard, E. (1940b) Protozoaires et Psychologie. *Archives des Sciences Physiques et Naturelles de Genève* **22**: 160–175.
- Penard, E. (1940c) Protozoaires et Psychologie. *Archives des Sciences Physiques et Naturelles de Genève* **22**: 203–226.
- Penard, E. (1940d) Protozoaires et Psychologie. *Archives des Sciences Physiques et Naturelles de Genève* **22**: 265–289.
- Sandon, H. (1927) *The Composition and Distribution of the Protozoan Fauna of the Soil*. Edinburgh, UK: Oliver and Boyd.
- Singer, D., Kosakyan, A., Pillonel, A., Mitchell, E.A.D., and Lara, E. (2015) Eight species in the *Nebela collaris* complex: *Nebela gimlii* (Arcellinida, Hyalospheniidae), a new species described from a Swiss raised bog. *Eur J Protistol* **51**: 79–85.