Protist, Vol. 166, 271–295, May 2015
http://www.elsevier.de/protis

Protist

ORIGINAL PAPER

Expansion of the ‘Reticulosphere’: Diversity of Novel Branching and Network-forming Amoebae Helps to Define Variosea (Amoebozoa)

Cédric Berney[a,1,2], **Stefan Geisen**[b,3], **Jeroen Van Wichelen**[c], **Frank Nitsche**[d], **Pieter Vanormelingen**[c], **Michael Bonkowski**[b], and **David Bass**[a,4]

[a]Department of Life Sciences, The Natural History Museum, Cromwell Road,
London SW7 5BD, United Kingdom
[b]Department of Terrestrial Ecology, Zoological Institute, University of Cologne,
Zülpicher Str. 47b, 50674 Cologne, Germany
[c]Research Unit Protistology and Aquatic Ecology, Biology Department, Ghent University,
Krijgslaan 281 (S8), 9000 Gent, Belgium
[d]Department of General Ecology, Zoological Institute, University of Cologne, Zülpicher Str.
47b, 50674 Cologne, Germany

Submitted September 3, 2014; Accepted April 6, 2015
Monitoring Editor: Sandra L. Baldauf

Amoebae able to form cytoplasmic networks or displaying a multiply branching morphology remain very poorly studied. We sequenced the small-subunit ribosomal RNA gene of 15 new amoeboid isolates, 14 of which are branching or network-forming amoebae (BNFA). Phylogenetic analyses showed that these isolates all group within the poorly-known and weakly-defined class Variosea (Amoebozoa). They are resolved into six lineages corresponding to distinct new morphotypes; we describe them as new genera *Angulamoeba* (type species *Angulamoeba microcystivorans* n. gen., n. sp.; and *A. fungorum* n. sp.), *Arboramoeba* (type species *Arboramoeba reticulata* n. gen., n. sp.), *Darbyshirella* (type species *Darbyshirella terrestris* n. gen., n. sp.), *Dictyamoeba* (type species *Dictyamoeba vorax* n. gen., n. sp.), *Heliamoeba* (type species *Heliamoeba mirabilis* n. gen., n. sp.), and *Ischnamoeba* (type species *Ischnamoeba montana* n. gen., n. sp.). We also isolated and sequenced four additional variosean strains, one belonging to *Flamella*, one related to *Telaepolella tubasferens*, and two members of the cavosteliid protosteloid lineage. We identified a further 104 putative variosean environmental clone sequences in GenBank, comprising up to 14 lineages that may prove to represent additional novel morphotypes. We show that BNFA are phylogenetically widespread in Variosea and morphologically very variable, both within and between lineages.

Key words: Network-forming amoebae; reticulate; Variosea; Amoebozoa; molecuar phylogeny; SSU rDNA.

---

[1] present address: UMR7144, groupe EPEP, CNRS - Station Biologique, Place Georges Teissier, 29680 Roscoff, France
[2] Corresponding author; e-mail cedric.berney@sb-roscoff.fr (C. Berney).
[3] present address: Department of Terrestrial Ecology, Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands
[4] present address: Centre for Environment, Fisheries & Aquaculture Science, Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom

http://dx.doi.org/10.1016/j.protis.2015.04.001
1434-4610/

Introduction

Naked heterotrophic reticulose and branching amoebae, characterized by more or less thin cytoplasmic extensions from, or as part of, their cell bodies, occur in several places across the eukaryote tree of life. The appearance of the cytoplasmic extensions varies considerably. In some lineages parts of the cells are able to anastomose, connecting their cytoplasm, whereas in others this never happens. While this characteristic is known for some taxa, in general the distinction is difficult to make because very little is known about most lineages. Some highly branching amoebae are not known to form networks (e.g. *Mesofila*; Bass et al. 2009), whereas others appear to form networks under some conditions but perhaps not others. Where networks occur they can be very fine, with or without distinct cell bodies (e.g. *Filoreta* and *Reticulamoeba*; Bass et al. 2009, 2012), or more compact with proportionally smaller lacunae (e.g. *Leptomyxa*, *Protomyxa*, and some vampyrellids; Berney et al. 2013; Goodey 1914; Hess et al. 2012; Rhumbler 1904; Smirnov et al. 2008), sometimes resembling 'sheets' of cytoplasm within which lacunae may occur, e.g. *Thalassomyxa* (Berney et al. 2013; Grell 1985). In these cases lacunae are often formed by cleavage of the cytoplasm into separate streams, so there is no clear distinction between cell body and cytoplasmic network. A large range of variants of the organization of the cell body (hereafter referred to as 'morphotypes') can be found between these main forms.

Despite their morphological distinctiveness, these branching and network-forming amoebae (BNFA) are remarkably poorly known. Ultrastructural and molecular phylogenetic studies have revealed that BNFA can be found in many places across the eukaryotic tree of life within major groups such as Amoebozoa, Rhizaria, and Stramenopiles. However, there are also many such amoebae described in the literature for which sequence data are not yet available (e.g. Adl et al. 2012; Bass et al. 2009, 2012; Lee et al. 2000). Table 1 lists formally described BNFA genera from the literature from before the advent of DNA sequencing (excluding those known to belong to the well characterized and in several ways distinct Mycetozoa and testate Foraminifera, as well as photosynthetic species such as chlorarachniophytes). This list is not exhaustive, but highlights two significant facts: (1) molecular data are still missing for most of these BNFA, and (2) many BNFA isolated and sequenced more recently were morphologically distinct enough from these characterised genera to be described as new taxa, for example *Acramoeba* (Smirnov et al. 2008), *Filoreta* (Bass et al. 2009), and *Telaepolella* (Lahr et al. 2012). Together these observations reinforce the idea that the morphological and molecular diversity of BNFA remains largely unexplored. Supplementary Material Table S1 provides further morphological and ecological information about different species and isolates of these BNFA where it is available. It also emphasizes the difficulty of species identification of BNFA in the absence of molecular data, which can lead to taxonomic confusion and probable misidentifications or incorrect lumping of unrelated organisms in the same genera.

The range of food items utilized by BNFA is strikingly wide, including bacteria, other protists, diatoms, other algae, fungi, and even small metazoans. In some cases feeding behaviour has been elusive, further emphasizing the interesting nutritional modes shown by BNFA. It is thought that cytoplasmic networks enable BNFA to be more efficient than many heterotrophic protists in finding and ingesting surface-attached prey, and exploiting food sources in interstitial spaces in particulate sediments (Butler and Rogerson 1997; Rogerson et al. 1996). Networks are often very thin and flat and maximize the cell-surface to volume ratio (i.e. foraging area), making them theoretically more energetically efficient in certain microhabitats in comparison with larger, rounder cells. Per cell volume they have been shown to consume bacteria more quickly than non-reticulose cells, and there is evidence that some lineages can digest bacteria or other prey such as diatoms within the pseudopodia themselves (Grell 1994, 1995; Rogerson et al. 1996).

The current state of knowledge of BNFA suggests that, although they can be found across the whole eukaryotic tree of life, there is a relatively small number of lineages with these morphological characteristics (compared to non-BNFA, heterotrophic flagellates, etc.), and that they are present in relatively low numbers in the environment. However, this perception is influenced by some important factors: (1) the cells are easily disrupted and broken by standard sample collection methods, (2) they are often very slow growing in culture or do not thrive in standard laboratory culturing conditions (which usually favour relatively fast-growing bacterivores and ecological generalists), and (3) they are not readily seen or recognized due to lack of dispersed expertise and because they are rarely the focus of experimental work. Rogerson et al. (1996) found that although they were not as numerous as other heterotrophic protists, direct