The Protistan Origins of Animals and Fungi

1

Martin Carr, Kayleigh Hopkins, and Michael L. Ginger

Abstract

Fungi and Metazoa (animals) are two major multicellular kingdoms of life and both are positioned in the eukaryotic Opisthokonta. Within the supergroup Fungi and Metazoa fall into either side of the opisthokont root, in the major sub-groups Holomycota and Holozoa. In this chapter, we cover recent advances in the understanding of opisthokont biology, in particular looking at their diversity and where opisthokonts fall in the eukaryotic tree. Although much uncertainty remains over how different eukaryotic supergroups are related to each other, the closest relatives of Opisthokonta are now widely recognised.

The composition of Opisthokonta has been revised due to the discovery of new species, as well as the reassignment of taxa on the basis of phylogenetic analyses. We consider common traits and characteristics found in opisthokonts. The explosion of genomic and transcriptomic sequencing since the turn of the century has allowed the identification of genes involved in multicellularity in both Metazoa and Fungi; molecular phylogenies show multicellularity has independently evolved in multiple lineages across the opisthokonts. Annotated gene complements from species spanning the group highlight that gene loss and gain is a dynamic process in the opisthokonts.

Keywords

Holomycota · Holozoa · Multicellularity · Opisthokonta · Taxonomy

1.1 Introduction

Fungi and Metazoa constitute two of the major multicellular eukaryotic lineages and a large body of robust data confirms that they are close relatives (Brown et al. 2018; Derelle et al. 2015; Wainright et al. 1993). Together, along with a number of clades comprising unicellular taxa, Fungi and Metazoa make up the eukaryotic supergroup Opisthokonta (Adl et al. 2019). This chapter sets out to describe our current knowledge on the species and biology of the opisthokonts, with a particular emphasis on the unicellular representatives.

The composition of Opisthokonta has been disputed since the existence of the group was first proposed; however, a more settled view has emerged within recent years, with between seven to ten major lineages recognised. In addition to the multicellular Fungi and Metazoa, Opisthokonta also contains the unicellular

M. Carr (✉) · K. Hopkins · M. L. Ginger
Department of Biological & Geographical Sciences, University of Huddersfield, Queensgate, Huddersfield, West Yorkshire, UK
e-mail: m.carr@hud.ac.uk; kayleigh.hopkins@hud.ac.uk; m.ginger@hud.ac.uk

S. Pöggeler, T. James (eds.), *Evolution of Fungi and Fungal-Like Organisms*, The Mycota 14,
https://doi.org/10.1007/978-3-031-29199-9_1

lineages Choanoflagellates, Filasterea, Ichthyosporea, and Opisthosporidia, as well as the nucleariid amoebae (Adl et al. 2019). The monophyly of Opisthosporidia has been questioned (Karpov et al. 2014a, b), with Aphelida potentially forming a clade independent of the other opisthosporidians (Torruella et al. 2018). A putative ninth taxon, Pluriformea, has also been proposed (Hehenberger et al. 2017), but, at present, the relationships between the two known pluriform species and other opisthokonts have not been resolved and recognition of the group is not universal (Torruella et al. 2015). The most recently discovered independent opisthokont group is the genus *Tunicaraptor*, currently only known by the type species *T. unikontum* (Tikhonenkov et al. 2020).

The relationships amongst the opisthokont groups, and of those of the opisthokonts with other eukaryotic supergroups, are slowly becoming clearer. We discuss here the major taxonomic groups within Opisthokonta and their relationships with each other. It is now clear that the deepest bifurcation within the opisthokonts resulted in two major lineages, referred to as Holozoa and Holomycota (the latter has also been labelled by authors as the holofungi (Lara et al. 2009) and Nucletmycea (Brown et al. 2009)). Holomycota is composed of the Fungi, Opisthosporidia, and nucleariid amoebae (Lara et al. 2009). Metazoa, choanoflagellates, filastereans, pluriformeans, *Tunicaraptor*, and the ichthyosporeans are collectively known as Holozoa (Lang et al. 2002; Shalchian-Tabrizi et al. 2008).

The lack of any universal diagnostic characteristics means that membership of Opisthokonta is often based upon phylogenetic trees. Early molecular phylogenetic studies of eukaryotes were prone to generating erroneous topologies, which lead to conflicting theories on how groups were related to each other. In particular, in many studies there was a paucity of species for which sequence data were available. Limited taxa sampling can lead to species being present on isolated long branches; this, in turn, may lead to problems when reconstructing phylogenies due to the phenomenon of long-branch attraction (Felsenstein 1978; Hendy and Penny 1989). When distantly related sequences share a relatively high number of homoplasies (shared characters, present due to convergence rather than common ancestry) the true phylogenetic signal may be overwhelmed and long-branched sequences incorrectly clustered together. Long-branch effects can also be produced by unequal rates of evolution; therefore, when possible, it is advisable to screen taxa and select those most suitable for phylogenetic reconstruction. This is not always possible, particularly in the case of less well-studied eukaryotes where some lineages are only represented by a single species. A further problem was that early phylogenetic studies mainly relied upon single gene phylogenies. This was often the small subunit ribosomal (SSU) RNA gene, which had the advantages of being ubiquitous across all domains of life, present in multiple copies per genome and amplifiable with universal PCR primers (Medlin et al. 1988). Limitations associated with single gene phylogenies include insufficient phylogenetically informative sites and lineage-specific rate changes which have the potential to produce long-branch artefacts.

Studies have subsequently shown that increasing the length of alignments, through concatenating multiple gene sequences, can reduce long-branch issues (reviewed in Bergsten 2005), as the effects of gene-specific homoplasies are diluted within the greater volume of data. Furthermore, increasing the number of taxa in a phylogenetic analysis reduces the average branch length across a tree, lessening the impact of long-branch attraction by dispersing homoplasies which would otherwise be concentrated on long internal branches (DeBry 2005; Zwickl and Hillis 2002).

With the advent of high-throughput sequencing it is now comparatively inexpensive to sequence an organism's genome or transcriptome, allowing the generation of phylogenetic datasets made up from hundreds of protein sequences (e.g. see Brown et al. 2018; Gawryluk et al. 2019; Derelle et al. 2016). This field of phylogenomics has resolved many previously unknown relationships within the

eukaryotic tree; however, artefacts remain a major issue (Betancur-R et al. 2014; Philippe et al. 2011) and conflicting topologies are still regularly published in academic papers. The use of sequences from multigene families can be problematic unless the chosen genes are carefully screened. Members of gene families that diverged when species diverged, termed orthologues, are suitable for species phylogenetic reconstruction. In contrast, paralogues, which are the product of gene duplication events, have different evolutionary histories from the species that encode them. The inclusion of paralogues in datasets used to create species trees can therefore result in misleading topologies. Paralogy may be difficult to identify when gene duplication events are ancient, or in cases where a gene family is evolving under weak selective constraint. Further complications in phylogenetic analyses may arise due to the choice of the amino acid substitution model used, as well as the partitioning of alignments, with incorrect models resulting in both erroneous relationships and inaccurate node support values (Philippe et al. 2011; Young and Gillung 2020).

A well-resolved phylogeny is essential in order to determine how traits and characteristics have evolved within groups of species. Due to the presence of both Fungi and Metazoa, the opisthokonts have been intensively studied with regard to the origins of multicellularity. Two forms of multicellularity are known to have evolved within eukaryotes. In clonal multicellularity a single initial cell undergoes rounds of cell division, with the daughter cells remaining adhered to each other, resulting in a multicellular organism made up from genetically identical cells. In aggregate multicellularity, exemplified by *Dictyostelium discoideum* (Schilde and Schaap 2013), genetically unrelated unicellular individuals of the same species assemble together to form a multicellular "superorganism". Although both metazoans and fungi exhibit clonal multicellularity, they have fundamentally different developmental pathways and early opisthokont phylogenies confirmed that multicellularity evolved independently in the two groups, as both groups are more closely related to unicellular relatives than each other (Ruiz-Trillo et al. 2004). As we will set out in this chapter, it is clear that multicellularity, both aggregate and clonal, has evolved independently in a diverse range of opisthokont lineages.

1.2 Opisthokonta

The original Opisthokont group, proposed by Vischer (1945), unified chytrid fungi with the choanoflagellates on the basis of both groups possessing a single posterior flagellum that pushes swimming cells through water. Gams (1947) subsequently expanded the group with the inclusion of Metazoa, under the name Opisthokonten. In contrast to modern views on opisthokonts, both Vischer and Gams placed uniflagellate algae with their groupings. Perhaps surprisingly, the next amendment of the group, proposed by Copeland (1956) under the phylum Opisthokonta, was not a further expansion but to restrict membership to only chytrid fungi. Copeland dismissed an evolutionary link between the chytrids with the choanoflagellates and metazoans as "far-fetched" and there was limited acceptance of the group, with debate continuing over the relationships between the multicellular kingdoms of animals, fungi, and plants for the next four decades. The taxon was revived, again on the basis of morphological characteristics, as the informal group Opisthokonta by Cavalier-Smith (1987) to encompass fungi, metazoans, and the choanoflagellates.

Molecular studies in the 1980s produced varied and equivocal results on the placement of both Fungi and Metazoa within the eukaryotic tree (Gouy and Li 1989; Sogin et al. 1986). Through a combination of a greater number of taxa and increasingly sophisticated phylogenetic analyses, robust molecular support for the opisthokont group emerged through a trio of papers in the early 1990s (Baldauf and Palmer 1993; Hasegawa et al. 1993; Wainright et al. 1993). The Wainright et al. (1993) study was of particular importance, as, in addition to highlighting the close relationship between fungi and metazoans, it confirmed, through the

phylogenetic position of choanoflagellates, the existence of unicellular opisthokonts. Whilst the Wainright phylogeny recovered a strongly supported Opisthokonta, the relationships between the three represented lineages remained unresolved.

As the volume of molecular data increased, it became clear that the opisthokonts harboured much greater diversity, with multiple unicellular lineages being closely related to the metazoans (Cavalier-Smith and Allsopp 1996; Herr et al. 1999; Kerk et al. 1995). The first indications of unicellular holomycotans were discovered at a similar time (Edlind et al. 1996; Keeling and Doolittle 1996); however, clear evidence for unicellular relatives of Fungi was later produced through the phylogenetic placement of species that were previously believed to belong to Amoebozoa and Holozoa (Amaral-Zettler et al. 2001; Brown et al. 2009; Ruiz-Trillo et al. 2004).

Early evidence for the existence of the opisthokont grouping was based upon morphological traits. However, as the depth of diversity in opisthokont lineages has been uncovered, it has become apparent that there are no recognised universal morphological characters unique to this group. The posterior flagellum is not present in all opisthokont groups; loss of the flagellum must have occurred on multiple occasions within both Holomycota and Holozoa (Adl et al. 2019; Galindo et al. 2021; James et al. 2006). Throughout the opisthokonts, the morphology of mitochondrial cristae is predominantly flat, but this appears to be a plastic trait, with tubular and discoidal cristae also present (Adl et al. 2019; Amaral-Zettler et al. 2001; Ragan et al. 1996; Wylezich et al. 2012). Nonetheless, the posterior flagellum and flat cristae are widespread across the opisthokonts and point to the ancestral states of the group. The abilities to produce amoeboid cells and also to engulf particles by phagocytosis are present in most of the major lineages. Moreover, Metazoa is the only major opisthokont lineage that does not contain species with cell walls, and it has been suggested that the last common ancestor of the opisthokonts also possessed the potential to produce a cell wall (Mendoza et al. 2002).

1.3 The Placement of Opisthokonta in the Eukaryotic Tree of Life

The improvements in phylogenetic analyses set out in Sect. 1.2 have also led to a much greater understanding of the overall eukaryotic tree. For much of the last two decades, most eukaryotes were believed to fall into one of seven supergroups, namely the Alveolata, Amoebozoa, Archaeplastida, Excavata, Opisthokonta, Rhizaria, and the stramenopiles. In addition to the supergroups, a number of minor, or orphan, groups of uncertain phylogenetic position were recognised (reviewed in Adl et al. 2005, 2012, 2019). Robust relationships between the supergroups have been less certain, but the stramenopiles, alveolates, and rhizarians were frequently recovered as clade known as the SAR group (Burki et al. 2007). Furthermore, a close relationship between the amoebozoans and opisthokonts was also reported (Stechmann and Cavalier-Smith 2003a, b). As both groups contain uniflagellate species, the combined Amoebozoa +Opisthokonta taxon was defined as unikont, a term originally coined by Cavalier-Smith (2002) to describe species which possess a single flagellum and single centriole. The remaining eukaryotic supergroups, which predominantly contain biflagellate taxa, were labelled as bikonts. It is now clear that these terms are no longer appropriate, since nested within the unikont grouping are a small number of biflagellate species such as *Apusomonas proboscidea* (Kim et al. 2006; Vickerman et al. 1974).

Recent findings have cast doubt on the simplicity of the supergroup system (Burki et al. 2020), with neither Archaeplastida nor Excavata being recovered as monophyletic in large-scale phylogenomic studies (Cavalier-Smith et al. 2014; Gawryluk et al. 2019; Heiss et al. 2018). Advances in phylogenetics have also resulted in the recovery of further novel lineages, such as Ancyromonadida, Hemimastigophora, and Malawimonadida, which appear to fall outside of the previously recognised supergroups (Fig. 1.1). The greatest issue currently hindering

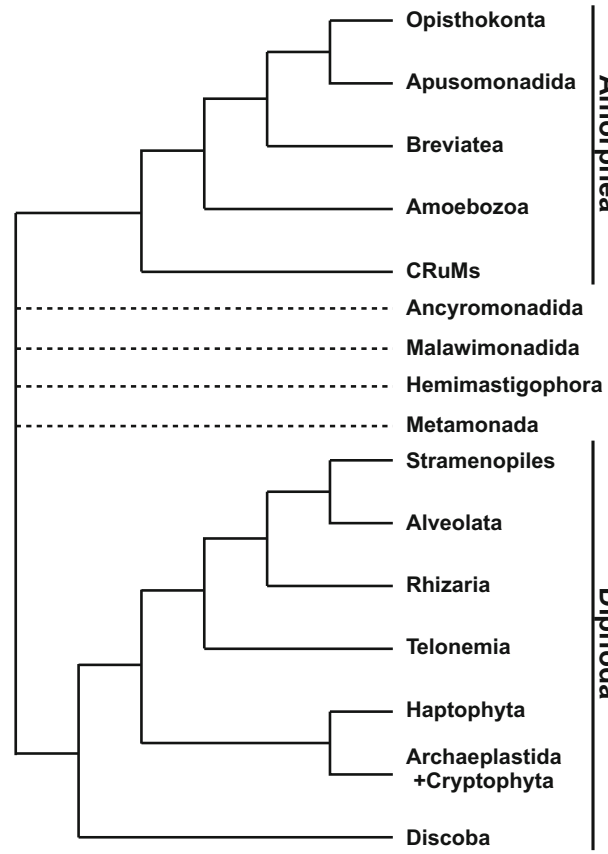

Fig. 1.1 Representative cladogram of Eukaryota. The Amorphea and Diphoda are written in black font. Due to the lack of a resolved root for the eukaryotic tree, four lineages, shown by dotted grey branches, are of uncertain position. The topology is based upon phylogenies presented in Brown et al. (2018), Heiss et al. (2018), Lax et al. (2018), and Strassert et al. (2019)

eukaryotic deep phylogenetics is the unknown position of the root, or earliest branching point, of the eukaryotes. Until the position of the root is established, determining the order of divergence events and establishing sister groups cannot be confidently achieved.

Eukaryotes appear to have evolved from a symbiosis between an alphaproteobacterium and an Asgard archaeon (Gray et al. 1999; Zaremba-Niedzwiedzka et al. 2017), with the eukaryotic crown group estimated to have arisen between 1.6 and 2.5 billion years ago (Parfrey et al. 2011). This great antiquity leads to long branches positioned between eukaryotes and archaea in phylogenetic trees, with long-branched artefacts pulling rapidly evolving species to the base of the eukaryotic group (Brinkmann et al. 2005; Williams and Embley 2014). Eukaryotic phylogenies created with bacterial genes tend to recover accepted eukaryotic supergroups but fail to find a consistent root (Derelle et al. 2015; He et al. 2014). A particularly contentious issue with deep eukaryotic phylogenetics, highlighted in the He and Derelle studies, is the position of Excavata. The supergroup is proposed to consist of three lineages, in Discoba, Malawimonadida, and Metamonada, all of which contain multiflagellate species that possess a ventral feeding groove (Adl et al. 2012, 2019). Multiple studies have both recovered (Hampl et al. 2009; He et al. 2014) and rejected (Brown et al. 2018; Heiss et al. 2018) the monophyly of the excavates. Trees which fail to recover excavate monophyly often place excavate lineages on either side of the eukaryotic root (Derelle et al. 2015; Heiss et al. 2018). This raises the possibility of the excavates being a paraphyletic grouping, with two flagella and a feeding groove being ancestral traits for all eukaryotes.

Whilst the position of the eukaryotic root, as well as the monophyly of the excavate lineages, remains uncertain, two major domains are now recognised. The Diaphoretickes consists of the SAR group, now unified with Telonemia to form the TSAR clade (Strassert et al. 2019), Archaeplastida, Cryptista, and Haptista (Adl et al. 2012, 2019). The relationships between latter three lineages have yet to be resolved; however, a number of recent phylogenies suggest that Cryptista may in fact be a clade within the archaeplastids (Gawryluk et al. 2019; Strassert et al. 2019). Derelle et al. (2015) further unified Diaphoretickes with the excavate Discoba lineage under the name Diphoda (Fig. 1.1).

The opisthokonts fall into the second major eukaryotic domain, Amorphea (Adl et al. 2012; Fig. 1.1). A clade of gliding, biflagellate heterotrophs termed Apusomonadida (Karpov and Mylnikov 1989) are robustly recovered as the sister group to the opisthokonts, highlighting that the flagellum loss to the uniflagellate state must have occurred in the opisthokont stem group. Breviatea (Cavalier-Smith et al. 2004) is a clade of gliding flagellated amoebae, which may possess either one or two flagella. The phylogenetic position of this group has had something of a troubled history, being placed initially with excavates (Cavalier-Smith et al. 2004) and then amoebozoans (Minge et al. 2009) before being finally recognised as an independent lineage related to both the opisthokonts and apusomonads in the taxon Obazoa (Brown et al. 2013). The earliest branching Amorphea lineage, and sister taxon to the Obazoa, is the Amoebozoa (Adl et al. 2019).

The recently proposed CRuMs supergroup, comprising free-swimming Collodictyonidae, amoeboid Rigifilida, and the gliding *Mantamonas* has been recovered as the putative closest relative to Amorphea (Brown et al. 2018; Lax et al. 2018); however, this proposition is controversial due to the use of unrooted phylogenetic trees and the uncertainty over the position of the eukaryotic root.

1.4 Holomycota

Molecular phylogenies in the early 1990s confirmed that Fungi is a member of Opisthokonta; however, it was later in the decade that unicellular close relatives of Fungi were identified. Due to weak phylogenetic support, early phylogenies could not differentiate between unicellular holomycotans being close relatives of, or being nested within, Fungi (Edlind et al. 1996; Fast et al. 1999; Gill and Fast 2006; Keeling and Doolittle 1996). The expansion of known unicellular holomycotan diversity has been mainly driven by the phylogenetic analysis, and subsequent taxonomic reassignment, of previously known taxa, such as microsporidians, nucleariids, and aphelids. Whilst not universally agreed upon (Richards et al. 2017), there has also been a movement of groups from Fungi, into sister lineages of the group. Two groups of early-branching holomycotans are recognised, in the nucleariid amoeba and the opisthosporidians; however, the latter may be paraphyletic and not a true clade (Fig. 1.2). At present, there is also no consensus upon whether opisthosporidians should be considered members of an enlarged Fungi kingdom, or whether they should be classified as the sister group to "true" or "classical" Fungi. Within this chapter, we refer to Opisthosporidia as the closest protistan relatives to Fungi and not as members of the group.

1.4.1 Nucleariid Amoebae

The nucleariid amoebae is the informal name assigned, initially, to two distinct genera, *Fonticula* and *Nuclearia*, of holomycotans that were unified through multigene amino acid phylogenies (Brown et al. 2009). The group is also known under the formalised synonym Rotosphaerida, which was originally proposed as a taxon within Heliozoa (Rainer 1968). As is the case with numerous unicellular opisthokont groups, both genera were previously erroneously

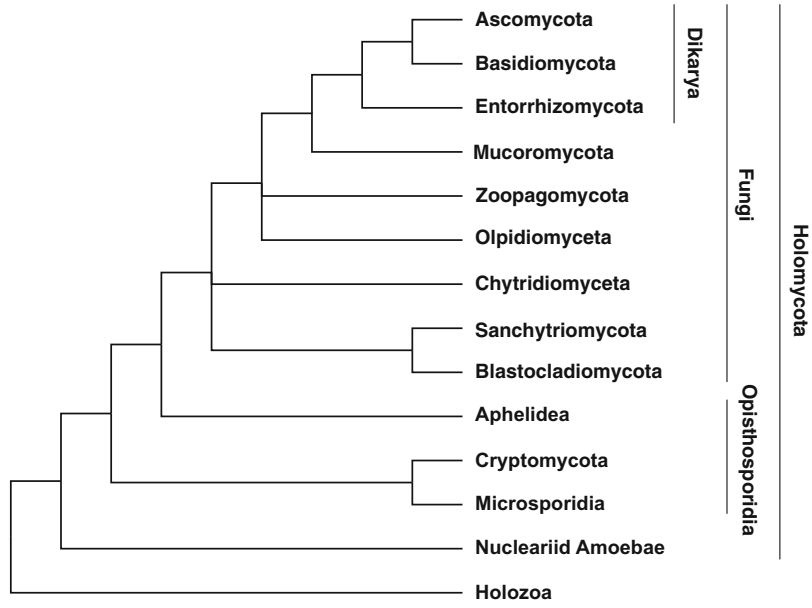

Fig. 1.2 Representative cladogram of Holomycota. The holomycotans are rooted with Holozoa. Polytomies highlight areas of uncertainty in fungal phylogenetic studies and Opisthosporidia is shown as paraphyletic. The topology is based upon phylogenies presented in Bauer et al. (2015), James et al. (2020), Tikhonenkov et al. (2020), and Torruella et al. (2018)

assigned to other taxonomic groups before molecular studies placed them within Holomycota. Nucleariids are slow grazers that can feed on a variety of organisms depending on the species; these include unicellular bacteria and algae. It is suggested that their growth rate may be directly proportional to the availability of their prey, with growth most observable after algal blooms (Dirren et al. 2017).

Discovered over 150 years ago (Cienkowski 1865), to date fewer than 10 nucleariid species have been described (López-Escardó et al. 2017). Thriving in both eutrophic and polluted environments, *Nuclearia* species can feed on toxic cyanobacteria owing to their bacterial endosymbionts which are believed to provide toxicity protection (Dirren and Posch 2016; Dirren et al. 2017). All known species have been isolated from freshwater habitats; individuals floating within the water column possess a protoplast (cell body) that is spherical and non-flagellated, whilst cells in contact with a substratum become amoeboid. Both forms of protoplast exhibit thin filopodia, which are used to contact bacterial and algal prey prior to phagocytosis (Yoshida et al. 2009). Mitochondrial cristae are either flattened or discoidal (Amaral-Zettler et al. 2001; Dyková et al. 2003), whilst species may be uninucleate, multinucleate, or capable of switching between the two states (Dirren and Posch 2016).

Page (1987) and Cavalier-Smith (1993) both placed *Nuclearia* into the order Cristidiscoidida, as members of the amoebozoan phylum Rhizopoda due to morphological characteristics. This placement was questioned by Patterson (1999), on the basis that branching filopodia are likely to have evolved on multiple occasions within eukaryotes as well as the unexpected presence of three mitochondrial cristae morphologies in the group. Through the use of rRNA phylogenetic trees, Amaral-Zettler et al. (2001) placed *Nuclearia* within Opisthokonta; however, their phylogenies could not resolve whether the group was more closely related to either Metazoa or Fungi. Ruiz-Trillo et al. (2004) finally robustly placed *Nuclearia* as members of Holomycota, as the then recognised sister group to Fungi.

Fonticula alba was isolated from dog faeces in 1960, but was not named for almost two decades, until Worley et al. (1979) described the species as a bacterivorous acrasiomycete slime mold. Individual cells are small (7–13 μm), enclosed in a mucosal glycocalyx, possess mitochondria with

discoidal cristae, and exhibit pseudopodia that may project several body lengths from the protoplast (Brown et al. 2009). Cells may produce either filose pseudopodia, when feeding, or a single lobose pseudopodium when migrating (Toret et al. 2022). Individual cells predominantly possess a single nucleus, but larger bi- and trinucleate cells are infrequently observed in laboratory cultures (Worley et al. 1979). In a form of multicellularity superficially similar to that observed in dictyostelid amoebozoans, trophic cells may form aggregates when local prey becomes depleted (Worley et al. 1979). Aggregates become enveloped in a slime-based extracellular matrix and form into a mound; the height of the mound increases and subsequently develops into a globular sorocarp (fruiting body) on volcano-like stalk. The majority of cells in the aggregate migrate to the sorocarp and encyst to become spores; however, a small number of cells remain at the base of the stalk. After spores have germinated the stalk structure subsides, allowing juvenile amoeboid cells to disperse (Worley et al. 1979).

It has recently been discovered that multicellular aggregates also develop when *F. alba* is presented with a new prey source (Toret et al. 2022). When encountering a bacterial biofilm a leader cell directs an elongated collective of connected cells into the prey. Cell-to-cell contacts are well defined with regions enriched with actin; however, individual cells may leave or join the predatory collective during its migration.

The original description in Acrasiomycetes was problematic, as *F. alba* did not appear to be a member of any known acrasiomycete group (Worley et al. 1979). Cavalier-Smith (1993) placed *F. alba* alongside *Nuclearia* in Cristidiscoidia within Filosea; however, Brown et al. (2009) showed that *Fonticula* clusters with *Nuclearia* as the sister group to Fungi. Morphological studies have associated a number of other filose amoebae with the nucleariids (Mikrjukov 1999; Patterson 1985; Patterson et al. 1987). The monotypic genus *Vampyrellidum* is similar to *Nuclearia* in that cells are surrounded by a mucosal glycocalyx. In contrast, species in the genera *Lithocolla* and *Pompholyxophrys* possess assemblages of scales surrounding the protoplast (Galindo et al. 2019); *Pompholyxophrys* taxa produce their own siliceous scales, whilst in *Lithocolla* cells acquire sand particles or diatom frustules (Gabaldón et al. 2022). Molecular phylogenies have confirmed *Lithocolla* and *Pompholyxophrys*, as well as *Parvularia* (originally deposited in the American Type Culture Collection as a *Nuclearia* taxon), as members of the nucleariid amoebae (Galindo et al. 2019). At present no sequence data are available for *Vampyrellidum perforans* so the taxonomic affinity of this species remains unclear.

Galindo et al. (2019) reconstructed states for ancestral nucleariid amoebae on the basis of a multigene phylogeny. They proposed that the ancestral opisthokont single flagellum was lost in the stem lineage of the nucleariids and that last common ancestor was a freshwater protist that possessed filose pseudopodia and a mucosal glycocalyx. Aggregate multicellularity evolved in the *Fonticula* lineage, whilst scale bearing evolved an ancestor of *Lithocolla* and *Pompholyxophrys*.

1.4.2 Opisthosporidia

The superphylum Opisthosporidia, which comprises three endoparasitic lineages in the Aphelida, Microsporidia, and Cryptomycota (the latter also described as Rozellida and the Rozellomycota), was only proposed in 2014 on the basis of molecular phylogenies (Karpov et al. 2014a). The taxon is a controversial one, as its placement in Fungi or alternatively the sister group to Fungi is disputed. Furthermore, the validity of Opisthosporidia is unclear, as it has been recovered as paraphyletic in some molecular phylogenies; whilst Cryptomycota and Microsporidia are recognised as sister groups, Aphelida has been recovered in a variety of positions in Holomycota (Corsaro et al. 2014; Galindo et al. 2019, 2021; James et al. 2006; Karpov et al. 2014a; Torruella et al. 2018).

Initially described in the 1880s by Balbiani (1882) microsporidians are a group of predominantly obligate intracellular parasites. Their

non-flagellated cells are distinguished by the presence of a unique structure, the polar tube, which is present within their spores. Upon infecting a new host the polar tube breaks through the chitin-based spore wall and penetrates the plasma membrane of a host cell, allowing the unwalled sporoplasm to pass down the polar tube and enter the cytoplasm of the infected cell. The sporoplasm, which may be uninucleate or binucleate, then proliferates within the new host cells, resulting in a new generation of infective spores (Franzen 2005; Wadi and Reinke 2020).

The first described microsporidians were parasites of metazoans, notable for their highly reduced nuclear genomes, small ribosomes, and lack of mitochondria (Fast et al. 1999). Cavalier-Smith (1983) proposed that they were amongst the earliest branching eukaryotes and placed them in the kingdom Archezoa along with other amitochondriate eukaryotes. Rapidly evolving gene sequences appeared to confirm their antiquity (Vossbrinck et al. 1987); however, the use of more functionally conserved genes showed that the phylogenetic recovery of Archezoa was an artefact due to long-branch attraction, with the microsporidia being either fungi or closely related to fungi (Edlind et al. 1996; Hirt et al. 1999).

Microsporidians are now known to associate with non-metazoan hosts, acting as endosymbionts and hyperparasites for multiple species in the SAR eukaryotic supergroup (Bass et al. 2018). Phylogenetic studies including environmental DNA (eDNA) have uncovered a far greater diversity of microsporidians than was previously known (Bass et al. 2018; Bojko et al. 2022). It is now clear that many of the traits present in the "canonical" or long-branch microsporidians, which infect metazoans, are absent in other lineages. In particular, mitochondria are widespread outside of the canonical microsporidians. The derived *Paramicrosporidium saccamoebae* possesses a mitochondrion which has a genome similar to those of typical fungi (Quandt et al. 2017). In contrast, the early-branching *Mitosporidium daphniae* has lost the genes for the mitochondrial respiratory chain complex I, highlighting independent mitochondrial reduction occurring across Microsporidia (Haag et al. 2014).

Despite being recognised in 2011, the Cryptomycota remain an enigmatic group within Holomycota with the composition of the group in a state of flux (Bass et al. 2018; Corsaro et al. 2014; Jones et al. 2011; Letcher et al. 2013). *Rozella* is the most extensively studied genus within Cryptomycota, with 27 species recognised by Letcher and Powell (2018). Species possess uniflagellated, unwalled zoospores and were for a long time considered to be chytrid fungi (Adl et al. 2005; James et al. 2006); however, rozellids differ from chytrids in that they employ phagocytosis to consume the cytoplasmic contents of their hosts, rather than osmotrophic absorption of nutrition (Powell 1984). Host species include early-branching fungi, as well as green algae and oomycete stramenopiles (Letcher and Powell 2018). Upon contact with a new host cell the *Rozella* zoospore withdraws its flagellum and forms a cyst which possesses a chitin-based wall. The infective cell then enters the host cell via a penetration tube (James and Berbee 2012). Sequencing of the *R. allomycis* mitochondrial genome revealed a 12 kb circular chromosome that has undergone extensive gene loss and a loss of functional constraint on the remaining genes (James et al. 2013), mirroring the reduction of mitochondrial genomes observed in the closely related microsporidians.

The initial proposal of Cryptomycota by Jones et al. (2011) was based in part upon a phylogenetic analysis which contained a large number of eDNA sequences. This tree, as well as a number of others published in the 2010s, indicated that Cryptomycota possessed a high level of species diversity (Corsaro et al. 2014; Karpov et al. 2014a, b, 2018). The inclusion of early-branching microsporidian sequences in phylogenetic trees, however, revealed that much of this diversity was not actually cryptomycotan but microsporidian (Bass et al. 2018; Bojko et al. 2022), suggesting that the diversity of the group may not be much greater than the *Rozella* taxa which have currently been described.

The aphelids are parasites of archaeplastid and stramenopile algae and show a number of similarities with rozellids with regard to their infection of host cells. Infective propagules make contact with host cells and their

pseudopodia search for a break in the algal cell wall. The propagule subsequently forms a cyst with a chitin-based wall and develops a penetration tube at the site of the hole in the host cell wall. The alga is breached by the tube, allowing the amoeboid trophic stage to pass from the cyst into the host cytoplasm. The aphelid phagocytoses the alga cytoplasm, ultimately killing the host cell, and undergoes nuclear division to become a plasmodium. Multiple rounds of cell division subsequently occur to produce the next generation of uninucleate zoospores which then emerge through the host cell wall via the hole produced by the penetration tube (reviewed in Karpov et al. 2014a; Letcher and Powell 2019; Torruella et al. 2018).

The type species *Aphelidium deformans* was described by Zopf (1885) and subsequently 14 further species have been described (Letcher and Powell 2019). Aphelid taxa have been assigned to four ecologically distinct genera: *Amoebaphelidium* and *Aphelidium* are freshwater genera and *Paraphelidium* and *Pseudaphelidium* form marine genera (Letcher and Powell 2019). Whilst all described rozellids possess flagellated zoospores, aphelid infective cells may be flagellated, amoeboflagellated, or amoeboid cells with an immobile flagellum (Letcher and Powell 2019). Studies suggest that mitochondrial cristae morphologies may vary across species and also between the different stages of lifecycles, with flat, tubular, and lamellar cristae reported (Karpov et al. 2014a; Letcher et al. 2013).

The taxonomic placement of aphelids has proved to be controversial, with varying authors considering them to be members of Rhizopoda, Phycomycetes, Mesomyceteozoea, Rozellidea, and Cryptomycota (Gromov 2000; Karpov et al. 2014a; Letcher et al. 2013; Letcher and Powell 2019). Karpov et al. (2013) recovered *Amoeboaphelidium protococcarum* in a clade with the cryptomycotan *Rozella allomycis* and nine microsporidians, with the taxon Opisthosporidia erected the following year (Karpov et al. 2014a). More recent phylogenomic studies have placed Aphelida as the sister group to Fungi and on this basis Galindo et al. (2022) proposed two novel holomycotan taxa. Phytophagea unifies Fungi with Aphelida, whilst Cryptomycota and Microsporidia make up Opisthophagea.

1.4.3 Fungi

Fungi are a highly diverse group of heterotrophs, which may be saprotrophs, commensal symbionts, or parasites; species employ osmotrophy, gaining nutrition through absorption from their environment. Fungi often produce multinucleate hyphae and possess cell walls that comprise both β-glucan and chitin (Adl et al. 2019; Cavalier-Smith 1998a). In a process that appears to be mirrored in Metazoa and Microsporidia the mitochondrial genomes of fungi are reduced in comparison to the ancestral state of opisthokonts, typically encoding 30–40 genes. This reduction in mitochondrial genome size and gene content has been shown to have begun in the fungal stem group, but is a continuing process in the crown group (Bullerwell and Lang 2005). Within the early-branching Neocallimastigomycota the capacity for oxidative phosphorylation has been completely lost and the mitochondrion has evolved into a hydrogenosome; this organelle produces ATP anaerobically and has convergently evolved in eukaryotes on multiple occasions (Embley et al. 2003). Some members of Chytridiomyceta have also undergone tRNA gene loss in their mitochondria and rely upon extensive tRNA editing or the import of tRNA molecules into mitochondria in order to facilitate localised protein translation (Bullerwell and Lang 2005).

The taxonomy of Fungi has undergone substantial revisions since the turn of the century, due to molecular phylogenetic studies. In particular, the number of phyla has increased considerably from the four generally recognised in the 1990s, in the Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota, to between 8 and 18 phyla (Galindo et al. 2021; James et al. 2020; Tedersoo et al. 2018). The disagreements in the number of phyla are predominantly due to the taxonomic rank assigned, either phylum or sub-phylum, to fungal groups, rather than the

validity of the groups themselves. However, disagreements over phylum number are also due to classification systems, such as that proposed by Tedersoo et al. (2018), which include opisthosporidian groups as fungal phyla.

The Ascomycota and Basidiomycota remain recognised as valid taxa, but the early-branching Chytridiomycota has now been split into Cryptomycota, Blastocladiomycota, Monoblepharidomycota, Neocallimastigomycota and Olpidiomyceta, as well as a reduced Chytridiomycota (James et al. 2020; Spatafora et al. 2016). The Chytridiomycota, Monoblepharidomycota, and Neocallimastigomycota have been shown to form a monophyletic grouping and make up the subkingdom Chytridiomyceta. The former Zygomycota has now been divided into the Mucoromycota and Zoopagomycota (Fig. 1.2; James et al. 2020; Spatafora et al. 2016). The branching order of the non-Dikarya fungi has yet to be resolved, with Blastocladiomycota, Chytridiomyceta, or a unified Blastocladiomycota+Chytridiomyceta clade being recovered as the earliest branching fungal lineage in different studies (Chang et al. 2015; Galindo et al. 2021; Tedersoo et al. 2018).

The subkingdom Dikarya, also described as Neomycota (Cavalier-Smith 1998b), is defined by species that possess cells with unfused haploid nuclei called dikaryons. Dikarya contains the two major fungal phyla Ascomycota and Basidiomycota and encompasses ~98% of known fungal diversity (James et al. 2006). A recent phylogenetic study erected a third dikaryon phylum in Entorrhizomycota, a group which had previously been considered a member of the Basidiomycota (Bauer et al. 2015). The Mucoromycota are now recognised as the sister clade to Dikarya (James et al. 2020).

A robust phylogeny of Fungi is vital in order to understand how the group evolved. Phagocytosis was ancestral to opisthokonts, and the trait is present in early-branching holomycotans, including the opisthosporidians, but absent from all fungi, indicating it was lost in the stem lineage after the divergence of Fungi from Aphelida. The ancestral single flagellum is present in early-branching fungi; however, it has been lost on multiple occasions across the kingdom. Within Chytridiomyceta, *Hyaloraphidium curvatum* appears to be aflagellate (Ustinova et al. 2000), highlighting a loss within early-branching taxa. Sanchytriomycota is a novel phylum erected to accommodate two recently discovered flagellated early-branching fungi (Karpov et al. 2018, 2019b). Unusually, their flagella are non-motile, with evidence indicating that the structure has evolved to become a light sensing organelle (Galindo et al. 2021). Species within Dikarya, Mucoromycota, and Zoopagomycota all appear to lack a flagellum (James et al. 2006); however, their relationships with the flagellated Olpidiomyceta remain uncertain. As a result, it is not clear if the ancestral flagellum was lost within these species on one or two occasions.

Whilst a comprehensive phylogeny of the deepest branches of Fungi has yet to be agreed upon, initiatives such as the Joint Genomes Initiative's MycoCosm program are generating the data required for an accurate reconstruction of ancestral fungal and holomycotan metabolism as well as a resolved phylogeny of the group.

1.5 Holozoa

The second major lineage of Opisthokonta, Holozoa, was proposed in 2002, on the basis of the phylogenetic unification of Metazoa with two unicellular groups, in the choanoflagellates and ichthyosporeans, to the exclusion of Fungi (Lang et al. 2002). Since this initial proposal the known diversity of the clade has expanded, with the identification of the filasterean group (Shalchian-Tabrizi et al. 2008). Two additional putative lineages have recently been described in Pluriformea (Hehenberger et al. 2017) and *Tunicaraptor* (Tikhonenkov et al. 2020); however, neither of these groups have robustly resolved phylogenetic positions and their validity has not been confirmed.

1.5.1 Ichthyosporea

The ichthyosporeans are an ecologically and morphologically diverse group of predominantly parasitic, unicellular holozoans. The first reports of this clade came from Ragan et al. (1996) and Spanggaard et al. (1996), with the former naming the group the DRIP clade as an acronym based on the first known members—*Dermocystidium*, rosette agent, *Ichthyophonus,* and *Psorospermium*. Cavalier-Smith (1998a) described these species as Ichthyosporea, due to the four known species all infecting fish hosts. Herr et al. (1999) noted, however, that non-fish hosts had subsequently been discovered and so recommended the name Mesomycetozoa to reflect the phylogenetic position of the group, that is holozoans located between Fungi and Metazoa. Adl et al. (2005) expanded Mesomycetozoa to include unicellular holozoans and holomycotans; however, this status, as a paraphyletic dustbin taxon, was not widely accepted and the term Mesomycetozoa has generally reverted to being a synonym of Ichthyosporea.

A lack of unifying morphological characters means that membership of Ichthyosporea is mainly based upon molecular phylogenies. Prior to the emergence of molecular phylogenetics, a number of ichthyosporean taxa, such as species in the Amoebidiidae and Eccrinidae, were considered to be trichomycete fungi; however, these earlier placements were considered somewhat controversial at the time due to the presence of amoeboid stages in their lifecycles and a lack of a chitinous cell wall (Lichtwardt 1986; Trotter and Whisler 1965).

All known ichthyosporeans are symbionts of metazoans, with relationships varying between commensalism, mutualism, and parasitism; however, there has also been speculation that some species have saprotrophic stages within their lifecycles (Glockling et al. 2013; Mendoza et al. 2002). Species are unicellular and frequently multinucleate, exhibiting a great variety of morphologies. Across the group mitochondrial cristae are flat, with a single known exception in *Ichthyophonus hoferi* which possesses tubular cristae (Ragan et al. 1996). Phylogenetic studies have consistently recovered the ichthyosporeans as comprising two robustly supported clades, labelled Ichthyophonida and Dermocystida (Cafaro 2005; Grau-Bové et al. 2017; Lohr et al. 2010; Pereira et al. 2005) that are both morphologically and ecologically distinct.

Erected by Cavalier-Smith (1998a), the order Dermocystida has also been described as the family Rhinosporidiaceae (Mendoza et al. 2001). Most described species are parasites of vertebrates; however, the host species and site of infection vary across dermocystid species. *Dermocystidium* and *Sphaerothecum* species infect fish, with the latter apparently restricted to infecting internal organs whilst the former may additionally be found on external structures, such as gills and fins (Glockling et al. 2013; Ramaiah 2006). *Rhinosporidium* species cause rhinosporidiosis (Thompson 2016), a disease resulting in granulated polyps in the sinonasal tract, conjunctiva, and urethra in mammals and birds (Kennedy et al. 1995; Seeber 1900). Six genera of dermocystids are known to infect amphibians, predominantly infecting the skin but also being associated with the heart and liver (reviewed in Borteiro et al. 2018).

The infectious agents of dermocystids are endospores. Fish parasites tend to have uniflagellate zoospores, whilst flagellum loss appears to have occurred in species with amphibian hosts (Glockling et al. 2013). Upon infecting a new host the endospore encysts and produces a walled sporangium (cyst) in the host. The sporangia then increase in size to 200–400 μm in diameter, whilst cells undergo division to produce thousands of zoospores. Endospores range across 7–15 μm diameter (Herr et al. 1999) and are either released directly into the tissue of the original host or into the environment to infect a new host (for a review of the life cycle, see Mendoza et al. 2002). Whilst Dermocystida is consistently recovered as monophyletic, the internal relationships within the clade are poorly resolved (Fig. 1.3; González-Hernández et al. 2010). As a result of this lack of phylogenetic

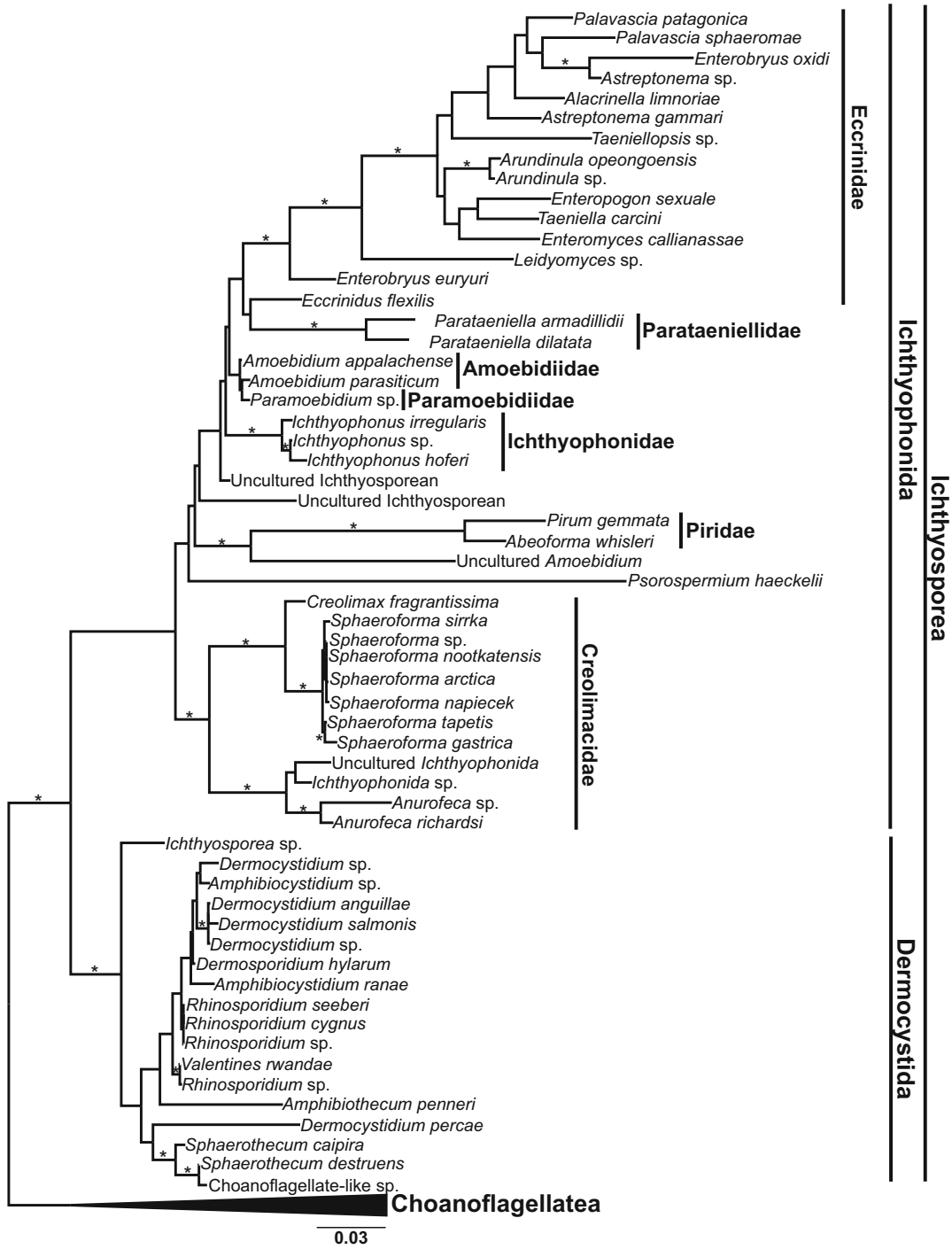

Fig. 1.3 Maximum likelihood phylogeny of Ichthyosporea. The phylogeny was created with RAxMLGUI 2.0 (Edler et al. 2020) from a SSU RNA alignment of 1538 sites sequences generated in MAFFT 7.309 (Katoh and Standley 2013). The phylogenetic analysis used the TIM2 model (Posada 2003) with a four-category gamma distribution and a proportion of invariant sites. Support values were calculated as bootstrap percentages from 1000 replicates. A Bayesian inference phylogeny was created using the same alignment with MrBayes 3.2.6 (Ronquist et al. 2012) The ichthyosporeans are rooted with SSU sequences from four choanoflagellates (*Diaphanoeca grandis*, *Monosiga brevicollis*, *Savillea parva*, and *Stephanoeca diplocostata*). Asterisks highlight nodes strongly supported with both methodologies (≥70% maximum likelihood bootstrap percentage, ≥0.97 Bayesian inference posterior probability). The scale bare represents the number of substitutions per site. Family names are taken from Reynolds et al. (2017)

definition, to date it has been difficult to reconstruct the evolution of observed traits within the order.

In contrast to Dermocystida, phylogenetic relationships within Ichthyophonida are well resolved and the order is made up from multiple recognised families (Fig. 1.3; Glockling et al. 2013). Whilst vertebrate parasitism is prevalent in the dermocystids, only two genera of ichthyophonids are known to have vertebrate hosts. *Anurofeca richardsi* is a gut pathogen of frogs and toads and infection results in inhibited larval growth (Baker et al. 1999; Beebee and Wong 1992); *Ichthyophonus* species are also known to parasitise a range of amphibians, as well as both freshwater and marine fish hosts (Herman 1984; Raffel et al. 2006; Rowley et al. 2013). The majority of the investigated ichthyophonids associate with arthropods and molluscs, although species also infect echinoderms, peanut worms, and tunicates (Lu et al. 2020; Marshall and Berbee 2011, 2013; Reynolds et al. 2017). Not all ichthyophonids are believed to be parasitic; despite its name, the arthropodophilous *Amoebidium parasiticum* is a non-pathogenic symbiont which attaches itself to the external exoskeleton of its insect hosts (Benny and O'Donnell 2000). A further contrast to dermocystids is found in the morphology of the motile dispersal stage. Flagellated cells have yet to be reported, with all motile dispersal cells showing an amoeboid morphology (Mendoza et al. 2002). However, amoeboid dispersal cells are not universal across the group and appear to have been lost on multiple occasions (Lord et al. 2012; Reynolds et al. 2017).

The well-resolved phylogeny of Ichthyophonida allows the evolutionary reconstruction of traits in the group. In particular, it can be seen that *Anurofeca* and *Ichthyophonus*, the two genera known to parasitise vertebrate hosts, are distantly related (Fig. 1.3), showing that ichthyophonids have undergone at least two transitions from non-vertebrate to vertebrate hosts. Based upon their phylogenetic trees, Reynolds et al. (2017) speculated that the ancestors of *Ichthyophonus* were externally attached commensals of non-vertebrate hosts that subsequently evolved into internal parasites of vertebrates.

Whilst the monophyly of Ichthyosporea is widely accepted, the relationships of the group with other holozoans are far from resolved. Since the recognition of the group in 1996 many studies have recovered the ichthyosporeans as both an independent lineage and the earliest branching group in Holozoa (Hehenberger et al. 2017; Paps et al. 2013; Ragan et al. 1996; Torruella et al. 2012). However, discoveries of novel holozoans in recent years have clouded this view and the identities of the most basal holozoan group and the closest relatives of the ichthyosporeans are not presently clear (Grau-Bové et al. 2017; Tikhonenkov et al. 2020; Torruella et al. 2015).

1.5.2 Pluriformea

One of two enigmatic holozoan taxonomic groups Pluriformea was only erected in 2017 and, at present, contains two highly disparate species (Hehenberger et al. 2017). The first species identified in the group, *Corallochytrium limacisporum*, is a unicellular saprotroph isolated from coral lagoons in the Arabian Sea (Raghukumar 1987). To date flagellated cells have not been observed; however, transcriptome analyses have shown presence of the genes required for flagellum assembly (Torruella et al. 2015), suggesting that the *C. limacisporum* may possess more morphological diversity than that witnessed in laboratory cultures. Like most opisthokonts the mitochondria of *C. limacisporum* possess flat cristae (Mendoza et al. 2002). Mature cells are spherical, ranging from 4.5 to 20.0 μm in diameter, and possess a filamentous cell wall that contains multiple pores (Cavalier-Smith and Allsopp 1996; Mendoza et al. 2002). Cell division occurs within the cell wall, resulting in the release of up to 32 elongated, amoeboid daughter cells (Mendoza et al. 2002). The juvenile cells show motility via a slow rocking movement (Cavalier-Smith and Allsopp 1996). Unlike many eukaryotes, cell division is decoupled

from nuclear division and *C. limacisporum* is binucleate for most of the lifecycle as observed in laboratory cultures. The majority of binucleate cells undergo cell division to produce uninucleate daughter cells; however, approximately 1% of observed binucleate cells continue to undergo nuclear division to produce multinucleate coenocytes. Coenocytes eventually bud off daughter cells to return to a uninucleate state (Kożyczkowska et al. 2021).

Upon its discovery *C. limacisporum* was described by Raghu-kumar (1987) as a thraustochytrid. Cavalier-Smith and Allsopp (1996) disputed this classification, on the basis that *C. limacisporum* lacks any of the diagnostic morphological characters of either thraustochytrids or fungi. With a SSU rRNA phylogeny, they recovered *C. limacisporum* as a holozoan and a close relative of the choanoflagellates. Later multigene phylogenies, based upon three or four genes, provided conflicting positions with *C. limacisporum* recovered as a close relative of either choanoflagellates or ichthyosporeans (Carr et al. 2008; Ruiz-Trillo et al. 2006; Steenkamp et al. 2006). Torruella et al. (2015) found a sister relationship between two strains of *C. limacisporum* and the ichthyosporeans, as the earliest branching group of Holozoa, and suggested the name Teretosporea (meaning "rounded spore") for this basal clade.

The discovery of *Syssomonas multiformis*, as a close relative of *C. limacisporum* in 2017, led to the proposal of the Pluriformea clade (Hehenberger et al. 2017). *S. multiformis*, a freshwater unicellular holozoan isolated from a pond in Vietnam, shares very little morphological or ecological similarities with *C. limacisporum*. *S. multiformis* can switch between amoeboid and amoeboflagellate cells, as well as form cysts and multicellular clusters. The species is a predator, which ingests the cytoplasmic content of eukaryotic prey cells (Hehenberger et al. 2017). A 225-gene phylogenetic analyses placed Ichthyosporea as an independent basal holozoan lineage, with Pluriformea being recovered in a more derived position; however, the authors acknowledged their datasets could not exclude the possibility of an enlarged Teretosporea clade made up from Ichthyosporea+Pluriformea (Hehenberger et al. 2017).

1.5.3 Tunicaraptor

The second, and most recently described, of the enigmatic holozoan lineages, *Tunicaraptor*, was only discovered in 2020 and is represented by a single species in *T. unikontum* (Tikhonenkov et al. 2020). Isolated from marine waters from the coast of Chile, *T. unikontum* is a small (3.5–5.1 μm in length) predator of unicellular eukaryotes. As with the pluriform *S. multiformis*, *T. unikontum* exhibits a broad range of morphologies. Observed cells are predominantly uniflagellate, with most of the cell enclosed in a rigid theca that possesses long hair-like structures. The flagellum protrudes from the posterior end of the theca, whilst an anterior aperture of the theca exposes a mouth-like structure. To date no other unicellular opisthokonts are known to possess a feeding mouth, although superficially similar structures are widespread amongst biflagellate eukaryotes (Steinert and Novikoff 1960; Verni and Gualtieri 1997). Short filopodia may exude from the protoplast and aggregations of 3–6 cells have been observed, particularly during feeding.

Despite the availability of a full transcriptome, the phylogenetic position of *T. unikontum* has not been established. Indeed, the inclusion of *T. unikontum* genes into phylogenomic datasets leads to instability in phylogenetic reconstructions of Holozoa. Tikhonenkov et al. (2020) presented a consensus Bayesian inference tree placing the *Tunicaraptor* genus as a sister group to a clade comprising Metazoa, Choanoflagellatea, and Filasterea. However, the authors acknowledged that this position was not well supported and their own maximum likelihood phylogeny using the same data united *Tunicaraptor* in a weakly supported clade with Filasterea, Ichthyosporea, and Pluriformea. Additional analyses by Tikhonenkov et al. (2020), varying both the number of genes and taxa, resulted in poorly resolved phylogenies with no consistent placement of *T. unikontum*.

1.5.4 Filasterea

As with most of the taxonomic groups within Holozoa, Filasterea is a clade based upon phylogenetic relationships, with member species harbouring a high level of morphological and ecological diversity. Erected in 2008 by Cavalier-Smith (Shalchian-Tabrizi et al. 2008) through the phylogenetic clustering of two *incertae sedis* holozoan genera, the group has expanded in recent years to its current membership of six taxa.

Capsaspora owczarzaki was the first species discovered, as an endosymbiont of the pulmonate snail *Biomphalaria glabrata* (Owczarzak et al. 1980; Stibbs et al. 1979); however, the species was not named and described for a further 23 years, when it was recognised as a member of Holozoa (Hertel et al. 2002). *C. owczarzaki* protoplasts are typically spherical and amoeboid (3–5 μm in diameter), possessing numerous thin, unbranching filopodia (Hertel et al. 2002; Stibbs et al. 1979). Cells encyst due to overcrowding (Hertel et al. 2002) and mature cells may form aggregates (Sebé-Pedrós et al. 2013). Flagellated cells have never been observed and the sequenced genome revealed the loss of over 80 genes necessary for flagellum formation (Suga et al. 2013). As with all known filastereans, mitochondria possess flattened cristae (Amaral-Zettler et al. 2001; Urrutia et al. 2022).

Within the *B. glabrata* host *C. owczarzaki* predates the sporocysts of the parasitic trematode flatworm *Schistosoma mansoni*, which uses *B. glabrata* as a vector in its lifecycle (Stibbs et al. 1979). A specialised filopodium extends from the protoplast and penetrates the sporocyst when *C. owczarzaki* comes into contact with *S. mansoni* (Stibbs et al. 1979). It is unknown if *C. owczarzaki* provides a pathogenic burden to its snail host, or if the symbiosis is one of mutualism; however, *C. owczarzaki* can be cultured in axenic media, highlighting the existence of nutritional mechanisms other than *S. mansoni* predation (Stibbs et al. 1979).

Two further filastereans were discovered in the mid-1990s, prior to the recognition of the group. *Ministeria marisola* (Patterson et al. 1993) and *M. vibrans* (Tong 1997) are both cosmopolitan marine bacteriovores, which capture prey through phagocytosis. Both species are characterised by a small spherical protoplast (1.5–3.6 μm in diameter) that possesses an array of straight, rigid microvilli (approximately 9 μm in length) that radiate symmetrically around the cell body (Mylnikov et al. 2019; Patterson et al. 1993). *M. vibrans* may attach to surfaces via a peduncle, previously believed to be a derived flagellum (Cavalier-Smith and Chao 2003); however, this appears to be a plastic trait with many *M. vibrans* cultures lacking the peduncle (Mylnikov et al. 2019). The presence of unequivocally flagellated protoplasts has recently been confirmed in *M. vibrans*; however, less than 1% of observed cells in culture were observed to possess a flagellum (Mylnikov et al. 2019).

Three recently discovered species have further expanded the known morphological and ecological diversity of filastereans. Hehenberger et al. (2017) isolated two freshwater unicellular, flagellated eukaryovores from Chile and Vietnam, which they assigned to the novel genus *Pigoraptor*. Whilst being distinct from *C. owczarzaki* as a flagellated species, both *Pigoraptor* species show clear similarities to *C. owczarzaki*, as all three species feed through the capture of the cytoplasmic contents of their eukaryotic prey and have the capacity to encyst as well as form multicellular aggregates (Hehenberger et al. 2017). A second symbiotic, and potentially parasitic, filasterean was recently identified by Urrutia et al. (2022). In contrast to *C. owczarzaki*, which habituates the haemolymph of its snail host, *Txikispora philomaios* is an intracellular symbiont of multiple genera of amphipod crustaceans. Individual infected host cells may harbour up to 10 *T. philomaios* cells. Host cells become necrotic as a result of infection, with the host individuals becoming lethargic and unresponsive to stimuli (Urrutia et al. 2022). *T. philomaios* cells are typically spherical (2–4 μm in diameter) and frequently are enclosed within a cell wall. Multicellular groupings of 3–4 cells are also observed within host cells; however, it is unclear if these are aggregates of unrelated

cells or the result of clonal cell division. Naked protoplasts, lacking a cell wall, may produce microvilli and a flagellum (Urrutia et al. 2022).

Phylogenetic analyses have consistently recovered Filasterea as a robust clade. The amoeboid symbiont *C. owczarzaki* has been shown to cluster with the flagellated *Pigoraptor* predators, whilst the parasitic *T. philomaios* appears to be the closest known relative of the free-living *Ministeria* species (Hehenberger et al. 2017; Urrutia et al. 2022).

1.5.5 Choanoflagellatea

With approximately 300 species described in over 50 recognised genera, the choanoflagellates are acknowledged as the most speciose group of unicellular holozoans. The first records of choanoflagellates came in the mid-nineteenth century (Ehrenberg 1831, 1838; von Fresenius 1858); however, due to the limitations of microscopy at the time, the microanatomy of the protoplast was not clearly visible. Choanoflagellate cells are characterised by a distinctive collar of 30–40 actin-based microvilli that surrounds a single apical flagellum (see Carr et al. 2008 for a review of choanoflagellate morphology). These features were not observed until the 1860s when James-Clark described three species, whilst also noting the morphological similarity between choanoflagellate cells and the choanocyte feeding cells of poriferans (James-Clark 1866, 1867). Independently Bütschli (1878), Kent (1878, 1880–1882) and von Stein (1878) all recognised that the various species of recently described collared flagellates could be assigned to a single taxonomic group, which they respectively named as Cylicomastiges, Choanoflagellata, and Craspedomonadina. The latter two names have both persisted in the literature, and Kent's Choanoflagellata has been adapted to provide the group's common name. In the most recent major taxonomic revision, Nitsche et al. (2011) raised the taxonomic rank of the group to the class Choanoflagellatea.

The choanoflagellates are aquatic protists, although some species may also be present in heavily hydrated soils, where they play an important role in microbial communities and food webs as filter feeders (Leadbeater 2015). The beating of their flagellum creates a water current that sweeps bacteria and eukaryotic picoplankton onto the collar of microvilli, which acts as a filter by trapping food particles on the outside of collar (Pettitt 2001). Pseudopodia, which emerge from the base of the collar, engulf trapped food particles; however, captured cells may also be translocated down the microvilli to be phagocytosed closer to the cell body. Food vacuoles formed within the pseudopodia move to the base of the cell where digestion occurs (Fig. 1.4; Leadbeater 1983).

Despite the choanoflagellates showing a similar phylogenetic diversity to Metazoa (Richter et al. 2018), the morphology of their protoplasts shows very little variation. Cells tend to be spherical or ovoid with a collar surrounding a posterior flagellum. All species have a periplast (extracellular coat) that encloses at least some part of the protoplast. One function of the periplast in many choanoflagellate species is to secure the cell to a surface. In motile cells the beating of the flagellum drives locomotion and reduces the volume of water flow over the collar (Lighthill 1976). As a result, swimming cells are less efficient feeders in comparison to sedentary cells, which can filter greater water volumes through each beat of the flagellum (Leadbeater 2008a).

Kent's description (1880–1882) of Choanoflagellata contained three families, namely the Codonosigidae, Phalansteriidae, and Salpingoecidae; however, of these, only the Salpingoecidae remains accepted as a valid choanoflagellate taxon (Nitsche et al. 2011). In addition to Salpingoecidae, the Acanthoecidae and Stephanoecidae are morphologically distinct families (Nitsche et al. 2011).

Three different forms of periplast have been described, which were traditionally the basis underpinning choanoflagellate taxonomy. Two forms of periplast are purely organic based in construction, in the glycocalyx and the theca (reviewed in Leadbeater 2015). The glycocalyx is a mucilaginous and flexible investment, which may be made from either one or two layers of fine

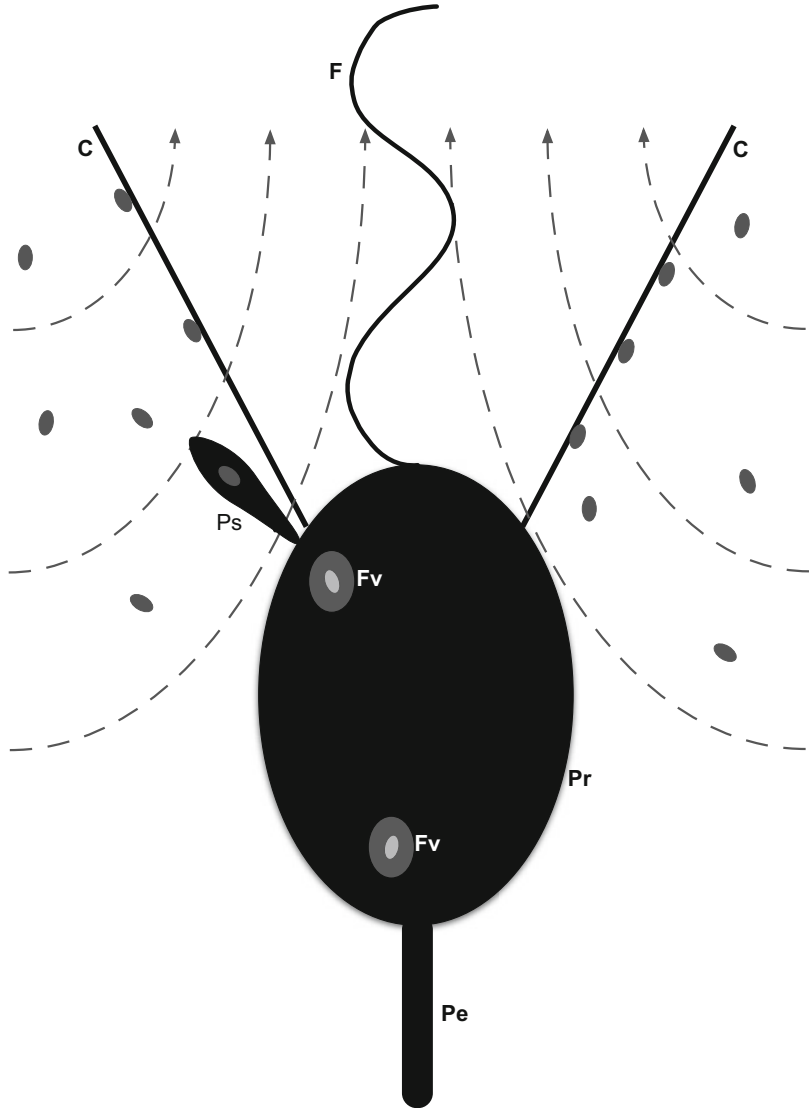

Fig. 1.4 Feeding mechanism of a sedentary choanoflagellate cell. The protoplast (Pr) is secured to a surface by a peduncle (Pe) which extends from an external cell coat. The beating of the flagellum (F) creates a water current (grey dotted arrows) through the microvilli of the collar (C). Food particles (grey ovals) are trapped on the outside of the collar and are phagocytosed by pseudopodia (Ps) which extend from the protoplast at the base of the collar. Food vacuoles (Fv) are transported from the apical pole of the cell to the base of the cell for digestion. Figure based upon Lapage (1925) and Pettitt et al. (2002)

fibrils (Carr et al. 2017). Species across the known diversity of choanoflagellates possess a glycocalyx and the structure appears to be both a universal and ancestral morphological trait of choanoflagellates (Leadbeater 2008a). In many species the glycocalyx extends into a peduncle which secures the protoplast to a surface (Fig. 1.4). Species which exhibit a single cell per peduncle are referred to as *monosigid*, whereas in *codosigid* species multiple clonal cells share the same peduncle. Phylogenetic studies have shown that both the monosigid and codosigid morphologies are polyphyletic, with convergent evolution occurring across the choanoflagellate tree (Carr et al. 2017; Nitsche et al. 2011; Fig. 1.5).

The theca is a more substantial and robust periplast than the glycocalyx, being composed of carbohydrate-based microfibrils (Leadbeater 2008a). The theca exhibits sufficient morphological variation across species for it to have been used as a taxonomic character, with cup, flask, ovoid, and tube shaped thecae being possessed by choanoflagellate species. However, species possessing particular thecae forms do not form monophyletic groups, indicating that theca

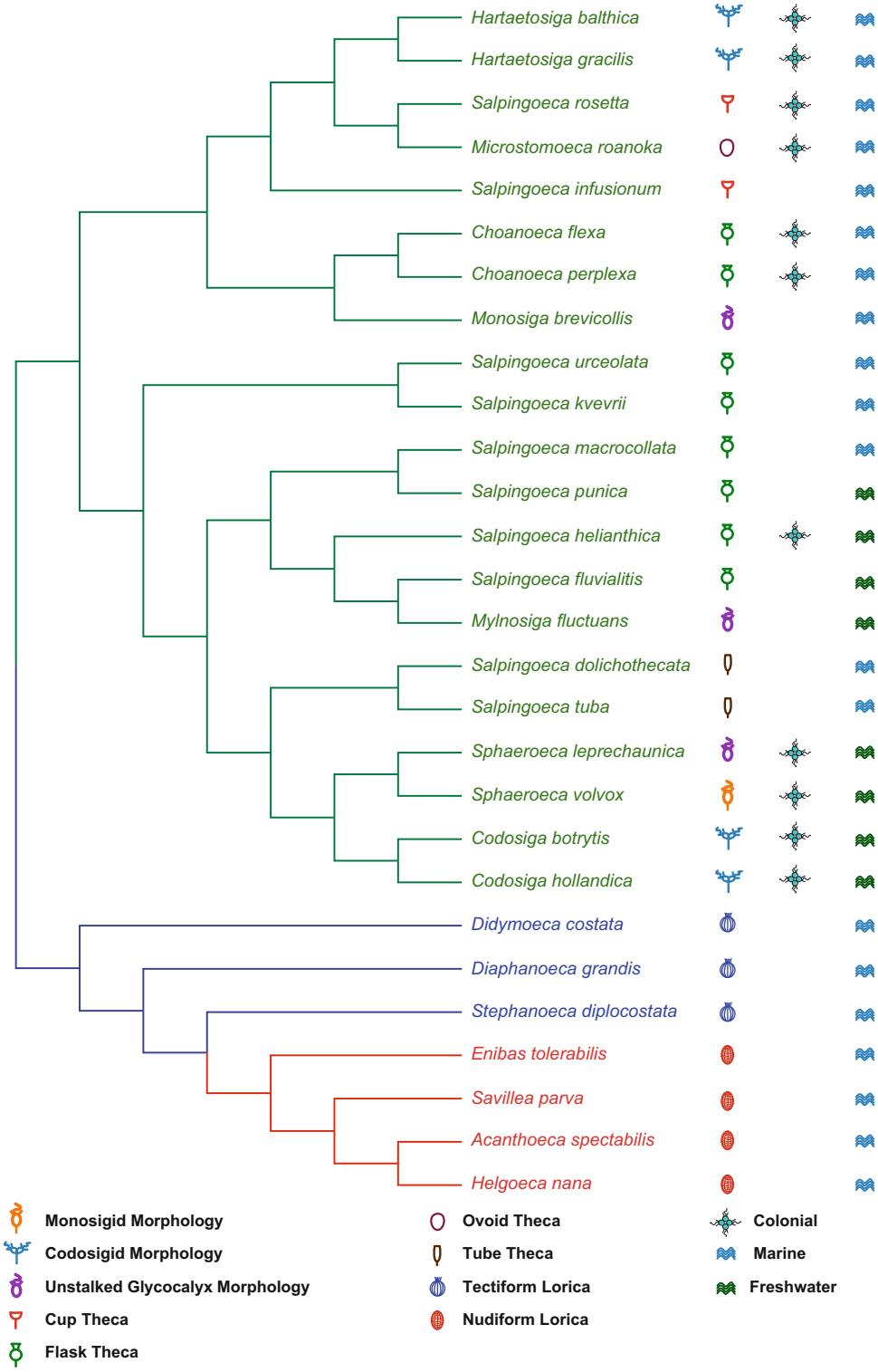

Fig. 1.5 Maximum likelihood 14-gene cladogram of 28 choanoflagellate species. The phylogeny was created with RAxMLGUI 2.0 using a mixed alignment of 4242 nucleotide positions, from partial sequences of SSU and LSU, and 5941 amino acid positions from 40S Ribosomal Protein S8, 60S Ribosomal Protein L10-B, Ribosomal

morphology is a plastic trait with multiple examples of convergent evolution and loss (Carr et al. 2017; Fig. 1.5). Whilst cell division may occur within the confines of the flexible glycocalyx, the rigid nature of the theca means that cells must become amoeboid and emerge before undergoing cell division outside of the thecae (Carr et al. 2008). After cell division, daughter cells from both glycocalyx and theca-bearing species may undergo a motile stage, with collared, flagellated swimming cells (Carr et al. 2008).

Species presenting only a glycocalyx were traditionally assigned to the family Codonosigidae, with theca-bearing species placed into the Salpingoecidae. Molecular phylogenies indicated neither family was monophyletic (Carr et al. 2008; Medina et al. 2003; Steenkamp et al. 2006), and Nitsche et al. (2011) subsumed the polyphyletic Codonosigidae into the paraphyletic Salpingoecidae. The Salpingoecidae is the lone recognised family within the choanoflagellate order Craspedida (originally described by Cavalier-Smith 1997), which contains all species that possess a purely organic-based periplast.

Codonosigidae and Salpingoecidae were both described in Kent's pioneering taxonomic work of the 1880s; however, the family Acanthoecidae was not described until 1965, under botanical nomenclature, with the name Acanthoecaceae (Norris 1965). Due to the diversity of species recognised within the group, the taxon was raised from a family to the order Acanthoecida by Nitsche et al. (2011). The order is characterised by the lorica, a distinctive periplast comprising two layers of silica costal strips which form a basket-like structure around the cell (Fig. 1.6a; Leadbeater et al. 2009). Organic microfibrils, similar to those present in the theca, are frequently arranged between the costal strips; these facilitate the adhesion of the protoplast to the lorica and, in some species, act to funnel water over the collar (Leadbeater 2008a; Leadbeater et al. 2009).

In contrast to the glycocalyx and theca, loricae show considerable morphological variation between species and are considered reliable diagnostic taxonomic characters (Nitsche et al. 2017; Schiwitza and Nitsche 2021). Loricate taxa can be divided into two groups based upon their mechanisms of cell division, as well as the structure of their loricae. *Nudiform* taxa are similar to craspedid species in that after cell division one daughter cell remains with the original periplast, whilst the second daughter cell will undergo a "naked" motile dispersal stage (Manton et al. 1981). The motile stage is relatively brief in nudiform species, compared to that observed in craspedids, and swimming cells rapidly settle onto a surface and construct a new lorica from costal strips that have accumulated in membrane-bound vesicles within the protoplast (Leadbeater 2008b). The loricae of nudiform species possess both longitudinal and helical costal strips but lack transverse rings of strips (Leadbeater et al. 2008). In contrast to the nudiforms, *tectiform* species do not have a naked dispersal stage. Prior to cell division the parental cell exocytoses all of the costal strips required to produce a new, second lorica and stores the strips at the top of its collar (Manton et al. 1981). Cell division occurs within the parental lorica and immediately after cytokinesis one daughter cell exits the lorica, taking the previously deposited costal strips and assembles

Fig. 1.5 (continued) Protein S5, Rps15A, EFL, EF1A, Hsp70, Hsp90, RNA Pol, RNA Pol II, TubA, and TubB. Each gene was aligned individually in MAFFT 7.309 and then concatenated. For nucleotide sites, the TN93 model (Tamura and Nei 1993) was employed with maximum likelihood-derived base frequencies and proportion of invariant sites, as well as a gamma distribution of rate variation. For amino acid sites, the LG model (Le and Gascuel 2008) was employed with maximum likelihood-derived base frequencies, as well as a gamma distribution of rate variation. Parameters were calculated through optimization in the RAxMLGUI. Craspedid species are shown in green and nudiform acanthoecid species are shown in red and tectiform acanthoecids are in blue. The key defines morphological and ecological traits

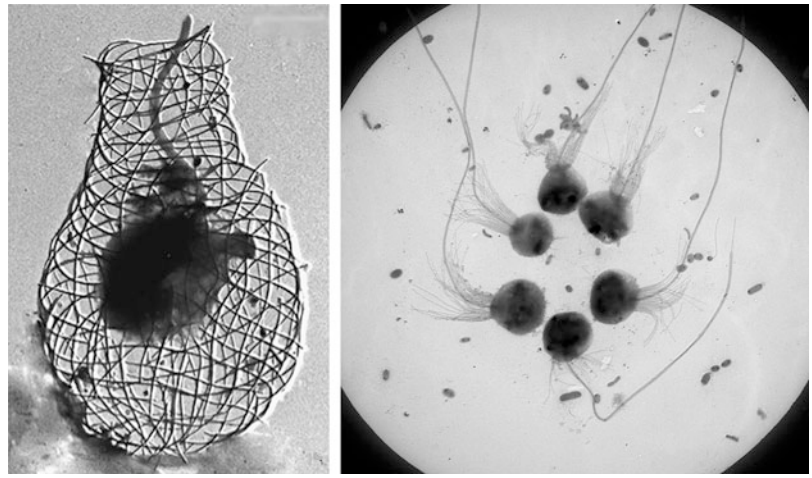

Fig. 1.6 Morphological variation in choanoflagellates. (**a**) The acanthoecid choanoflagellate *Savillea parva* surrounded by a lorica of silica strips. (**b**) Clonal colony of the choanoflagellate *Salpingoeca punica*. The flagella and collars of all cells face outward to maximise prey capture. Pictures presented with kind permission from Barry Leadbeater

its own new lorica (Leadbeater 2010; Manton et al. 1981). Tectiform loricae are often more elaborate than nudiform loricae in their construction, with costal strips being organised in longitudinal, helical, and transverse ring arrangements. The presence of transverse rings provides greater structural stability than helical strips, which has resulted in many tectiform species evolving lightweight, open baskets, allowing species to become planktonic and free-floating within the water column (Leadbeater 2010).

An excess of 100 tectiform species have been described; however, fewer than 10 nudiform species are recognised (Nitsche et al. 2017; Schiwitza and Nitsche 2021). Within Acanthoecida, the nudiforms and tectiforms have both been assigned family status, with the former described as Acanthoecidae and the latter as Stephanoecidae (Nitsche et al. 2011). Whilst both taxa are recognised as distinct families, the phylogenetic relationship between the two is far from clear. The use of nucleotide or amino acid datasets, as well as different phylogenetic methodologies, may result in either two monophyletic sister families or a monophyletic Acanthoecidae being nested within a paraphyletic Stephanoecidae (Carr et al. 2008, 2017). Carr and Leadbeater (2022) proposed that the monophyly of Stephanoecidae is an artefact produced due to convergent phylogenetic signals present in synonymous third codon positions and through a 14-gene phylogeny showed that the nudiform species evolved within a lineage of tectiform choanoflagellates.

Over 70 species of choanoflagellate have publicly available gene sequences; however, the majority of described species lack sequence data and cannot be placed into molecular phylogenies. Environmental DNA studies have indicated that a number of diverse choanoflagellate clades exist that currently have no known representative species (del Campo and Massana 2011; del Campo and Ruiz-Trillo 2013). However, as eDNA surveys tend to be based upon a single gene, the phylogenetic support for the novel groups is frequently weak and fewer novel lineages are recovered when more stringent analyses are undertaken (Carr et al. 2017).

A large number of choanoflagellate species have been observed to switch from a unicellular state to colonial forms (Fig. 1.6b). The number of species known to have the capacity to form colonies is probably an underestimate of the true number, as environmental conditions appear to play a major role in colony formation and laboratory culture conditions may not always provide the necessary cues for coloniality (Carr et al. 2017; Ireland et al. 2020). The morphologies of colonies vary between choanoflagellate taxa and may even vary within the same species (Dayel et al. 2011). Sedentary species possessing either a glycocalyx or theca may generate colonies on a single peduncle, whilst free-swimming species may produce spherical globular colonies or raft-

like chains of cells (Dayel et al. 2011). Such colonies are clonal, rather than aggregate, in nature (Dayel et al. 2011) and also show evidence for cellular differentiation (Laundon et al. 2019). The presence of a spacious, restrictive lorica appears to limit the capacity of acanthoecid species to form connections between individual protoplasts (Carr et al. 2017). However, *Diaphanoeca sphaerica* and *Parvicorbicula socialis* produce colonies through the linkage of the loricae of individual cells (Leadbeater 2015; Thomsen 1982).

Whilst James-Clark (1867) speculated on a close relationship between choanoflagellates and metazoans, the exact relationship between choanoflagellates and other eukaryotic groups was a long source of considerable debate. Since their discovery, it has been speculated that the choanoflagellates may be highly simplified metazoans (Maldonado 2004), a paraphyletic grouping ancestral to both Metazoa and Fungi (Cavalier-Smith 1987), the sister group to Ichthyosporea or *Corallochytrium* (Cavalier-Smith and Chao 2003; Medina et al. 2003; Mendoza et al. 2002), or even a form of algae (Bourrelly 1968; Chadefaud 1960). Early molecular phylogenies lacked phylogenetic support or omitted important holozoan groups (Baker et al. 1999; Jiménez-Guri et al. 2007; Medina et al. 2003; Steenkamp et al. 2006; Wainright et al. 1993). The first phylogenetic analysis to include the major recognised lineages within the Holozoa recovered the choanoflagellates as the sister group of Metazoa (Carr et al. 2008), and this relationship has been consistently recovered in subsequent phylogenomic studies (Hehenberger et al. 2017; Tikhonenkov et al. 2020). Molecular clock analyses indicate that the last common ancestor of choanoflagellates and metazoans existed approximately one billion years ago (Berbee and Taylor 2010; Parfrey et al. 2011).

Based upon traits shared between both Choanoflagellatea and Metazoa, a number of putatively ancestral characteristics have been recovered. Carr et al. (2008) proposed the last common ancestor of both groups was a marine organism that possessed a microvilli collar and apical flagellum employed for filter-feeding. Due to the widespread nature of coloniality in choanoflagellates, it is also possible that the ancestor of both metazoans and choanoflagellates may also have been able to transition between unicellular and colonial states (Carr et al. 2008). The cytoplasmic bridges formed between cells in choanoflagellate colonies resemble those present between metazoan cells and similar bridges may have been present in a putative colonial ancestor of both groups (Dayel et al. 2011).

Based upon 16 species, Carr et al. (2008) highlighted a marine origin of the choanoflagellates, with freshwater species falling into a single phylogenetic group. Subsequent phylogenetic studies have confirmed one major freshwater invasion occurred early in the evolutionary history of choanoflagellates. There have been a limited number of more recent, minor freshwater incursions which appear to involve individual species (Carr et al. 2017; Nitsche et al. 2011; Paul 2012). Marine-freshwater transitions in the group appear to be rare, which is not uncommon in unicellular eukaryotes (Logares et al. 2009), with only a single species, *Salpingoeca macrocollata*, known to have reverted to a marine habitat from freshwater ancestors (Carr et al. 2017).

1.5.6 Metazoa

Uniquely amongst the opisthokonts, Metazoa only contains species that have multicellular stages within their lifecycles. Species exhibit multiple epithelial layers which are linked by connective tissue that generally contains collagen fibres (Cavalier-Smith 1998b). Mitochondrial cristae are flat and metazoans typically possess a 13–19 kb circular mitochondrial chromosome which encodes a suite of 37 highly conserved protein-coding and non-coding RNA genes (Burger et al. 2003). The reduction in mitochondrial genome size must have occurred in the metazoan stem group and contrasts with the large genomes present in Choanoflagellatea, Filasterea, and Ichthyosporea (Burger et al. 2003; Suga et al. 2013). As noted in Sect. 1.4, a similar reduction in the mitochondrial genome

has also occurred within fungi; however, fungal mitochondrial genomes are far more variable in length and gene content in comparison to metazoan genomes (Bullerwell and Lang 2005).

Despite being highly conserved across most metazoans, the ancestral state of a reduced circular mitochondrial chromosome has been independently lost in a number of cnidarian lineages. Linear mitochondrial chromosomes, with telomeres, have evolved in both medusozoan and anthozoan cnidarians (Smith et al. 2011; Stampar et al. 2019). Furthermore, the anthozoan *Protanthea simplex* has its mitochondrial genome divided into two circular mito-chromosomes (Dubin et al. 2019), whilst the myxozoan *Henneguya salminicola* possesses a mitochondria-related organelle but lacks both a mitochondrial genome and many of the genes required for aerobic respiration (Yahalomi et al. 2020).

The number of recognised phyla within Metazoa is disputed, with between 30 and 35 being recognised (Erwin and Valentine, 2013). A clear division in phyla is observed in the number of cell layers present within organisms. In four phyla, the Porifera (sponges), Ctenophora (comb jellies), Placozoa (a phylum consisting of three described marine species possessing highly simplified bodyplans), and Cnidaria (corals, jellyfish, sea anemones, and sea pens), species possess two layers of cells, generally referred to as the endoderm (internal layer) and ectoderm (outer layer), giving rise to the informal name *diploblasts* (Kobayashi et al. 1996).

All other metazoan phyla have body plans that contain a third cell layer, the mesoderm, located between the endoderm and ectoderm, resulting in them being referred to as *triploblasts* (Christen et al. 1991). In at least part of their lifecycles triploblasts show left:right (bilateral) symmetry across the sagittal plane of their body plans, resulting in an alternative, formalised, name of Bilateria (Hatschek 1888). Bilateral symmetry is also present in a number of anthozoan and hydrozoan cnidarians, whilst radial symmetry is observed in ctenophores, as well as some cnidarians and poriferans (Hyman 1940; Malakhov 2016).

Within diploblasts, the internal and external cells layers are separated by a gelatinous matrix termed mesophyll in poriferans (Bonasoro et al. 2001) and mesoglea in both cnidarians and ctenophores (Hyman 1940). The mesoglea of cnidarians and ctenophores contains both muscle cells and a simple neural network (Hyman 1940), but neither of these tissue types are present in either placozoans or poriferans (Burkhardt and Sprecher 2017). A further similarity between cnidarians and ctenophores is the presence of a blind gut, whereas both the placozoans and poriferans lack any form of gut (Hyman 1940). The *Hox* gene family plays a major role in body patterning during bilaterian development, establishing cell identity along the anterior-to-posterior axis (Ferrier and Holland 2001). Cnidarians and placozoans also possess a limited repertoire of *Hox* or *Hox*-like genes, but orthologues have not been identified to date in any poriferan or ctenophore (Ramos et al. 2012; Moroz et al. 2014; Pastrana et al. 2019; Srivastava et al. 2010).

Molecular phylogenetics has yet to produce an unequivocal tree of metazoan phyla; however, it is clear the bilaterians form a robustly supported clade. The Porifera, Ctenophora, Placozoa, and Cnidaria are recovered at the base of the metazoan tree and, as such, can be described as early-branching metazoans. The four early-branching phyla do not form a single clade and their branching order remains unresolved. Cnidaria is now recognised as the sister taxon to Bilateria (Philippe et al. 2019; Rouse et al. 2016; Cannon et al. 2016). Similarities in embryonic gene expression patterns between the groups, as well as the presence of bilateral symmetry in a number of cnidarian species, have led to speculation that the common ancestor of Bilateria and Cnidaria exhibited bilateral symmetry and that the condition has been lost on multiple occasions within cnidarians (Matus et al. 2006). Most phylogenies that contain Placozoa only include one of the three described species, in *Trichoplax adhaerens*; however, the phylum is consistently recovered in phylogenomic studies as the sister group to the

clade of Bilateria+Placozoa (Philippe et al. 2019; Pisani et al. 2015).

Molecular phylogenies of the earliest branching metazoan group have proved to be extremely controversial (Telford 2016; Moroz and Halanych 2016). Recent large phylogenomic studies have recovered either the poriferans (Kapli and Telford 2020; Pick et al. 2010; Pisani et al. 2015; Simion et al. 2017) or the ctenophores (Dunn et al. 2008; Moroz et al. 2014; Ryan et al. 2013; Whelan et al. 2015) as the first branching metazoan lineage. The internal branch leading to Ctenophora in phylogenetic trees is considerably longer than the branches leading to other metazoan phyla, giving rise to concerns that the recovery of ctenophores at the base of the metazoans may be due to long-branch attraction (Kapli and Telford 2020; Simion et al. 2017; Telford 2016). The effects of long-branch artefacts can be weakened through the use of more appropriate substitution models in phylogenetic reconstruction. In general, site-homogenous amino acid substitution models tend to place ctenophores as the basal lineage, whilst more sophisticated site-heterogenous models recover the poriferans as the first branching group. At present, however, it is not clear whether either the homogenous or heterogenous models more accurately estimate amino acid changes within deep metazoan evolution.

This controversy is not simply one of taxonomy, as the correct identification of the first branching metazoan group has a considerable impact on the reconstruction of ancestral traits. The ctenophore-first scenario indicates either that neuronal cells, musculature, and possibly a gut were present in the metazoan last common ancestor (LCA) or that these characters have evolved independently in different metazoan phyla. The distinctive nature of neurons and striated muscle in ctenophores may indicate convergent evolution within metazoans (Moroz and Halanych 2016); however, such convergence could have occurred under both the ctenophore-first and poriferan-first scenarios. The poriferan-first model would suggest that the metazoan LCA was a relatively simple organism which existed prior to the evolution of true tissue layers. The similar morphologies of choanoflagellate protoplasts and choanocytes have been argued to be homologous and therefore present in the metazoan LCA. However, a detailed study of cell ultrastructure and flagella beating, albeit based only upon two species, has raised the possibility that the collared flagellated cells may not be homologous but could have independently evolved in the ancestors of both extant choanoflagellates and poriferans (Mah et al. 2014). Furthermore, gene expression profiles across different poriferan cell types and unicellular holozoans also failed to recover evidence for homology between choanoflagellates and choanocytes (Sogabe et al. 2019). However, as remarked upon by Laundon et al. (2019), the two cell types have been evolving under different evolutionary pressures since the choanoflagellate and metazoan lineages diverged; therefore, differences between choanoflagellates and choanocytes should not be unexpected even if they are homologous.

The majority of metazoan diversity and phyla are assigned to Bilateria. Based upon embryonic development patterns, Grobben (1908) divided Bilateria in protostomes and deuterostomes. During gastrulation, in the former group the primary opening (blastopore) typically develops into the mouth, whilst in the latter group the blastopore develops into the anus. Multigene phylogenies have generally recovered both Deuterostomia and Protostomia as monophyletic (Bourlat et al. 2008; Dunn et al. 2008; Laumer et al. 2019); however, a number of recent phylogenomic studies indicate that the deuterostomes may be paraphyletic (Kapli and Telford 2020; Kapli et al. 2021; Philippe et al. 2019). In these studies, the Chordata (metazoans that possess a notochord) can either cluster with Protostomia, resulting in deuterostome paraphyly, or within a monophyletic Deuterostomia that is separated from Protostomia by a very short internal branch in trees. If the latter topology is correct, it indicates that the deuterostome radiation commenced only a very short time after the origin of Bilateria. In contrast, deuterostome paraphyly would point to the ancestral bilaterian possessing deuterostome traits, such as blastopore fate and the presence of pharyngeal slits, which would

have been subsequently lost in the derived protostomes.

1.6 Genomic Evolution

The advent of high-throughput technologies has made sequencing whole genomes relatively rapid and low cost. RNA-Seq, the sequencing of transcriptomes, is an alternative approach that may be employed when obtaining pure genomic DNA is technically challenging or when sequencing intergenic DNA is not required. As a result, a large body of data now exists on the genomes of the unicellular relatives of both fungi and metazoans. These data are providing new insights into the origins of multicellularity.

Fungi are characterised by the presence of both chitin and 1,3-β-glucan in their cell walls, and the gene complements of unicellular holomycotans have allowed the evolution of these cell wall components to be reconstructed. Chitin synthases are present in fungi and all opisthosporid lineages, indicating chitin walls are an ancestral character (James and Berbee 2012; Torruella et al. 2018). The early-branching opisthosporids, Microsporidia and Cryptomycota, possess chitin, but only in the walls of their infective or resting cyst stages (Torruella et al. 2018). The infective cysts of aphelids also contain chitin and their genomes encode a broad range of genes involved in chitin synthesis, modification, and degradation, some of which may have been acquired through horizontal gene transfer (Torruella et al. 2018). Microsporidians and cryptomycotans lack 1,3-β-glucan, as do chytrid fungi. However, the presence of 1,3-β-glucan synthase genes in the transcriptome of the aphelid *Paraphelidium tribonemae* (Torruella et al. 2018) indicates that 1,3-β-glucan was present in the cell walls of the ancestor of fungi and aphelids, with its subsequent loss in the chytrids. Chang et al. (2015) highlighted a major expansion in pectinase gene diversity within fungal lineages, which they proposed occurred after the divergence of the Cryptomycota lineage in Holomycota. Their explanation for this expansion was that ancient fungi evolved to digest the cell walls of algae. The putative sister group to Fungi is Aphelida, a group made up of algal parasites, and the findings from the Chang et al. (2015) study would indicate that the last common ancestor of Aphelida and Fungi was a flagellated, freshwater parasite of archaeplastid algae. The aphelids have retained this ancestral nutritional mode, as have some extant fungal lineages, whilst other fungi have diversified to parasitise other host groups or become saprotrophs and symbionts.

The parasitic lifecycles of microsporidians and cryptomycotans appear to have resulted in genome reduction; this is seen in terms of genome size and gene content, with both groups showing a loss of genes involved in metabolism (Bass et al. 2018; James et al. 2013). Gene loss has occurred across the microsporidian crown group, with different lineages having lost different ancestral genes; genes involved in processes such as lipid metabolism, glycolysis, and mRNA splicing have been independently lost on multiple occasions across the group (Wadi and Reinke 2020). Microsporidian genomes have also undergone considerable expansions of copies in some gene families, through both individual gene and whole-genome duplication events, resulting in species within the same genus having large numbers of different genes (Reinke et al. 2017). Despite being closely related parasites, the aphelids do not appear to have undergone a similar loss to those observed in cryptomycotans and microsporidians, with *P. tribonemae* showing a similar diversity of metabolic genes to fungi (Torruella et al. 2018); however, further genome sequences are required to determine if *P. tribonemae* is typical of aphelids. In general, holomycotan genomes are characterised by gene reduction, with notable losses of genes involved in signal transduction. The repertoire of metabolism genes began to expand in the common ancestors of Fungi and Aphelida, a process which continued within the fungal stem lineage (Ocaña-Pallarès et al. 2022).

Gene complements from all major holozoan lineages have been accrued over the last decade (Denbo et al. 2018; Fairclough et al. 2013; Hehenberger et al. 2017; López-Escardó et al. 2019; Richter et al. 2018; Suga et al. 2013). One

striking finding from these studies is that many gene families previously believed to be metazoan-specific are now known to have much greater antiquity with origins deeper in Holozoa. Both the metazoan and choanoflagellate stem groups underwent considerable expansions of gene number, as both lineages gained approximately 2000 novel families (Richter et al. 2018), with a burst of innovation in genes involved in both gene transcription and signal transduction occurring in the metazoan stem group (Ocaña-Pallarès et al. 2022). The expansion of the metazoan gene complement appears to have been through both gene duplication and the rearrangement of existing domains, rather than a result of evolving novel protein domains (López-Escardó et al. 2019; Richter et al. 2018). Holozoan genome evolution is a dynamic process, as, in addition to the large-scale gene gain that occurred, over 1500 gene families, including many involved in energy production, as well as the metabolism of amino acids, carbohydrates, and lipids, were lost in premetazoan genomes (Ocaña-Pallarès et al. 2022). Gene loss has continued in metazoans as, in a screen of crown-group taxa, fewer than 40 of the novel genes that evolved in the stem group were found to be universally retained (Richter et al. 2018). As a result of these changes, extant metazoans, unlike fungi, have gene complements which are distinct from their protistan relatives (Ocaña-Pallarès et al. 2022). As the number of genomes from early-branching holomycotan and holozoan genomes increases, ancestral reconstructions of gene loss and gain will become more accurate and fine-tuned in comparison to current studies.

The increased volume of genomic data now available has highlighted the role that horizontal gene transfer has played in the holozoan evolution. Hundreds of genes identified in choanoflagellates, filastereans, ichthyosporeans, and pluriformeans have been shown to be acquired from donor species outside of Opisthokonta (Betat et al. 2015; Carr et al. 2010; Matriano et al. 2021; Southworth et al. 2019; Yue et al. 2013). The identified donor species consist of bacteria and algal alveolates, archaeplastids, haptophytes, rhizarians, and stramenopiles, all of which are prey items of predatory unicellular holozoans. Horizontal transfer may occur if partially degraded chromosomal DNA escapes from the food vacuoles of holozoan predators, passes into the nucleus, and then integrates into chromosomes. Transferred genes have been shown to provide new functions to the recipient cell and, on some occasions, replace ancestral holozoan homologues (Carr and Leadbeater 2022; Yue et al. 2013). Highlighting the dynamic nature of genome evolution, horizontally acquired genes may subsequently be lost in some descendent lineages whilst retained in others (Carr et al. 2010; Suga et al. 2013).

1.7 Conclusions

1.7.1 Molecular Phylogenetic Analyses of Opisthokonta and the Eukaryotic Tree of Life

Advances in both DNA sequencing technology and phylogenetic methodologies have allowed many of the deeper branches in the eukaryotic tree to be resolved. This greater resolution has been somewhat offset by the discovery that a number of orphan lineages, such as Hemimastigophora and Malawimonadida, appear to fall outside of the generally recognised supergroups (Brown et al. 2018; Lax et al. 2018). The ongoing failure to locate the root of eukaryotic tree means that the composition of the Amorphea clade, to which the opisthokonts belong, remains unclear. Despite this uncertainty, the placements of the biflagellate Apusomonadida and uniflagellate Breviatea as the closest relatives of Opisthokonta are now broadly recognised.

Within Opisthokonta, the view that the root lies between Holomycota and Holozoa remains unchallenged, despite numerous novel lineages being identified since Holomycota and Holozoa were originally proposed. Areas of uncertainty remain in Holomycota, with the monophyly or paraphyly of Opisthosporidia still to be robustly resolved (Karpov et al. 2014a, b; Torruella et al. 2018). As a result, the sister group to Fungi is not universally agreed upon. Despite major revisions

to the groups positioned in Holozoa, the choanoflagellates appear to be the closest relatives of Metazoa. The recent inclusion of the predatory *Tunicaraptor* in holozoan phylogenies disrupts the support for the deeper branches in Holozoa (Tikhonenkov et al. 2020); however, it is at present unclear if *Tunicaraptor* sequences are introducing phylogenetic artefacts or allowing a weak, but genuine, phylogenetic signal to be observed within trees. Furthermore, the earliest branching lineages in Metazoa and "classical" Fungi have not been identified, preventing reliable ancestral state reconstruction in both of these major multicellular kingdoms.

1.7.2 Determining Opisthokont Diversity

Like most, if not all, eukaryotic supergroups, the phylogenetic diversity of the opisthokonts remains uncertain. Since the turn of the century, novel lineages have been identified in both Holomycota and Holozoa, with the discovery of small predatory holozoans and slowly evolving microsporidians (Bass et al. 2018; Hehenberger et al. 2017; Tikhonenkov et al. 2020). Furthermore, eDNA studies have highlighted putative enigmatic clades of opisthokonts that have no recognised representatives. Our increasing knowledge of opisthokont diversity is well exemplified by the class Filasterea. Since its description in 2008, the number of known species has doubled; however, phylogenies which include eDNA sequences have identified clades containing unknown aquatic and terrestrial filastereans (Urrutia et al. 2022), highlighting the presence of taxa that have yet to be isolated and described.

The phylogenetic support of eDNA trees is however often weak; therefore, whilst such studies are useful for highlighting novel diversity, their potential for producing accurate phylogenetic placements is more limited. Novel approaches, such as the use of eDNA sequences as probes to identify cells of unknown species (Jones et al. 2011) and single-cell genomics (López-Escardó et al. 2019), may assist in expanding knowledge of opisthokont diversity.

1.7.3 Reconstructing Opisthokont Evolution and the Multiple Origins of Multicellularity

The last decade has seen an explosion in the sequencing of opisthokont genomes and transcriptomes. Whilst the emphasis has been on the multicellular Fungi and Metazoa, the increasing data available from their unicellular relatives have begun to shine a light on the origins of both kingdoms. Candidates for the closest relatives of Fungi and Metazoa have now appear to have been identified, in the Aphelida and Choanoflagellatea, respectively (Carr et al. 2008; Galindo et al. 2021). With two whole genomes and 20 transcriptomes, a greater volume of gene data is currently available for the choanoflagellates (Richter et al. 2018), whilst at the time of writing only a single publicly available transcriptome, from *Paraphelidium tribonemae*, has been generated for the aphelids (Torruella et al. 2018). Comparative genomic and morphological studies have highlighted previously unidentified ancestral traits (Booth et al. 2018; Karpov et al. 2019a; Southworth et al. 2018; Torruella et al. 2018); however, many uncertainties remain. For example, whilst coloniality is widespread across craspedid choanoflagellates it is not clear if this trait was ancestral to choanoflagellates. Morphological similarities between their cytoplasmic bridges, as well as choanoflagellate rosette colonies and metazoan larval blastulae, raise the possibility that the common ancestor of metazoans and choanoflagellates may have exhibited facultative coloniality (Carr et al. 2008; Brunet and King 2017). Intriguingly, bacterial-mediated coloniality is observed in both Holomycota and Holozoa, whilst bacteria have been shown to be essential to the normal development of some metazoans (Fraune and Bosch 2010). Future work may uncover whether this was an ancestral character, or if similar mechanisms have evolved convergently in the two major branches of Opisthokonta.

Functional studies are required to show if there are any common genetic pathways in the development of metazoan bodies and choanoflagellate colonies. Furthermore, such studies may also identify shared developmental pathways across multicellularity in Choanoflagellatea, Filasterea, Fungi, Ichthyosporea, Metazoa, and nucleariid amoebae which may have evolved in the opisthokont stem. The advent of transfection techniques in unicellular opisthokonts (Booth et al. 2018; Koźyczkowska et al. 2021; Parra-Acero et al. 2018) promises to reveal the function of fungal and metazoan multicellularity genes in their protistan relatives.

The ongoing drive to sequence genomes across the eukaryotic tree of life will in the next few years provide accurate gene complements for the LCAs of Holomycota, Holozoa, and Opisthokonta. In combination with studies of gene expression and function, these gene complements can be expected to identify the origins of multicellularity in Fungi and Metazoa, as well as colony formation in opisthokont protists.

References

Adl SM, Simpson AGB, Farmer MA et al (2005) The new higher level of classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol 52:399–451

Adl SM, Simpson AGB, Lane CE, Lukeš J, Bass D et al (2012) The revised classification of eukaryotes. J Eukaryot Microbiol 59:429–493

Adl SM, Bass D, Lane CE et al (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. J Eukaryot Microbiol 66:4–119

Amaral-Zettler LA, Nerad TA, O'Kelly J, Sogin ML (2001) The nucleariid amoebae: more protists at the animal–fungal boundary. J Eukaryot Microbiol 48: 293–297

Baker GC, Beebee TJ, Ragan MA (1999) *Prototheca richardsi*, a pathogen of anuran larvae, is related to a clade of protistan parasites near the animal–fungal divergence. Microbiology 145:1777–1784

Balbiani G (1882) Sur les microsporidies ou psorospermies des Articulés. C R Acad Sci 95:1168–1171

Baldauf SL, Palmer JD (1993) Animals and fungi are each others closest relatives: congruent evidence from multiple proteins. Proc Natl Acad Sci U S A 90:11558–11562

Bass D, Czech L, Williams BAP et al (2018) Clarifying the relationships between Microsporidia and Cryptomycota. J Eukaryot Microbiol 65:773–782

Bauer R, Garnica S, Oberwinkler F et al (2015) Entorrhizomycota: A new fungal phylum reveals new perspectives on the evolution of Fungi. PLoS One 10: e0128183

Beebee TJC, Wong AL-C (1992) *Prototheca*-mediated interference competition between anuran larvae operates by resource diversion. Physiol Zool 65:815–831

Benny GL, O'Donnell K (2000) *Amoebidium parasiticum* is a protozoan, not a Trichomycete. Mycologica 92: 1133–1137

Berbee ML, Taylor JW (2010) Dating the molecular clock in fungi – how close are we? Fungal Biol Rev 24:1–16

Bergsten J (2005) A review of long-branch attraction. Cladistics 21:163–193

Betancur-R R, Naylor GJP, Ortí G (2014) Conserved genes, sampling error, and phylogenomic inference. Syst Biol 63:257–262

Betat H, Mede T, Tretbar S et al (2015) The ancestor of modern Holozoa acquired the CCA-adding enzyme from Alphaproteobacteria by horizontal gene transfer. Nucleic Acids Res 43:6739–6746

Bojko J, Reinke AW, Stentiford GD et al (2022) Microsporidia: a new taxonomic, evolutionary, and ecological synthesis. Trends Parasitol 38:642–659

Bonasoro F, Wilkie IC, Bavestrello G et al (2001) Dynamic structure of the mesohyl in the sponge *Chondrosia reniformis* (Porifera, Demospongiae). Zoomorphology 121:109–121

Booth DS, Szmidt-Middleton H, King N (2018) Transfection of choanoflagellates illuminates their cell biology and the ancestry of animal septins. Mol Biol Cell 29: 3026–3038

Borteiro C, Baldo D, Maronna MM et al (2018) Amphibian parasite of the Order Dermocystida (Ichthyosporea): current knowledge, taxonomic review and new records from Brazil. Zootaxa 4461:499–518

Bourlat SJ, Nielsen C, Economou AD, Telford MJ (2008) Testing the new animal phylogeny: a phylum level molecular analysis of the animal kingdom. Mol Phylogenet Evol 49:23–31

Bourrelly P (1968) Les algues d'eau douce. Tome II: Les algues jaunes et brunes. N. Boubée et Cie, Paris

Brinkmann H, van der Giezen M, Zhou Y et al (2005) An empirical assessment of long-branch attraction artefacts in deep euakaryotic phylogenomics. Syst Biol 54:743–757

Brown MW, Spiegel FW, Silberman JD (2009) Phylogeny of the "forgotten" cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. Mol Biol Evol 26:2699–2709

Brown MW, Sharpe SC, Silberman JD et al (2013) Phylogenomics demonstrates that breviate flagellates are related to opisthokonts and apusomonads. Proc R Soc B 280:20131755

Brown MW, Heiss AA, Kamikawa R et al (2018) Phylogenomics places orphan protistan lineages in a novel eukaryotic super-group. Genome Biol Evol 10: 427–433

Brunet T, King N (2017) The origin of animal multicellularity and cell differentiation. Dev Cell 43:124–140

Bullerwell CE, Lang BF (2005) Fungal evolution: the case of the vanishing mitochondrion. Curr Opin Microbiol 8:362–369

Burger G, Forget L, Zhu Y et al (2003) Unique mitochondrial genome architecture in unicellular relatives of animals. Proc Natl Acad Sci U S A 100:892–897

Burkhardt P, Sprecher SG (2017) Evolutionary origin of synapses and neurons – bridging the gap. BioEssays 39:1700024

Burki F, Shalchian-Tabrizi K, Minge M et al (2007) Phylogenomics reshuffles the eukaryotic supergroups. PLoS One 8:e790

Burki F, Roger AJ, Brown MW, Simpson AGB (2020) The new tree of eukaryotes. Trends Ecol Evol 35:43–55

Bütschli O (1878) Beiträge zur Kenntnis der Flagellaten und einiger verwandter Organismen. Z Wiss Zool Abt A 30:219–281

Cafaro MJ (2005) Eccrinales (Trichomycetes) are not fungi, but a clade of protists at the early divergence of animals and fungi. Mol Phylogenet Evol 35:21–34

Cannon JT, Cossermelli Vellutini B, Smith J III et al (2016) Xenacoelomorpha is the sister group to Nephrozoa. Nature 530:89–93

Carr M, Leadbeater BSC (2022) Re-evaluating loricate choanoflagellate phylogenetics: molecular evidence points to the paraphyly of tectiform species. Protist 173:125924. https://doi.org/10.1016/j.protis.2022.125924

Carr M, Leadbeater BSC, Hassan R et al (2008) Molecular phylogeny of choanoflagellates, the sister group to Metazoa. Proc Natl Acad Sci U S A 105:16641–16646

Carr M, Leadbeater BSC, Baldauf SL (2010) Conserved meiotic genes point to sex in the choanoflagellates. J Eukaryot Microbiol 57:56–62

Carr M, Richter D, Fozouni P et al (2017) A six-gene phylogeny provides new insights into choanoflagellate evolution. Mol Phylogenet Evol 107:166–178

Cavalier-Smith T (1983) A 6-kingdom classification and a unified phylogeny. In: Schenk HEA, Schwemmler WS (eds) Endocytobiology. II. Intracellular space as oligogenetic. Walter de Gruyter, Berlin, pp 1027–1034

Cavalier-Smith T (1987) The origin of fungi and pseudofungi. In: Rayner ADM, Brasierand M, Moore D (eds) Evolutionary biology of fungi. Cambridge University Press, Cambridge, pp 339–353

Cavalier-Smith T (1993) Kingdom protozoa and its 18 phyla. Microbiol Rev 57:953–994

Cavalier-Smith T (1997) Amoeboflagellates and mitochondrial cristae in eukaryote evolution: megasystematics of the new protozoan subkingdoms Eozoa and Neozoa. Arch Protistenkd 147:237–258

Cavalier-Smith T (1998a) Neomonada and the origin of animals and fungi. In: Coombs GH, Vickerman K, Sleigh MA, Warren A (eds) Evolutionary relationships among Protozoa. Chapman & Hall, London, pp 375–407

Cavalier-Smith T (1998b) A revised six kingdom system of life. Biol Rev 73:203–266

Cavalier-Smith T (2002) The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. Int J Syst Evol Biol 52:297–354

Cavalier-Smith T, Allsopp MTEP (1996) *Corallochytrium*, an enigmatic non-flagellate protozoan related to choanoflagellates. Eur J Protistol 32: 306–310

Cavalier-Smith T, Chao EE-Y (2003) Phylogeny of Choanozoa, Apusozoa, and other protozoa and early eukaryote megaevolution. J Mol Evol 56:540–563

Cavalier-Smith T, Chao EE-Y, Oates B (2004) Molecular phylogeny of Amoebozoa and the evolutionary significance of the unikont *Phalansterium*. Eur J Protistol 40:21–48

Cavalier-Smith T, Chao EE, Snell EA et al (2014) Multigene eukaryote phylogeny reveals the likely protozoan ancestors of opisthokonta (animals, fungi, choanozoans) and Amoebozoa. Mol Phylogenet Evol 81:71–85

Chadefaud M (1960) Les végétaux non vasulaires (Cryptogamie). In: Chadeauf M, Emberger I (eds) Traité de botanique systématique, vol 3. Masson et Cie, Paris, pp 1–1018

Chang Y, Wang S, Sekimoto S et al (2015) Phylogenomic analyses indicate that early fungi evolved digesting cells walls of algal ancestors of land plants. Genome Biol Evol 7:1590–1601

Christen R, Ratto A, Baroin A et al (1991) An analysis of the origin of metazoans, using comparisons of partial sequences of the 28S RNA, reveals an early emergence of triploblasts. EMBO J 10:499–503

Cienkowski L (1865) Beiträge zur Kenntniss der Monaden. Arch mikr Anat 1:203–232

Copeland HF (1956) The classification of lower organisms. Pacific Books, Palo Alto, CA

Corsaro D, Walochnik J, Venditti D et al (2014) Rediscovery of *Nucleophaga amoebae*, a novel member of the Rozellomycota. Parasitol Res 113:4491–4498

Dayel MJ, Alegado RA, Fairclough SR et al (2011) Cell differentiation and morphogenesis in the colony-forming choanoflagellate *Salpingoeca rosetta*. Dev Biol 357:73–82

DeBry RW (2005) The systematic component of phylogenetic error as a function of taxonomic sampling under parsimony. Syst Biol 54:432–440

del Campo J, Massana R (2011) Emerging diversity within chrysophytes, choanoflagellates and bicosoecids based upon molecular surveys. Protist 162:435–448

del Campo J, Ruiz-Trillo I (2013) Environmental survey meta-analysis reveals hidden diversity among unicellular opisthokonts. Mol Biol Evol 30:802–805

Denbo S, Aono K, Kai T et al (2018) Revision of the *Capsaspora* genome using read mating information adjusts the view on premetazoan genome. Dev Growth Differ 61:34–42

Derelle R, Torruella G, Klimeš V et al (2015) Bacterial proteins pinpoint a single eukaryotic root. Proc Natl Acad Sci U S A 112:E693–E699

Derelle R, López-Garcia P, Timpano H, Moreira D (2016) A phylogenomic framework to study the diversity and evolution of stramenopiles. Mol Biol Evol 33:2890–2898

Dirren S, Posch T (2016) Promiscuous and specific bacterial symbiont acquisition in the amoeboid genus *Nuclearia* (Opisthokonta). FEMS Microbiol Ecol 92: fiw105

Dirren S, Pitsch G, Silva MOD, Posch T (2017) Grazing of *Nuclearia thermophila* and *Nuclearia delicatula* (Nucleariidae, Opisthokonta) on the toxic cyanobacterium *Planktothrix rubescens*. Eur J Protistol 60:87–101

Dubin A, Chi SI, Emblem Å et al (2019) Deep-water sea anemone with a two-chromosome mitochondrial genome. Gene 692:195–200

Dunn CW, Hejnol A, Matus DQ et al (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452:745–749

Dyková I, Veverkov M, Fiala I et al (2003) *Nuclearia pattersoni* sp. n. (Filosea), a new species of amphizoic amoeba isolated from gills of roach (Rutilus rutilus), and its rickettsial endosymbiont. Folia Parasitol 50: 161–170

Edler D, Klein J, Antonelli A, Silvestro D (2020) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol 12:373–377

Edlind TD, Li J, Visvesvara GS et al (1996) Phylogenetic analysis of β-tubulin sequences from amitochondrial protozoa. Mol Phylo Evol 5:359–367

Ehrenberg CG (1831) Über die Entwickelung und Lebensdauer der Infusionthiere: nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Système, vol 1831. Abh Königl Akad Wiss Berlin, pp 1–154

Ehrenberg CG (1838) Die Infusionsthierchen als vollkommene Organismen. Voss, Leipzig

Embley TM, van der Giezen M, Horner DS et al (2003) Hydrogenosomes, mitochondria and early eukaryotic evolution. IUBMB Life 55:387–395

Erwin DH, Valentine JW (2013) The Cambrian explosion: the construction of animal biodiversity. Roberts & Company, Calgary, AB

Fairclough SR, Chen Z, Kramer E et al (2013) Premetazoan genome evolution and the regulation of cell differentiation in the choanoflagellate *Salpingoeca rosetta*. Genome Biol 14:R15

Fast NM, Logsdon JM Jr, Doolittle WF (1999) Phylogenetic analysis of the TATA box binding protein (TBP) gene from *Nosema locustae*: evidence for a microsporidia-fungi relationship and spliceosomal intron loss. Mol Biol Evol 16:1415–1419

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. Syst Biol 27:401–410

Ferrier DEK, Holland PWH (2001) Ancient origin of the *Hox* gene cluster. Nat Rev Genet 2:33–38

Franzen C (2005) How do microsporidia invade cells. Folia Parasitol 52:36–40

Fraune S, Bosch TCG (2010) Why bacteria matter in animal development and evolution. BioEssays 32: 571–580

Gabaldón T, Völcker E, Torruella G (2022) On the biology, diversity and evolution of nucleariid amoebae (Amorphea, Obazoa, Opisthokonta). Protist 173: 125895

Galindo LJ, Torruella G, Moreira D et al (2019) Combined cultivation and single-cell approaches to the phylogenomics of nucleariid amoebae, close relatives of fungi. Philos Trans R Soc B 374:20190094

Galindo LJ, López-García P, Torruella G et al (2021) Phylogenomics of a new fungal phylum reveals multiple waves of reductive evolution across Holomycota. Nat Commun 12:4973

Galindo LJ, Torruella G, López-García P et al (2022) Phylogenomics supports the monophyly of aphelids and fungi and identifies new molecular synapomorphies. Syst Biol. https://doi.org/10.1093/sysbio/syac054

Gams H (1947) Die Protochlorinae als autotrophe Vorfahren von Pilzen und Tieren? Mikroskopie 2: 383–387

Gawryluk RMR, Tikhonenkov DV, Hehenberger E et al (2019) Non-photosynthetic predators are sister to red algae. Nature 572:241–243

Gill EE, Fast NM (2006) Assessing the microsporidia-fungi relationship: combined phylogenetic analysis of eight genes. Gene 375:103–109

Glockling SL, Marshall WL, Gleason FH (2013) Phylogenetic interpretations and ecological potentials of the Mesomycetozoae (Ichthyosporea). Fungal Ecol 6: 237–247

González-Hernández M, Denoël M, Duffus AJL et al (2010) Dermocystid infection and associated skin lesions in free-living palmate newts (*Lissotriton helveticus*) from Southern France. Parasitol Int 59: 344–350

Gouy M, Li W-H (1989) Molecular phylogeny of the kingdoms Animalia, Plantae and Fungi. Mol Biol Evol 6:109–122

Grau-Bové X, Torruella G, Donachie S et al (2017) Dynamics of genomic innovation in the unicellular ancestry of animals. elife 6:e26036

Gray MW, Burger G, Lang BF (1999) Mitochondrial evolution. Science 283:1476–1481

Grobben K (1908) Die systematische Einteilung des Tierreiches, vol 58. Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien, Vienna, pp 491–511

Gromov BV (2000) Algal parasites of the genera *Aphelidium*, *Amoeboaphelidium* and *Pseudoaphelidium* from the Cienkovski's "Monadinea" group as representatives of new class. Zool Zhurn 79:517–525

Haag KL, James TY, Pombert J-F, Larsson R, Schaer TMM, Refardt D, Ebert D (2014) Evolution of a morphological novelty occurred before genome

compaction in a lineage of extreme parasites. Proc Natl Acad Sci U S A 111:15480–15485
Hampl V, Hug L, Leigh JW et al (2009) Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups". Proc Natl Acad Sci U S A 106:3859–3864
Hasegawa M, Hashimoto T, Adachi J et al (1993) Early branchings in the evolution of eukaryotes: ancient divergence of Entamoeba that lacks mitochondria revealed by protein sequence data. J Mol Evol 36: 380–388
Hatschek B (1888) Lehrbuch der Zoologie: eine morphologische Übersicht des Thierreiches zur Einführung in das Studium dieser Wissenschaft. Georg Fischer, Jena
He D, Fiz-Palacios O, Fu C-J et al (2014) An alternative root for the eukaryotic tree of life. Curr Biol 24:456–470
Hehenberger E, Tikhonenkov DV, Kolisko M et al (2017) Novel predators reshape Holozoan phylogeny and reveal the presence of a two-component signaling system in the ancestor of animals. Curr Biol 27:2043–2050
Heiss AA, Kolisko M, Ekelund F et al (2018) Combined morphological and phylogenomic re-examination of malawimonads, a critical taxon for inferring the evolutionary history of eukaryotes. R Soc Open Sci 5: 171707
Hendy MD, Penny D (1989) A framework for the quantitative study of evolutionary trees. Syst Zool 38:297–309
Herman RL (1984) Ichthyophonus-like infection in newts (*Notophthalmus viridescens* Rafinesque). J Wildl Dis 20:55–56
Herr RA, Ajello L, Taylor JW et al (1999) Phylogenetic analysis of *Rhinosporidium seeberi's* 18S small-subunit ribosomal DNA groups this pathogen among members of the protoctistan Mesomycetozoa clade. J Clin Microbiol 37:2750–2754
Hertel LA, Bayne CJ, Loker ES (2002) The symbiont *Capsaspora owczarzaki*, nov. gen. nov. sp., isolated from three strains of the pulmonate snail *Biomphalaria glabrata* is related to members of the Mesomycetozoa. Int J Parasitol 32:1183–1191
Hirt RP, Logsdon JM Jr, Healy B et al (1999) Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc Natl Acad Sci U S A 96:580–585
Hyman LH (1940) The invertebrates: protozoa through ctenophora. McGraw Hill, New York
Ireland EV, Woznica A, King N (2020) Synergistic cues from diverse bacteria enhance multicellular development in a choanoflagellate. Appl Environ Microbiol 86:e02920
James TY, Berbee ML (2012) No jacket required – new fungal lineage defies dress code. BioEssays 34:94–102
James TY, Kauff F, Schoch L et al (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822
James TY, Pelin A, Bonen L et al (2013) Shared signatures of parasitism and phylogenomics unite Cryptomycota and Microsporidia. Curr Biol 23:1548–1553
James TY, Stajich JE, Hittinger CT, Rokas A (2020) Toward a fully resolved fungal tree of life. Annu Rev Microbiol 74:291–313
James-Clark H (1866) Note on the Infusoria Flagellata and the Spongiae Ciliatae. Am Sci 1:113–114
James-Clark H (1867) On the Spongiae Ciliatae as Infusoria Flagellata; or observations on the structure, animality and relationship of *Leucosolenia botryoides*, Bowerbank. Ann Mag Nat Hist 1:133–142
Jiménez-Guri E, Philippe H, Okamura B, Holland PWH (2007) *Buddenbrockia* is a cnidarian worm. Science 317:116–118
Jones MDM, Forn I, Gadelha C et al (2011) Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474:200–203
Kapli P, Telford MJ (2020) Topology-dependent asymmetry in systematic errors affects phylogenetic placement of Ctenophora and Xenacoelomorpha. Sci Adv 6: eabc5162
Kapli P, Natsidis P, Leite DJ et al (2021) Lack of support for Deuterostomia prompts reinterpretation of the first Bilateria. Sci Adv 7:eabe2741
Karpov SA, Mylnikov AP (1989) Biology and ultrastructure of colorless flagellates Apusomonadida ord. n. Zool Zhurn 68:5–17
Karpov SA, Mikhailov KV, Mirzaeva GS et al (2013) Obligately phagotrophic aphelids turned out to branch with the earliest-diverging Fungi. Protist 164:195–205
Karpov SA, Mamkaeva MA, Aleoshin VV et al (2014a) Morphology, phylogeny, and ecology of the aphelids (Aphelidae, Opisthokonta) and proposal for the new superphylum Opisthosporidia. Front Microbiol 5:112
Karpov SA, Mamkaeva MA, Benzerara K et al (2014b) Molecular phylogeny and ultrastructure of *Aphelidium aff. Melosirae* (Aphelida, Opisthosporidia). Protist 165:512–526
Karpov SA, López-Garcia P, Mamkaeva MA et al (2018) The chytrid-like parasites of algae *Amoeboradix gromovi* gen. et sp. nov. and *Sanchytrium tribonematis* belong to a new fungal lineage. Protist 169:122–140
Karpov SA, Cvetkova VS, Annenkova NV, Vishnyakov AE (2019a) Kinetid structure of *Aphelidium* and *Paraphelidium* (Aphelida) suggests the features of the common ancestor of Fungi and Opisthosporidia. J Eukaryot Microbiol 55:911–924
Karpov SA, Vishnyakov AE, Moreira D, López-García P (2019b) The ultrastructure of *Sanchytrium tribonematis* (Sanchytriaceae, Fungi *incertae sedis*) confirms its close relationship to *Amoeboradix*. J Eukaryot Microbiol 66:892–898
Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780
Keeling PJ, Doolittle WF (1996) Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. Mol Biol Evol 13:1297–1305

Kennedy FA, Buggage RR, Ajello L (1995) Rhinosporidiosis: a description of an unprecedented outbreak in captive swans (*Cygnus* spp.) and a proposal for revision of the ontogenic nomenclature of *Rhinosporidium seeberi*. J Med Vet Mycol 33:157–165

Kent WS (1878) A new field for the microscopist. Pop Sci Rev 2:113–132

Kent WS (1880–1882) A manual of the Infusoria, vol 1–3. D Bogue, London

Kerk D, Gee A, Standish M, Wainwright PO et al (1995) The rosette agent of chinook salmon (*Oncorhynchus tshawytscha*) is closely related to choanoflagellates, as determined by the phylogenetic analyses of its small ribosomal subunit RNA. Mar Biol 122:187–192

Kim E, Simpson AGB, Graham LE (2006) Evolutionary relationships of apusomonads inferred from taxon-rich analyses of 6 nuclear encoded genes. Mol Biol Evol 23:2455–2466

Kobayashi M, Wada H, Satoh N (1996) Early evolution of the Metazoa and phylogenetic status of diploblasts as inferred from amino acid sequence of Elongation Factor-1. Mol Phylo Evol 5:414–422

Kożyczkowska A, Najle S, Ocaña-Pallarès E et al (2021) Stable transfection in protist *Corallochytrium limacisporum* identifies novel cellular features among unicellular animals relatives. Curr Biol 31:1–7

Lang BF, O'Kelly C, Nerad T et al (2002) The closest unicellular relatives of Animals. Curr Biol 12:1773–1778

Lapage G (1925) Notes on the choanoflagellate, *Codosiga botrytis*, Ehrbg. Q J Microsc Sci 69:471–508

Lara E, Moreira D, López-Garcia P (2009) The environmental clade LKM11 and *Rozella* form the deepest branching clade of Fungi. Protist 161:116–121

Laumer CE, Fernández R, Lemer S et al (2019) Revisiting metazoan phylogeny with genomic sampling of all phyla. Proc R Soc B 286:20190831

Laundon D, Larson BT, McDonald K et al (2019) The architecture of cell differentiation in choanoflagellates and sponge choanocytes. PLoS Biol 17:e3000226

Lax G, Eglit Y, Eme L et al (2018) Hemimastigophora is a novel supra-kingdom-level lineage of eukaryotes. Nature 564:410–414

Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. Mol Biol Evol 25:1307–1320

Leadbeater BSC (1983) Distribution and chemistry of microfilaments in choanoflagellates, with special reference to the collar and other tentacle systems. Protistologica 19:157–166

Leadbeater BSC (2008a) Choanoflagellate evolution: the morphological perspective. Protistology 5:256–267

Leadbeater BSC (2008b) Choanoflagellate lorica construction and assembly: The nudiform condition. I. *Savillea* species. Protist 159:259–268

Leadbeater BSC (2010) Choanoflagellate lorica construction and assembly: The tectiform condition. *Volkanus costatus* (=*Diplotheca costata*). Protist 161:160–176

Leadbeater BSC (2015) The Choanoflagellates: evolution, biology and ecology. Cambridge University Press, Cambridge, UK

Leadbeater BSC, Hassan R, Nelson M et al (2008) A new genus, *Helgoeca* gen. nov., for a nudiform choanoflagellate. Eur J Protistol 44:227–237

Leadbeater BSC, Yu QB, Kent J, Stekel DJ (2009) Three-dimensional images of choanoflagellate loricae. Proc R Soc B 276:3–11

Letcher PM, Powell MJ (2018) A taxonomic summary and revision of *Rozella* (*Cryptomycota*). IMA Fungus 9: 383–399

Letcher PM, Powell MJ (2019) A taxonomic summary of *Aphelidiaceae*. IMA Fungus 10:4

Letcher PM, Lopez S, Schmieder R et al (2013) Characterization of *Amoeboaphelidium protococcarum*, an algal parasite new to the Cryptomycota isolated from an outdoor algal pond used for the production of biofuel. PLoS One 8:e56232

Lichtwardt RW (1986) The Trichomycetes: Fungal associates of arthropods. Springer, New York

Lighthill J (1976) Flagellar hydrodynamics. SIAM Rev 18:161–230

Logares R, Bråte J, Bertilsson S et al (2009) Infrequent marine-freshwater transitions in the microbial world. Trends Microbiol 17:414–422

Lohr JN, Laforsch C, Koerner H, Wolinsk J (2010) A *Daphnia* parasite (*Caullerya mesnili*) constitutes a new member of the Ichthyosporea, a group of protists near the animal-fungi divergence. J Eukaryot Microbiol 57:328–336

López-Escardó D, López-García P, Moreira D et al (2017) *Parvularia atlantis* gen. et sp. nov., a nucleariid filose amoeba (Holomycota, Opisthokonta). J Eukaryot Microbiol 65:170–179

López-Escardó D, Grau-Bové X, Guillaumet-Adkins A et al (2019) Reconstruction of protein domain evolution using single-cell amplified genomes of uncultured choanoflagellates sheds light on the origin of animals. Philos Trans R Soc B 374:20190088

Lord JC, Hartzer KL, Kambhampati S (2012) A nuptially transmitted ichthyosporean symbiont of *Tenebrio molitor* (Coleoptera: Tenebrionidae). J Eukaryot Microbiol 59:246–250

Lu Y, Ocaña-Pallarès E, López-Escardó D et al. (2020) Revisiting the phylogenetics position of *Caullerya mesnili* (Ichthyosporea), a common *Daphnia* parasite, based on 22 protein-coding genes. Mol Phylogenet Evol 151:106891

Mah JL, Christensen-Dalsgaard C, Leys SP (2014) Choanoflagellate and choanocyte collar-flagellar systems and the assumption of homology. Evol Dev 16:25–37

Malakhov VV (2016) Symmetry and the tentacular apparatus in Cnidaria. Russ J Mar Biol 42:287–298

Maldonado M (2004) Choanoflagellates, choanocytes, and animal multicellularity. Invert Biol 123:1–22

Manton I, Bremer G, Oates K (1981) Problems of structure and biology in a large collared flagellate (*Diaphanoeca*

grandis Ellis) from Arctic Seas. Proc R Soc B 213:15–26

Marshall WL, Berbee ML (2011) Facing unknowns: living cultures (*Pirum gemmate* gen. nov., sp. nov., and *Abeoforma whisleri*, gen. nov., sp. nov.) from invertebrate digestive tracts represent an undescribed clade within the unicellular opisthokont lineage Ichthyosporea (Mesomycetozoea). Protist 162:33–57

Marshall WL, Berbee ML (2013) Comparative morphology and genealogical delimitation of cryptic species of sympatric isolates of *Sphaeroforma* (Ichthyosporea, Opisthokonta). Protist 164:287–311

Matriano DM, Alegado RA, Conaco C (2021) Detection of horizontal gene transfer in the genome of the choanoflagellate *Salpingoeca rosetta*. Sci Rep UK 11: 5993

Matus DQ, Pang K, Marlow H et al (2006) Molecular evidence for deep evolutionary roots of bilaterality in animal development. Proc Natl Acad Sci U S A 103: 11195–11200

Medina M, Collins AG, Taylor JW et al (2003) Phylogeny of Opisthokonta and the evolution of multicellularity and complexity in Fungi and Metazoa. Int J Astrobiol 2:203–211

Medlin L, Elwood HJ, Stickel S et al (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71:491–499

Mendoza L, Ajello L, Taylor JW (2001) The taxonomic status of *Lacazia loboi* and *Rhinosporidium seeberi* has been finally resolved with the use of molecular tools. Rev Iberoam Micol 18:95–98

Mendoza L, Taylor JW, Ajello L (2002) The Class Mesomycetozoa: a heterogeneous group of microorganisms at the animal–fungal boundary. Annu Rev Microbiol 56:315–344

Mikrjukov KA (1999) Taxonomic revision of scale- bearing Heliozoon-like amoebae (Pompholyxophryidae, Rotosphaerida). Acta Potozool 38:119–131

Minge MA, Silberman JD, Orr RJS et al (2009) Evolutionary position of breviate amoebae and the primary eukaryote divergence. Proc R Soc B 276:597–604

Moroz LL, Halanych KM (2016) A sisterly dispute. Nature 529:286–287

Moroz LL, Kocot KM, Citarella MR et al (2014) The ctenophore genome and the evolutionary origins of neural systems. Nature 510:109–114

Mylnikov AP, Tikhonenkov DV, Karpov SA, Wylezich C (2019) Microscopical studies on *Ministeria vibrans* Tong, 1997 (Filasterea) highlight the cytoskeletal structure of the common ancestor Filasterea, Metazoa and Choanoflagellata. Protist 170:385–396

Nitsche F, Carr M, Arndt H, Leadbeater BSC (2011) Higher level taxonomy and molecular phylogenetics of the Choanoflagellatea. J Eukaryot Microbiol 58: 452–462

Nitsche F, Thomsen HA, Richter DJ (2017) Bridging the gap between morphological species and molecular barcodes – exemplified by loricate choanoflagellates. Eur J Protistol 57:26–37

Norris RE (1965) Neustonic marine Craspedomonadales (Choanoflagellata) from Washington and California. J Protozool 12:589–612

Ocaña-Pallarès E, Williams TA, López-Escardó D et al (2022) Divergent genomic trajectories predate the origin of animals and fungi. Nature 609:747–753

Owczarzak A, Stibbs HH, Bayne CJ (1980) The destruction of *Schistosoma mansoni* mother sporocysts in vitro by amoebae isolated from *Biomphalaria glabrata*: an ultrastructural study. J Invertebr Pathol 35:26–33

Page FC (1987) The classification of 'naked' amoebae (phylum rhizopoda). Arch Protistenkd 133:199–217

Paps J, Medina-Chacón LA, Marshall W, Suga H, Ruiz-Trillo I (2013) Molecular phylogeny of unikonts: new insights into the position of apusomonads and ancyromoands and the internal relationships of opisthokonts. Protist 164:2–12

Parfrey LW, Lahr DJG, Knoll AH, Katz LA (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc Natl Acad Sci U S A 108:13624–13629

Parra-Acero H, Ros-Rocher N, Perez-Posada A et al (2018) Transfection of *Capsaspora owczarzaki*, a close unicellular relative of animals. Development 145:dev162107

Pastrana CC, DeBiasse MB, Ryan JF (2019) Sponges lack ParaHox genes. Genome Bio Evol 11:1250–1257

Patterson DJ (1985) On the organization and affinities of the amoeba, *Pompholyxophrys punicea* Archer, based on ultrastructural examination of individual cells from wild material. J Eukaryot Microbiol 32:241–246

Patterson DJ (1999) The diversity of eukaryotes. Am Nat 154(Suppl):S86–S124

Patterson DJ, Surek B, Melkonian M (1987) The ultrastructure of *Vampyrellidium perforans* Surek & Melkonian and its taxonomic position among the naked filose Amoebae. J Protozool 34:63–67

Patterson DJ, Nygaard K, Steinberg G, Turley CM (1993) Heterotrophic flagellates and other protists associated with oceanic detritus throughout the water column in the mid North Atlantic. J Mar Biol Assoc UK 73:67–95

Paul M (2012) *Acanthocorbis mongolica* nov. spec. – description of the first freshwater loricate choanoflagellate (Acanthoecida) from a Mongolian lake. Eur J Protistol 48:1–8

Pereira CN, Di Rosa I, Fagotti A et al (2005) The pathogen of frogs *Amphibiocystidium ranae* is a member of the order Dermocystida in the class Mesomycetozoea. J Clin Microbiol 43:192–198

Pettitt ME (2001) Prey capture and ingestion in choanoflagellates. PhD thesis, University of Birmingham

Pettitt ME, Orme BAA, Blake JR, Leadbeater BSC (2002) The hydrodynamics of filter feeding in choanoflagellates. Eur J Protistol 38:313–332

Philippe H, Brinkmann H, Lavrov DV et al (2011) Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol 9:e1000602

Philippe H, Poustka AJ, Chiodin M et al (2019) Mitigating anticipated effects of systematic errors supports sister-group relationship between Xenacoelomorpha and Amubulacraria. Curr Biol 29:1818–1826

Pick KS, Philippe H, Schreiber F et al (2010) Improved phylogenomic taxon sampling noticeably affects nonbilaterian relationships. Mol Biol Evol 27:1983–1987

Pisani D, Pett W, Dohrmann M et al (2015) Genomic data do not support comb jellies as the sister group to all other animals. Proc Natl Acad Sci U S A 112:15402–15407

Posada D (2003) Using Modeltest and PAUP* to select a model of nucleotide substitution, current protocols in bioinformatics. Wiley, New York

Powell MJ (1984) Fine structure of the unwalled thallus of *Rozella polyphagia* in its host *Polyphagus euglenae*. Mycologia 76:1039–1048

Quandt CA, Beaudet D, Corsaro D et al (2017) The genome of an intracellular parasite, *Paramicrosporidium saccamoebae*, reveals alternative adaptations to obligate intracellular parasitism. eLife 6: e29594

Raffel TR, Dillard JR, Hudson PJ (2006) Field evidence for leech-borne transmission of amphibian *Ichthyophonus* sp. J Parasitol 92:1256–1264

Ragan MA, Goggins CL, Cawthorn RJ et al (1996) A novel clade of protistan parasites near the animal–fungal divergence. Proc Natl Acad Sci U S A 93:11907–11912

Raghu-kumar S (1987) Occurrence of the thraustochytrid, *Corallochytrium limacisporum* gen. et sp. nov. in the coral reef lagoons of the Lakshadweep Islands in the Arabian Sea. Bot Mar 30:83–89

Rainer H (1968) Urtiere, Protozoa, Wurzelfu ?ßler, Rhizopoda, Sonnentierchen, Heliozoa. Systematik und Taxonomie, Biologie, Verbreitung und Ökologie der Arten der Erde. In: Dahl M, Peus F (eds) Die Tierwelt Deutschlands und der angrenzenden Meeresteile, vol 56. VEB Gustav Fischer Verlag, Jena

Ramaiah N (2006) A review on fungal diseases of algae, marine fishes, shrimps and corals. Indian J Mar Sci 35: 380–387

Ramos OM, Barker D, Ferrier DEK (2012) Ghost loci imply *Hox* and *ParaHox* existence in the last common ancestor of animals. Curr Biol 22:1951–1956

Reinke AW, Balla KM, Bennett EJ et al (2017) Identification of microsporidia host-exposed proteins reveals a repertoire of rapidly evolving proteins. Nat Commun 8: 14023

Reynolds NK, Smith ME, Tretter ED et al (2017) Resolving relationships at the animal-fungal divergence: a molecular phylogenetic study of the protist trichomycetes (Ichthyosporea, Eccrinida). Mol Phylogenet Evol 109:447–464

Richards TA, Leonard G, Wideman JG (2017) What defines the "kingdom" Fungi. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.FUNK-0044-2017

Richter DJ, Fozouni P, Eisen MB, King N (2018) Gene family innovation, conversation and loss on the animal stem lineage. eLife 7:e34226

Ronquist F, Teslenko M, van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Rouse GW, Wilson NG, Carvajal JI et al (2016) New deep-sea species of *Xenoturbella* and the position of Xenacoelomorpha. Nature 530:94–97

Rowley JJL, Gleason FH, Andreou D et al (2013) Impacts of mesomycetozoean parasites on amphibian and freshwater fish populations. Fungal Biol Rev 27:100–111

Ruiz-Trillo I, Inagaki Y, Davis LA et al (2004) *Capsaspora owczarzaki* is an independent opisthokont lineage. Curr Biol 14:R946–R947

Ruiz-Trillo I, Lane CE, Archibald JM et al (2006) Insights into the evolutionary origin and genome architecture of the unicellular opisthokonts *Capsaspora owczarzaki* and *Sphaeroforma arctica*. J Eukaryot Microbiol 53: 379–384

Ryan JF, Pang K, Schnitzler CE et al (2013) The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. Science 342: 1242592

Schilde C, Schaap P (2013) The Amoebozoa. Methods Mol Biol 983:1–15

Schiwitza S, Nitsche F (2021) A needle in the haystack – mapping sequences to morphology exemplified by the loricate choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida, Acanthoecidae). Protist 172:125782

Sebé-Pedrós A, Irimia M, del Campo J et al (2013) Regulated aggregative multicellularity in a close unicellular relative of metazoa. eLife 2:e01287

Seeber GR (1900) Un nuevo esporozoario parásito del hombre. Dos casos encontrados en pólipos nasales. Graduation thesis, Facultad de Ciencias Médicas, Universidad Nacional de Buenos Aires, Buenos Aires

Shalchian-Tabrizi K, Minge MA, Espelund M et al (2008) Multigene phylogeny of Choanozoa and the origin of animals. PLoS One 3:e2098

Simion P, Philipe H, Baurain D et al (2017) A large and consistent phylogenomic dataset supports sponges as the sister group to all other animals. Curr Biol 27:958–967

Smith DR, Kayal E, Yanagihara AA et al (2011) First complete mitochondrial genome sequence from a box jellyfish reveals a highly fragmented linear architecture and insights into telomere evolution. Genome Biol Evol 4:52–58

Sogabe S, Hatleberg WL, Kocot KM et al (2019) Pluripotency and the origin of animal multicellularity. Nature 570:519–522

Sogin ML, Elwood HJ, Gunderson JH (1986) Evolutionary diversity of eukaryotic small-subunit rRNA genes. Proc Natl Acad Sci U S A 83:1383–1387

Southworth JS, Armitage P, Fallon B et al (2018) Patterns of ancestral animal codon usage bias revealed through holozoan protists. Mol Biol Evol 35:2499–2511

Southworth JS, Grace CA, Marron AO et al (2019) A genomic survey of transposable elements in the choanoflagellate *Salpingoeca rosetta* reveals selection on codon usage. Mob DNA 10:44

Spanggaard B, Skouboe P, Rossen L et al (1996) Phylogenetic relationships of the intercellular fish pathogen *Ichthyophonus hoferi*, and fungi, choanoflagellates and the rosette agent. Mar Biol 126:109–115

Spatafora JW, Chang Y, Benny GL et al (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1047

Srivastava M, Simakov O, Chapman J et al (2010) The *Amphimedon queenslandica* genome and the evolution of animal complexity. Nature 466:720–726

von Fresenius G (1858) Beiträge zur Kenntnis mikroskopischer Organismen. Abh Senckenb Naturforsch Ges 2:211–242

Stechmann A, Cavalier-Smith T (2003a) The root of the eukaryote tree pinpointed. Curr Biol 13:R665–R666

Stechmann A, Cavalier-Smith T (2003b) Phylogenetic analysis of eukaryotes using heat-shock protein Hsp90. J Mol Evol 57:408–419

Stampar SN, Broe MB, Macrander J et al (2019) Linear mitochondrial genoe in Anthozoa (Cnidaria): a case study in Ceriantharia. Sci Rep UK 9:6094

Strassert JFH, Jamy M, Mylnikov AP et al (2019) New phylogenomic analysis of the enigmatic phylum Telonemia further resolves the eukaryote tree of life. Mol Biol Evol 36:757–765

Steenkamp ET, Wright J, Baldauf SL (2006) The protistan origins of animals and fungi. Mol Biol Evol 23:93–106

von Stein FR (1878) Der Organismus der Infusionthiere. W. Engelmann, Leipzig

Steinert M, Novikoff AB (1960) The existence of a cytostome and the occurrence of pinocytosis in the trypanosome, *Trypanosoma mega*. J Biophys Biochem Cytol 8:563–569

Stibbs HH, Owczarzak A, Bayne CJ, DeWan P (1979) Schistosome sporocyst-killing amoebae isolated from *Biomphalaria glabrata*. J Invertebr Pathol 33:159–170

Suga H, Chen Z, de Mendoza A et al (2013) The *Capsaspora* genome reveals a complex unicellular prehistory of animals. Nat Commun 4:2325

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Tedersoo L, Sánchez-Ramírez S, Köljalg U et al (2018) High-level classification of the Fungi and a tool for evolutionary ecological analyses. Fungal Divers 90:135–159

Telford MJ (2016) Fighting over a comb. Nature 529:286

Thompson LD (2016) Rhinosporidiosis. Ear Nose Throat J 95:101

Thomsen HA (1982) Planktonic choanoflagellates from Disco Bugt, West Greenland, with a survey of the marine nanoplankton of the area. Meddelelser om Gronland Bioscience 8:3–35

Tikhonenkov DV, Mikhailov KV, Hehenberger E et al (2020) New lineage of microbial predators adds complexity to reconstructing the evolutionary origin of animals. Curr Biol 30:4500–4509

Tong SM (1997) Heterotrophic flagellates and other protists from Southampton Water, U.K. Ophelia 47:71–131

Toret C, Picco A, Boiero-Sanders M et al (2022) The cellular slime mold *Fonticula alba* forms a dynamic, multicellular collective while feeding on bacteria. Curr Biol 32:1961–1973

Torruella G, Derelle R, Paps J et al (2012) Phylogenetic relationships within the Opisthokonta based on phylogenomic analyses of conserved single-copy protein domains. Mol Biol Evol 29:531–544

Torruella G, Grau-Bové X, Moreira D et al (2018) Global transcriptome analysis of the aphelid *Paraphelidium tribonemae* supports the phagotrophic origin of fungi. Commun Biol 1:231

Torruella G, Mendoza A, Grau-Bové X et al (2015) Phylogenomics reveals convergent evolution of lifestyles in close relatives of animals and fungi. Curr Biol 25:2404–2410

Trotter MJ, Whisler HC (1965) Chemical composition of the cell wall of *Amoebidium parasiticum*. Can J Bot 43:869e876

Urrutia A, Mitsi K, Foster R et al (2022) *Txikispora philomaios* n. sp., n. g., a micro-eukaryotic pathogen of amphipods, reveals parasitism and hidden diversity in Class Filasterea. J Eukaryot Microbiol 69:e12875

Ustinova I, Krienitz L, Huss VA (2000) *Hyaloraphidium curvatum* is not a green alga, but a lower fungus; *Amoebidium parasiticum* is not a fungus, but a member of the DRIPs. Protist 151:253–262

Verni F, Gualtieri P (1997) Feeding behaviour in ciliated protists. Micron 28:487–504

Vickerman K, Darbyshire JF, Ogden CG (1974) *Apusomonas proboscidae* Aléxéieff 1924, an unusual phagotrophic flagellate from soil. Arch Protistenkd 116:254–269

Vischer W (1945) Über einen pilzähnlichen, autotrophen Mikroorganismus, *Chlorochytridion*, einige neue Protococcales und die systematische Bedeutun der Chloroplasten. Verhandlungen der Naturforschenden Gesellschaft in Basel 6:41–49

Vossbrinck CR, Maddox JV, Friedman S et al (1987) Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature 326:411–414

Wadi L, Reinke AW (2020) Evolution of microsporidia: An extremely successful group of eukaryotic intracellular parasites. PLoS Pathog 16:e1008276

Wainright PO, Hinkle G, Sogin ML, Stickel SK (1993) Monophyletic origins of the Metazoa: an evolutionary link with fungi. Science 260:340–342

Whelan NV, Kocot KM, Moroz LL, Halanych KM (2015) Error, signal, and the placement of Ctenophora sister to all other animals. Proc Natl Acad Sci U S A 112:5773–5578

Williams TA, Embley TM (2014) Archaeal "Dark Matter" and the origin of eukaryotes. Genome Biol Evol 6: 474–481

Worley AC, Raper KB, Hohl M (1979) *Fonticula alba*: a new cellular slime mold (Acrasiomycetes). Mycologia 71:746–760

Wylezich C, Karpov SA, Mylnikov AP et al (2012) Ecologically relevant choanoflagellates collected from hypoxic water masses of the Baltic Sea have untypical mitochondrial cristae. BMC Microbiol 12:271

Yahalomi D, Atkinson SD, Neuhof M et al (2020) A cnidarian parasite of salmon (Myxozoa: *Henneguya*) lacks a mitochondrial genome. Proc Natl Acad Sci U S A 117:5358–5363

Yoshida M, Nakayama T, Inouye I (2009) *Nuclearia thermophila* sp. nov. (Nucleariidae), a new nucleariid species isolated from Yunoko Lake in Nikko (Japan). Eur J Protistol 45:147–155

Young AD, Gillung JP (2020) Phylogenomics – principles, opportunities and pitfalls of big-data phylogenetics. Syst Entomol 45:225–247

Yue J, Sun G, Hu X, Huang J (2013) The scale and evolutionary significance of horizontal gene transfer in the choanoflagellate *Monosiga brevicollis*. BMC Genomics 14:729

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH et al (2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541:353–358

Zopf W (1885) Zur Morphologie und Biologie der niederen Pilztiere (Monadinen). University of Strasbourg, Leipzig

Zwickl DJ, Hillis DM (2002) Increased taxon sampling greatly reduces phylogenetic error. Syst Biol 51:588–598