**Protist Research**

**Which molecular phylogenies confirm/support the (paraphyletic) excavate hypothesis??**

**Euglenozoan original placement?**

**Parabasalids – original placement, description etc.**

**SAR-Discoba?**

**KEY PAPERS**

***From Simpson (1999):***

Note mitochondrial presence of Jakobids and Heteroloboseids vs other excavates.

Argued that *Carpediemonas* does not fit into any of the excavate taxa currently recognised.

*‘Malawimonas* has been referred to as a ‘Jakobid’….because of the common possession of a single vane on the posterior flagellum….character state is almost certainly not homologous across *Malawimonas* and Jakobids.’ 🡪 ‘No strong evidence for a common Jakobid ancestor of both core Jakobids and *Malawimonas*, ‘Jakobids’ may well be polyphyletic.’

‘The density of the matrix and the lack of cristae give the organelles in *Carpediemonas* a greater resemblance to hydrogenosomes than normal mitochondria’ 🡪 hydrogenosomal function would be consistent with the low oxygen environment of the organism.’ 🡪 Shares the double membrane-bound hydrogenosome character with the Heteroloboseids *Lyromonas* and *Psalteriomonas,* AND the Preaxostyla *Trimastix,* which also has ‘homogenous, dense contents.’

‘Barren basal body’ no. 3 of *Carpediemonas* is similarly positioned to that of *Chilomastix,* and *Retortamonas,* whose 3rd and 4th are also barren(morphological support for Fornicata) and equivalently positioned in *Trimastix* too (Support for Metamonada as a whole).

*Percolomonas* has 3 flagella AND the Diplomonad *Trigonomonas* has a pair of ‘trikinetids’ BUT ‘both probably otherwise fall well within tetrakont clades and so consequently are likely to have acquired trikinetids independently of *Carpediemonas*.’

However Ruinen (1938) presents several accounts (in German) of *Triflagellum* spp., otherwise undocumented protists with ventral feeding grooves.(!)

Recessing of flagellar axonemes observed in some excavate taxa: *Carpediemonas*, *Retortomonas*, *Chilomastix*, and some Diplomonads (Also in *Malawimonas*) 🡪 More morphological support for Metamonada.

Flagellar vanes present – single in *Malawimonas* (located ventrally) and core jakobids (located dorsally), double in *Chilomastix* and *Trimastix*, and one major vane in *Giardia*. Also, ‘discrete vane origin’ in *Malawimonas* vs diffuse in jakobids. ALSO differences in ‘circular support’ vs unsupported origin 🡪 ‘single vane state of *Malawimonas* and jakobids is not homologous and poor evidence of monophyly /paraphyly…’ – these characters appear to be quite confounding, not useful!

Split right root appears common across groups, most similar in *Carpediemonas* and *Malawimonas*. Feature also similar in *Reclinomonas* and *Jakoba (*both Jakobid*)*. May be present in some form in some Diplomonads. But not observed in *Monocercomonoides* (Simpson *et al.,* 2002b).

Singlet root common to most if not all excavate (excepting possibly Diplomonads and Heteroloboseids).

Left root similar in *Malawimonas* and *Carpediemonas*. Also similar in Retortamonads and Jakobids. Poorly defined in Heteroloboseids. Differ in Diplomonads left root homologues swap sides from their parent kinetid to support other side.

Anterior root similar in *Carpediemonas*, *Malawimonas* and *Trimastix*. Absent in core jakobids. Also observed in *Chilomastix*, Diplomonads and Heteroloboseids, but different form in each.

I fibre and A fibre again most similar in *Malawimonas* and *Carpediemonas*, yet present throughout group in some form.

B fibre is distinct in *Carpediemonas*, similar in *Malawimonas* and jakobids, probably present in some form in *Trimastix* and Retortamonads, and absent in Heteroloboseids.

C fibre most similar in *Carpediemonas* and core jakobids. Present in most groups except Heteroloboseids and Diplomonads.

Cytopharynx support by longitudinal microtubules originating from the right root occurs in retortamonads, the Diplomonad *Entermonas*, *Trimastix* *marina*, possibly not *Trimastix pyriformis* (!), and in some form in the Heteroloboseid *Tetramitus*. Present in some form in most other groups, probably.

Summary from paper: closest linked morphologically are *Carpediemonas* and *Malawimonas*. BUT relatively few similarities are exclusive, and possibility that the are retained plesiomorphies. Argues the characters of *Carpediemonas* appear to unite the different excavate taxa, and is evidence for a monophyly/paraphyly.

Worth noting that few spp. from each excavate group have been examined in this morphological analysis 🡪 entirely possible that not all members of each group possess the stated characters.

***From Simpson et al. (2002a) [Carpediemonas is related to diplomonads]:***

Molecular analysis of excavates (SSU, alpha and beta tubulin) scatters excavata across the Eukaryote tree, with weakly supported nodes.

Does away with suggestions that Diplomonads are a basal branch of Eukaryotic tree: assuming single mitochondrial endosymbiotic origin in Eukaryotes, from which *Carpediemonas* hydrogenosome is descended, along with other probable homologs within the Eukaryotes, and considering the clustering of *Carpediemonas* with Diplomonads and retortamonads, makes the premitochondrial Diplomonad scenario unlikely. This was originally thought due to highly divergent Diplomonad SSUs.

Study proposes the Diplomonads in fact nest within *Carpediemonas* and the retortamonads, with Carpediemonas an intermediate stage in the loss of mitochondria apparent in Diplomonads (although they have since been discovered to have mitosomes, the least functional manifestation of all). 🡪 support for monophyly within the Metamonada BUT does not necessarily suggest nonmonophyly of Excavata as a whole. In fact may be easier to explain presence of hydrogenosomes in Heteroloboseids if these were reduced from mitochondria in the common ancestor of them and Carpediemonas. However, may not be most useful character phylogenetically if reduction of mitochondria to hydrogenosome can readily occur depending on O2 content of the organism’s environment. See Roger *et al.,* (2017) fig. 4.

***From Simpson et al. (2002b) [Comparison of an oxymonad and excavate taxa]:***

Notes the primitive status given to oxymonads (specifically *Monocercomonoides*) due to lack of mitochondria and a ‘typical dictyosomal golgi apparatus’.

Lack flagellar microtubular roots, apart from the ‘enigmatic’ preaxostyle, which is unique to the group (Brugerolle, 1991), hence the name Preaxostyla in Simpson (2003) (although only small in *Monocercomonoides*, and previous studies have observed several flagellar microtubular roots…).

‘excavate taxa…all have similar flagellar apparatuses, differing more in the substructures of various components than in the absolute presence or absence of features.’

Single outer sheet of the I fibre seen in oxymonads is also present in *Trimastix*, arguably some diplomonads, AND Heterolobosea. This is in contrast to the bi-laminar sheet seen in typical excavates.

Essentially, most excavate characters are present, also some of the substructures of *Monocercomonoides* are proposed as equivalent homologs rather than identical.

***From Cavalier-Smith (2002):***

‘Most taxa above “corticoflagellate bar” have a rigid cell cortex often supported by microtubules.’ Excavates are directly above this bar but is only a schematic, not necessarily accurate…

Corticate character is here identified as a synapomorphy, and the Corticoflagellata clade (Excavata plus plants and chromists).

‘Excavata are characterised ancestrally by having two cilia, a single broad anterior centriolar microtubular fan and two lateral posterior centriolar bands.’

‘Characteristic excavate three ciliary roots…’

Link to SAR:

‘Ciliophora with bikinetids have two roots associated with mature cilium, and one with the younger cilium, as in excavates.’ 🡪 ‘excavate-like pattern of three (total, ciliate) roots in bikinetids, simplest interpretation is that this was ancestral state for Alveolata…’ 🡪 cilium roots could be significant character? Predicts Alveolata evolved from ancestral excavate, does not mention presence of character in contemporary excavates however…

Rhizaria shown as outgroup to excavates, suggestion of ancestral biciliate state – here entire group are termed bikonts – states that Alveolates are nested within the excavates, which are themselves nested within the Rhizaria (???)

BUT Fig. 4 schematic indicates that chromalveolates and Euglenids became photosynthetic through two separate endosymbioses.

Green-plant-like H+pyrophosphatase gene present in Kinetoplastids and Alveolata 🡪 tempting to think it may be an ortholog of the common ancestor of these and Euglenids, but so far has not been found in cyanobacterial-containing Euglenid genome. Could new data reveal otherwise?

Presence of 6-phosphogluconate dehydrogenase gene (gnd) of cyanobacterial affinity in *Naegleria* (Percolozoa) 🡪 potentially a relic of an early implantation of a green-algal plastid prior to the divergence of Percolozoa and Euglenozoa (p.340). Is found in Euglenozoa but does NOT branch within protist clade in optimal gnd trees (odd). Also present in *Giardia* and *Trichomonas*. Molecular evolution/endosymbiotic events still unclear (Andersson and Roger, 2002).

Presence of cyanobacterial-like glucokinase genes in Diplomonads and Parabasalia (Wu et al., 2001)…probably lateral transfer from bacterial food of ancestral Metamonad…

Hydrogenosomes evolved from mitochondria separately in many different groups as necessitated by environmental conditions – polyphyletic.

Describes the acquisition of green algal plastids by the Euglenoids as ‘one of the 5 major symbiogenetic events in the history of life’ in fig. 5.

***From Simpson (2003):***

***Excavate ultrastructure is well-characterised.***

***(Simpson 2003): ‘typical excavates’ have a ventral suspension-feeding groove, supported primarily by two multi-microtubular roots, positioned to the left and right, that are associated with the posterior basal body. The right root splits into two soon after its origin. A singlet root is also associated with the posterior basal body, and runs along the groove between the left root and inner right root. There are also usually three conspicuous nonmicrotubular fibres (‘I’, ‘B’, and ‘C’).***

Excavata first named by Thomas Cavalier-Smith (2002), based primarily on morphological characters.

7 taxa recognised: Jakobida, *Malawimonas, Trimastix, Carpediemonas*, Retortamonads, Diplomonads and Heterolobosea, PLUS the ‘potentially related organisms’ Parabasalids, Oxymonads (containing *Monocercomonoides*) and Euglenozoa (these lack feeding groove).

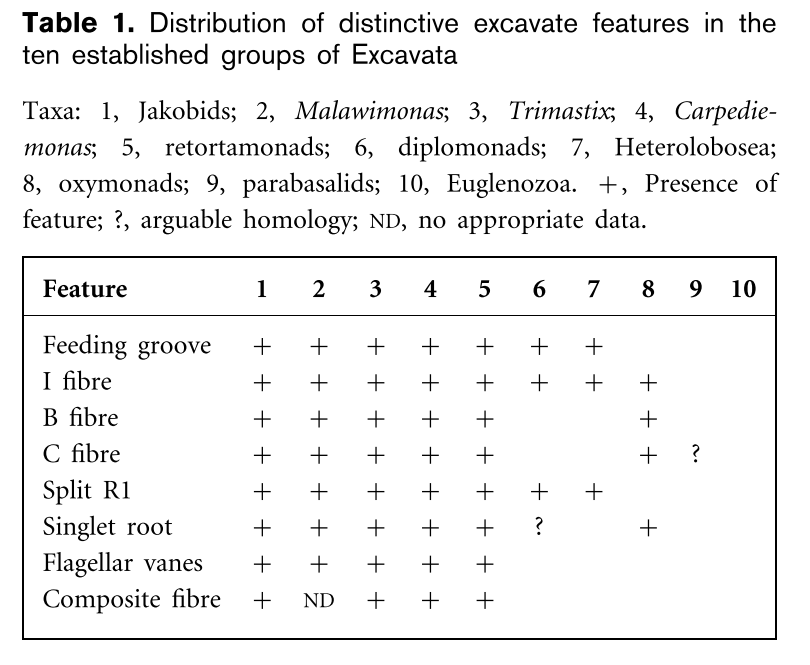
‘Taxon Heterolobosea usually considered to be related to Euglenozoa based on ultrastructural features (Patterson 1988).’

Trypanosomes and Bodo saltans both considered here to be Euglenids, but both subsequently classified as Kinetoplastids – what is development here – see Simpson et al. (2006)

Collodictyonids also contain ventral grooves, considered homologous by Cavalier-Smith (2002) but unrelated in most other aspects of morphology, and don’t cluster with excavates in molecular analyses. BUT see Zhao et al., (2012).

Excavate Hypothesis contends that all ventral feeding grooves are homologous, and that the Excavate comprise either a monophyly, OR, a paraphyly in which non-excavate groups have subsequently lost the ancestral Excavate characters. (Note polyphyly not mentioned).

‘It is clear that striking morphological characters can evolve convergently in distantly related Protists, e.g. Amoebae, Heliozoa.’



Above table indicates common excavate features thought to be synapomorphies(?), although in Simpson and Patterson’s (1999) study these are not necessarily found to be particularly similar across the group.

Excavata not recovered as a monophyly in SSu-rRNA phylogeny with representative Eukaryotes included.

*Carpediemonas* often clusters with Diplomonads/Parabasalids in molecular analyses.

‘Striking non-monophyly of Excavata in most molecular phylogenies is a serious barrier to acceptance of the excavate hypothesis.’

*Trimastix*/Oxymonads possess distinct ‘I fibres’. Here named Preaxostyla.

***From Cavalier-Smith and Chao (2010):***

The ancestral split right root of typical excavates has become completely separated in two in Collodictyonids/Diphylleids – ‘cytoskeleton shows features derivable from typical excavates’.

Microtubules of Left Root, medial root homology to outer RR of excavates, plus ventral groove = remarkable similarities of Apusomonadida to excavates.

‘ventral microtubular skeletons of Apusomonads and excavates are effectively identical’ (excepting the absence of posterior singlet microtubule in Apusomonads). Also suggest (as with Heiss et al., 2011) that these characters are ancestral and have been lost in other eukaryotes.

Within this ancestral excavate model, it is further suggested that the split right root has been inherited by probable descendants of excavates – the Bicoecea (Stramenopiles).

Of all the excavates, Apusomonads most similar to *Malawimonas* morphologically.

***From Heiss et al., (2011):***

Both groups (Ancyromonads and excavates) possess ventral grooves/channels used in feeding.

Also note similarity of ultrastructure to Apusomonada

Number of similarities seen in *A. sigmoides*:

crescent root occupies similar position relative to posterior basal body and channel. Also splits close to the origin, as with the right root. Non-microtubular component of crescent structure itself is similarly positioned to I fibre of excavates, although crescent structure is far smaller.

Presence and position of posterior singlet corresponds to that of singlet root in excavates.

Position and direction of anterior root is similar in both excavates and *A. sigmoides*, and both consist of small number of microtubules. BUT excavates lack the second anterior root found in *A. sigmoides*. BUT THEN there are short ‘protoroots’ present in some species (*Chilomastix – Bernard et al., 1997; Trimastix – Simpson et al., 2000*).

Caution against over-interpreting features of the cytoskeleton as strong evidence of specific relationships. Cite the bicosoecid stramenopiles as having similar features (namely the right/root/crescent root equivalent splitting into two main components, and S tubule similarity to posterior singlet in location of origin). 🡪 Discuss a couple of possibilities: the characters are a result of convergent evolution, OR, they are plesiomorphies retained in these groups, which, given the deep-branching positions they occupy on the eukaryote tree (in some analyses) makes this possibility somewhat more plausible.

***From Heiss et al, (2018):***

New *Gefionella* data, inc. morphology 🡪 possesses flagellar vanes and a composite fibre, PLUS ‘system of microtubular roots and supporting fibres that are general to typical excavates   
(R1, split R2, singlet root, fibres A, B, I and C)’ , i.e. classic excavate morpho.

B fibre most significant – no unambiguous homologue of this structure has been positively identified outside of excavate taxa.

Possession of opposed vanes is shared by Malawimonadidae and Metamonada, to the exclusion of Jakobida.

Support for Metamonada+Discoba monophyly begins to break down with the removal of fast-evolving sites in phylo analysis.

**LESS KEY BUT STILL RELEVANT**

***From O’Kelly (1993):***

Jakobids:

*Jakoba* characterised by aloricate trophic cells containing a branching mitochondrion with prominent nucleiods and flattened cristae.

*Reclinomonas* and *Histiona* characterised by loricate trophic cells, each with an unbranched mitochondrion without prominent nucleiods and with tubular cristae.

***From Atkins et al. (2000):***

Propose *Ancyromonas* as ancestor to the Opisthokonts on the basis of features of mitochondrial cristae.

***From Andersson and Roger (2002):***

6-Phosphogluconate dehydrogenase present in many Eukaryote and Prokaryote taxa, pattern of endosymbiosis unclear from results. However, one possible series of events is the secondary then tertiary endosymbiosis across the Eukaryote tree, resulting in the transfer of the gnd gene into the common ancestor of Euglenozoa and Heterolobosea (see Fig. 4 B!).

***From Brugerolle et al., (2002):***

*Diphylleia* possesses a deep ventral groove similar to that of Collodictyonids.

Have compared above two to the excavate taxa: ‘Without going into the details of the ultrastructure of the flagellar apparatus, it is obvious that Collodictyonids differ from each of these excavate taxa.’

***From Cavalier-Smith (2003):***

Original concept of Metamonada (Grasse 1952) included Parabasalia due to presence of exonuclear spindle, together with the axostyle (lack of which led Grasse to exclude Diplomonads from Metamonada). Parabasalids subsequently removed and reinstated.

Suggests ancestor of the Metamonada is *Malawimonas* or *Malawimonas*-like on the basis of ciliary root structural similarities.

***From Simpson et al. (2006):***

Kinetoplastids contain unusual mitochondrial features inc. RNA editing (as with Euglenids in Yang et al. (2017)), and unique mitochondrial DNA architecture.

Contain ultrastructural features indicating that their closest relatives are not Euglenids as was thought, but a group called the Diplonemids.

Trypanosomatids unique in their possession of kinetoplasts.

***From Zhao et al., (2012):***

Ventral feeding groove of Collodictyonidae may have some significance! Recovered as sister taxon to *Malawimonas* in some of the analyses of this study (as with Celine’s data!) However, warns that position of *Malawimonas* may be unstable…

Fusion of DHFR and TS genes may be a valuable genomic marker in the distinction between unikonts and bikonts (bikonts has fusion, unikonts have an insertion).

***From Karnkowska et al., (2016):***

*Monocercomonoides* unique amongst Eukaryotes in that it completely lacks a mitochondrion. This was lost secondarily in recent ancestor.

Reductive evolution of mitochondria occurred in common ancestor of Metamonads (see fig. 4).

Metamonada and Discoba nonmonophyletic in analysis (although an ML tree inferred from same supermatrix recovered them as sister taxa).

***From Roger, Munoz-Gomez, and Kamikawa (2017):***

*Monocercomonoides* completely lack mitochondria (see fig. 4).

***From Yang et al. (2017):***

Some Discoban mitochondrial genomes are remarkable – those of Jakobids are most bacterial-like and gene-rich discovered to date (Lang et al., 1997; Burger et al., 2013).

Mitochondria of Euglenids also have unusual features: gene fragmentation and editing of mitochondrial transcripts (Flegontov et al., 2011).

***From Lax. et al. (2018):***

Hemimastigophora (containing *Hemimastix*) firmly placed as new supra-kingdom. Metamonada unstable in Eukaryote tree (although only one representative, *Trimastix*).