The crustacean parasites *Ellobiopsis* Caullery, 1910 and *Thalassomyces* Niezabitowski, 1913 form a monophyletic divergent clade within the Alveolata

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Abstract The Ellobiopsidae are enigmatic parasites of crustaceans that have been grouped together exclusively on the basis of morphological similarities. Ultrastructural studies have revealed their affiliation within the alveolates, which was confirmed by the phylogenetic analysis of the ribosomal RNA gene (SSU rDNA) sequences of two species of *Thalassomyces* Niezabitowski, 1913. However, their precise systematic position within this group remains unresolved, since they could not be definitively allied with any particular alveolate group. To better determine the systematic position of ellobiopsids by molecular phylogeny, we sequenced the SSU rDNA from the type-species of the Ellobiopsidae, *Ellobiopsis chattoni* Caullery, 1910. We found *E. chattoni* infecting various copepod hosts, *Acartia clausi* Giesbrecht, *Centropages typicus* Kröyer and *Clausocalanus* sp., in the Bay of Marseille, NW Mediterranean Sea, which allowed us to study several stages of the parasite development. A single unicellular multinucleate specimen provided two different sequences of the SSU rDNA gene, indicating the existence of polymorphism at this locus within single individuals. *Ellobiopsis* Caullery, 1910 and *Thalassomyces* formed a very divergent and well-supported clade in phylogenetic analyses. This clade appears to be more closely related to the dinoflagellates (including the Syndiniales/ Marine Alveolate Group II and the Dinokaryota) and Marine Alveolate Group I than to the other alveolates (Ciliophora, Perkinsozoa and Apicomplexa).

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Introduction

The ellobiopsids, parasites of crustaceans, are multinucleate protists with a trophomere that possesses an absorbing 'root'. The trophomere root penetrates the host and reproductive structures, the gonomeres, protrude through, or are attached to, the host carapace. They look superficially like fungi, each individual consisting of one or several tubes which are generally transversally septate and ramified. Ellobiopsid parasites consist of five genera grouped on the basis of morphological similarities and distinguished by criteria, including the presence or absence of the attachment organs, the number, size and shape

of trophomeres and gonomeres, the host type, and the position where they settle on the host. Four genera, *Ellobiocystis* Coutière, 1911, *Ellobiopsis* Caullery, 1910, *Parallobiopsis* Collin, 1913 and *Thalassomyces* Niezabitowski, 1913 (syns *Amallocystis* Fage, 1936; *Staphylocystis* Coutière, 1911), are chiefly ectoparasites of pelagic crustaceans, although they also include epibionts (some species of *Ellobiocystis*), whereas the type-species of the monotypic *Rhizellobiopsis* Zachs, 1923 parasitises a benthic polychaetous worm. Currently, the group consists of about 20 species, most of them belonging to *Thalassomyces* (see Shields, 1994).

The first ellobiopsid described, the type-species, *Ellobiopsis chattoni* Caullery, 1910, was an ectoparasite of a calanoid copepod in the NW Mediterranean Sea (Caullery, 1910). *Ellobiopsis* spp. are widespread, infecting several marine and freshwater copepod species; they adversely affect fertility in females (Albaina & Irigoien, 2006) and cause feminisation in males (Théodoridès, 1989; Shields, 1994). This genus consists of three species, *E. chattoni*, *E. elongata* Steuer, 1932 and *E. fagei* Hovasse, 1951, which share a characteristic morphology with a well-defined stalk, a trophomere and one (*E. chattoni*) or two (*E. elongata*) gonomeres. *E. fagei*, with features intermediate between the other two species, has been suggested as being synonymous with *E. chattoni* (see Shields, 1994). The *Ellobiopsis* life-cycle follows several steps. Firstly, a dispersion phase, consisting of spores which settle onto the setae of the new host's appendages, where they metamorphose into trophomeres that extrude a root-like organelle through the copepod's cuticle. When the parasite body reaches a certain size, it becomes transversally septate and forms the gonomere in the distal segment. The distal gonomere becomes granulated and leads progressively to the formation of small groups of pre-spores that fall from the segregating mass. Each bud undergoes a series of divisions to form spores. Although the spores were reported to be flagellate (Shields, 1994), there is no evidence with regard to the number, and type of insertion, of the flagella in *Ellobiopsis* (see Hovasse, 1952).

Ellobiopsis was tentatively placed within the parasitic dinoflagellates (Caullery, 1910; Chatton, 1920; Reichenow, 1930). Hovasse (1926) observed that *Parallobiopsis coutieri* Collin, 1913 produce uniflagellate zoospores, which led him to conclude that ellobiopsid parasites were not dinoflagellates but a separate group in the Flagellata *incertae sedis*. Niezabitowski (1913) described *Thalassomyces* as fungi, and other authors agreed that the whole group was probably fungal (Jepps, 1937; Grassé, 1952; Dick, 2001), but ultrastructural studies have shown that the trophomere is not surrounded by a cell wall, as might be expected for a fungus. In contrast, *Thalassomyces* is bounded by a complex pellicle occasionally interrupted by flask-shaped organelles resembling mucocysts (Galt & Whisler, 1970; Whisler, 1990). Both the pellicle and the zoospore differentiation suggest an affiliation within the alveolates, the entire group being characterised by the presence of membrane-bound flattened vesicles named alveoli (Cavalier-Smith, 1993). Galt & Whisler (1970) placed the ellobiopsid parasites among the dinoflagellates, because the spores of *Thalassomyces marsupii* Kane, 1964 possess one flagellum directed posteriorly and the other circumferentially, which is reminiscent of dinoflagellate flagellar structure. However, the spore of *Thalassomyces* lacks an obvious sulcus and cingulum and also the highly organised interphasic chromosomes of the dinokaryotic dinoflagellates. Schweikert & Elbrächter (2006) observed in *Ellobiopsis* unique ultrastructural features, such as a peculiar organisation of the centrioles that is unknown in other protist groups, so that they discarded any relationship with the dinoflagellates. Cavalier-Smith & Chao (2004) proposed that the infraphylum Ellobiopsa Cavalier-Smith, which, together with the Dinoflagellata Bütschli, comprise the subphylum Dinozoa Cavalier-Smith.

The Alveolata Cavalier-Smith, 1991, one of the major eukaryotic lineages, is composed of three major classes: the Ciliophora Doflein, 1901, the Apicomplexa Levine, 1970 and the Dinoflagellata Bütschli, 1885, and minor groups, such as the Perkinsozoa Norén, Moestrup & Rehnstam-Holm, 1999 (*Perkinsus* Levine, 1978/*Parvilucifera* Norén & Moestrup, 1999), *Colpodella* Cienkowski, 1865 and *Rastrimonas* Brugerolle, 2003, among others (Cavalier-Smith & Chao, 2004). The Apicomplexa and the Perkinsozoa are obligate parasites, while ciliates and dinoflagellates include both parasitic and free-living species. In general, the parasites tend to simplify their morphologies and lose diagnostic morphological characters used for classification. The advent of molecular techniques has provided new tools to clarify the

evolutionary relationships among protist species, including parasites, with the small subunit ribosomal RNA gene (SSU rDNA) as the most popular marker. In the last few years, environmental molecular surveys of SSU rRNA genes has revealed the existence of novel alveolate sequences indicating two large and diverse clades that were initially named Marine Alveolate Groups I and II (MAGI and MAGII, respectively) (López-García et al., 2001; Moreira & López-García, 2002). Subsequently, it has been shown that these groups correspond to parasitic dinoflagellates previously placed within the order Syndiniales Loeblich based on a few morphologically characterised representatives for which SSU rDNA sequences are available (*Amoebophrya* Koeppen, 1894, *Syndinium* Chatton, 1920, *Hematodinium* Chatton & Poisson, 1931, *Duboscquella* Chatton, 1920 and *Ichthyodinium* Hollande & Cachon, 1952) (Skovgaard et al., 2005; Harada et al., 2007; Mori et al., 2007). Silberman et al. (2004) placed *Thalassomyces* within the alveolates using SSU rDNA phylogenetic analysis. However, they concluded that their analyses were unable to resolve whether *Thalassomyces* belonged to a described lineage (e.g. Perkinsozoa or Dinoflagellata) or represented a novel phylum within the alveolates. Moreover, it remains unclear whether the different ellobiopsids form a monophyletic assemblage or not. In order to determine the phylogenetic position of *Ellobiopsis* and to test whether this genus is phylogenetically related to *Thalassomyces*, we amplified, cloned and sequenced the SSU rDNA from the type-species, *E. chattoni*, collected from its type-locality, the NW Mediterranean Sea.

Materials and methods

Sampling and isolation

Infected copepods were collected in the SOMLIT-Marseille station in the Bay of Marseille (43°14′30″N, 05°17′30″E; bottom depth 60 m), using a 200 μm WP2 plankton net mounted with filtering cod ends. Hauls were carried out between 55 m and the surface at 1 m s^{-1}. The host copepods were identified according to Rose (1933). Infected copepods were isolated and placed individually in vials with absolute ethanol. The specimens of *Ellobiopsis* and host were photographed with a digital camera (Nikon Coolpix E995) connected to an inverted microscope (Nikon Eclipse

TE200). In order to avoid contamination with copepod DNA, the parasite was separated from the host by cutting off the root or separating the gonomere from the trophont. Then, this was micropipetted individually using a fine capillary into another chamber and washed three times with ethanol. Finally, the specimen was picked up and placed into a 1.5 ml Eppendorf tube filled with absolute ethanol. Samples were kept at laboratory temperature and in darkness until the molecular analysis could be performed.

PCR amplification of small subunit rRNA genes (SSU rDNAs) and sequencing

The ethanol-fixed specimen was centrifuged for 5 minutes at 3,000 rpm. Ethanol was removed by evaporation in a vacuum desiccator and the specimen resuspended directly in 50 μl of Ex TaKaRa (TaKaRa) PCR reaction mix containing 10 pmol of the eukaryotic-specific SSU rDNA primers EK-42F (5′-CTC AARGAYTAAGCCATGCA-3′) and EK-1520R (5′-CYGCAGGTTCACCTAC-3′). PCR reaction conditions were: 2 min denaturation at 94°C; 10 cycles of 'touch-down' PCR (denaturation at 94°C for 15 s; a 30-s annealing step at decreasing temperature from 65 down to 55°C (1°C decrease with each cycle), extension at 72°C for 2 min); 20 additional cycles with 55°C of annealing temperature; and a final elongation step of 7 min at 72°C. A nested PCR reaction was then carried out using 2 μl of the first PCR reaction in a GoTaq (Promega) polymerase reaction mix containing the eukaryotic-specific primers EK-82F (5′-GAAACTGCGAATGGCTC-3′) and EK-1498R (5′-CACCTACGGAAACCTTGTTA-3′) and similar PCR conditions as above except for an increase in the total number of cycles from 30 to 35. The amplified product was subsequently cloned using the Topo TA Cloning system (Invitrogen) following the instructions provided by the manufacturers. Twelve clones were picked and the corresponding insert amplified using vector primers. Amplicons of the expected size were fully sequenced (Cogenics, Meylan, France) with vector primers.

Phylogenetic analyses

Sequences were compared by BLAST (Altschul et al., 1997) to those in the GenBank database. Using the profile alignment option of MUSCLE 3.7 (Edgar, 2004), sequences were aligned to a large multiple sequence alignment containing 1,200 published

alveolate complete or nearly complete SSU rDNA sequences, which included representatives of the major alveolate groups available in public databases. The resulting alignment was manually inspected with the program ED of the MUST package (Philippe, 1993). Ambiguously aligned regions and gaps were excluded from phylogenetic analyses. Preliminary phylogenetic trees with all sequences were constructed using the Neighbor Joining (NJ) method (Saitou & Nei, 1987) implemented in the MUST package (Philippe, 1993). Phylogenetic trees enabled identification of the closest relatives of our sequences, which were selected, together with a sample of other alveolates species and some environmental sequences, to carry out computationally-intensive Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. The extremely divergent *Oxyrrhis marina* Dujardin, 1841 sequence was omitted from phylogenetic analyses in order to avoid long-branch attraction artefacts. A selection of 50 sequences representing different alveolates was thus determined to reconstruct the ML and BI trees. ML analyses were conducted using the program TREEFINDER (Jobb et al., 2004) by applying a GTR + Γ + I model of nucleotide substitution, taking into account a proportion of invariable sites, and a Γ-shaped distribution of substitution rates with four rate categories. BI analyses were carried out using both the program PHYLOBAYES, through the application of a GTR + CAT Bayesian mixture model (Lartillot & Philippe, 2004), and MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) with the model GTR (Lanave et al., 1984; Rodríguez et al., 1990), with the number of invariable sites being estimated, and a gamma-shaped distribution of variable sites with four rate categories (GTR + Γ + I). Four chains were run up to 1,000,000 generations from a random starting tree well beyond convergence. The first 5,000 trees were discarded as the burn in. Sequences were deposited in GenBank with the following accession numbers: FJ593705-FJ593708.

Results

In late spring of 2008, we observed several copepod species infected with ellobiopsid parasites. Since the phylogenetic position of these parasites remains uncertain, we collected ten individual specimens to carry out molecular phylogenetic studies (see below).

In addition, we completed previous observations concerning the life-cycle of these parasites. The copepod assemblage of the Bay of Marseille (NW Mediterranean) was dominated by *Acartia clausi* Giesbrecht, *Centropages typicus* Kröyer and *Pseudocalanus* sp. in the late spring of 2008. Both copepodites and adult stages of the three species appeared infected with specimens of *Ellobiopsis.* On May 29th, *c.*15% of the 200 examined specimens of *A. clausi* appeared infected with this parasite. Parasites were attached to various parts of the host's body, with a higher occurrence on the anterior appendices. Up to five parasites at different developmental stages were found on a single host. This number may be even higher, because younger specimens of *Ellobiopsis* may go unnoticed under optical microscopy due to their small size. The presence of one gonomere suggested that the parasite found in all the infected copepod species corresponded to the type-species, *E. chattoni*.

The *Ellobiopsis* life-cycle

Ellobiopsis chattoni first appeared as a small knob, and then developed into an oval test with a stalk which pierces the host's body (Fig. 1). The shape of the parasite changes throughout its life-cycle. The youngest cells are pyriform, while mature stages may be ellipsoidal or cylindrical (Figs. 2–4). An ellipsoidal or spherical gonomere (sporogenetic stage) is formed from the trophomere by means of a more or less marked constriction at the distal end (Figs. 5–8). In contrast to the live cells, the protoplast of ethanol-fixed specimens shrank and separated from the external membrane. A tube-like structure connected the membrane and protoplast in the distal extreme of the gonomere (Figs. 5–6). This structure seems to be related to the release of the spores. In mature specimens, the gonomere had a knobbly surface with small buds of pre-spores that underwent a series of divisions to give rise to non-flagellate spores (Figs. 9–14). We were unable to determine whether the spores required a post-maturation process to develop the flagella. On a live infected copepod, a specimen of *Ellobiopsis* was observed under the microscope to produce spores after half an hour. Spore formation did not apparently require the differentiation of the gonomere (Figs. 15–17).
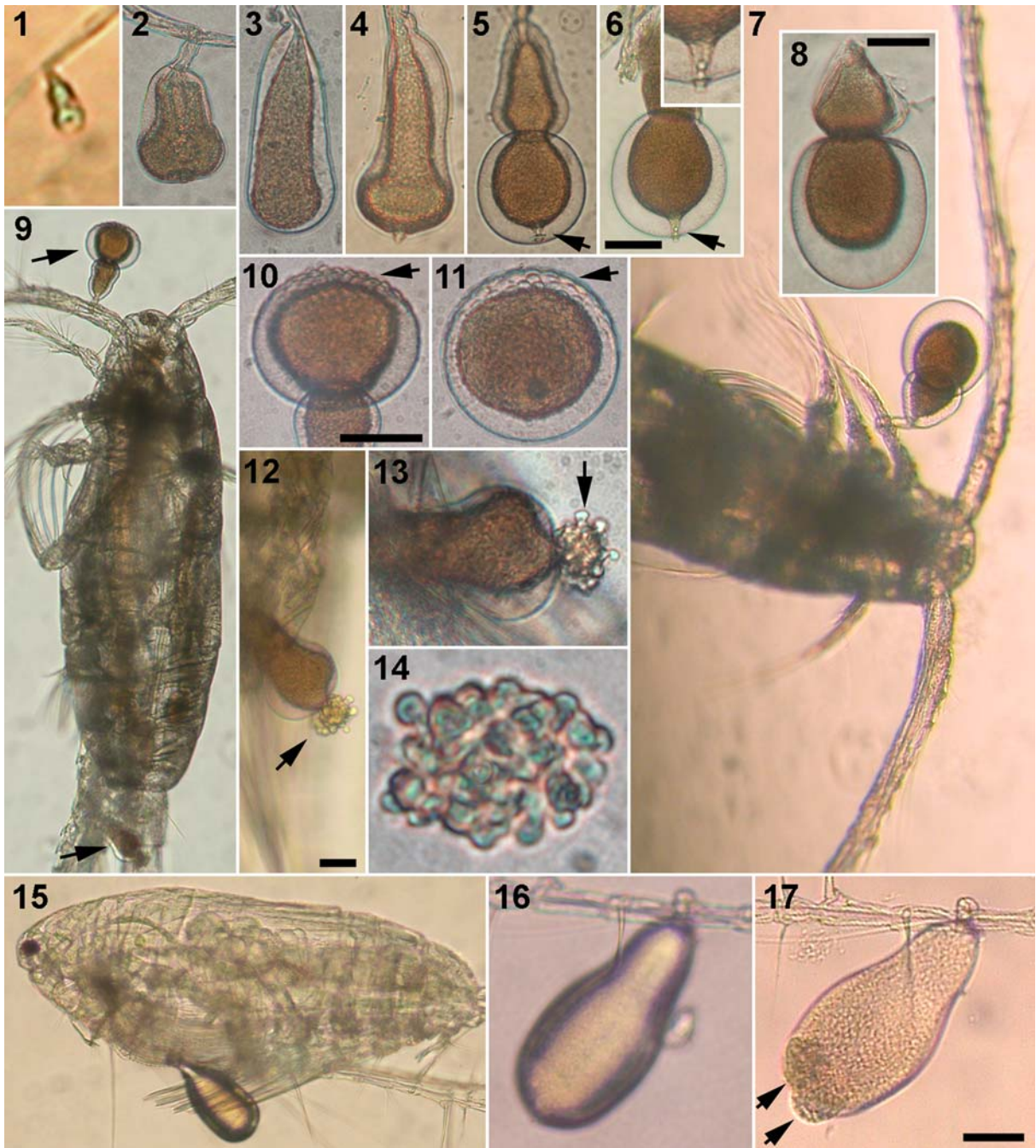
Figs. 1–17 Light micrographs of different life-cycle stages of *Ellobiopsis chattoni* parasitising copepods collected from the Bay of Marseille, NW Mediterranean Sea. 1–6. Different stages of the development; 5–6. the *arrows* indicate a tube-like structure in the distal part of the gonomere. 7–8. Specimen infecting *Acartia clausi* used for single-cell PCR. 9–14. Two parasites at different degrees of maturation in the same host; 10–11. the *arrows* indicate the irregular surface on the distal part of the gonomere; 12–13. the *arrows* indicate the budding of immature spores. 15–17. Live infected copepod; the *arrows* indicate the budding of spores formed after half an hour of observation. 1–14. Ethanol-fixed specimens collected on May 29th, 2008. 15–17. Live specimen collected on June 10th, 2008. *Scale-bar*: 50 µm

Molecular phylogenetic analysis of *Ellobiopsis chattoni*

We attempted to amplify the SSU rRNA gene from the ten *Ellobiopsis* specimens collected. Only four of them yielded DNA fragments of the expected size. Direct sequencing of the amplified products either failed or yielded copepod sequences, except for one partial sequence of poor quality that had *Thalassomyces* as its closest relative (specimen FG144, 87% identity). We then chose one different specimen that had yielded an amplicon of the expected size, FG141, and we cloned the PCR product as a means to discriminate copepod or other potential contaminant amplicons from parasite sequences. The specimen FG141 came from a multinucleate gonomere and a partial trophomere of *E. chattoni* that infected a copepodite stage IV of *Acartia clausi* (Figs. 7–8). We sequenced several clone inserts and consistently obtained two slightly different sequences (Fig. 18). The two SSU rDNA copies differed in 14 substitutions for a length of 1,739 characters. Substitutions occurred all along the sequences in the SSU rDNA variable regions. Since co-infection by two different *Ellobiopsis* species producing a single infecting structure is highly improbable, the presence of two different SSU rDNA sequences suggests the existence of two polymorphic copies of this gene in *Ellobiopsis* cells.

Initial BLAST comparisons showed that, with the exception of the partial environmental sequence MB07.44 (accession EF539153, retrieved from the western Pacific coast, which shared 99% identity with our *Ellobiopsis* sequences, but was not included in our phylogenetic analyses because of its much shorter length), the closest relatives in the database were alveolate sequences that had only low similarity values (<90%). Thus, the second closest relative was *Thalassomyces* sp. JDS-2003 (AY340591) with only 82% identity at the SSU rDNA locus.

We carried out phylogenetic analyses using various reconstruction methods (see Materials and methods). All phylogenetic analyses were congruent in showing that *Ellobiopsis chattoni* formed a monophyletic lineage with *Thalassomyces fagei* Boschma, 1948 and *Thalassomyces* sp. (100% bootstrap support (BS) and posterior probabilities (PP) of 1). The long-branch sequences revealed both ellobiopsid genera as members of the same highly divergent alveolate group.

Fig. 18 Maximum likelihood phylogenetic tree of alveolate ▶ SSU rDNA sequences, based on 1,096 aligned positions. Names in *bold* represent the four sequences of different clones from the same isolate of *Ellobiopsis chattoni* obtained in this study. Numbers at the nodes are bootstrap proportions (values <50% are omitted). Nodes supported by posterior probability values >0.90 in Bayesian Inference analyses are indicated by black circles. Several branches leading to fast-evolving species have been shortened to a half or a third (indicated by 1/2 or 1/3). Accession numbers are provided in brackets. The scale-bar represents the number of substitutions for a unit branch length

The inclusion of the ellobiopsids in the SSU rDNA phylogeny of the major alveolate lineages yielded a group that diverged after the Perkinsozoa (with 82% BS and PPs, for the two Bayesian methods used, of 0.94 and 0.86), formed by three clades: the ellobiopsids, the Marine Alveolate Group I and the dinoflagellates (Dinokaryota, Noctilucea and Syndinea). The order of emergence of these three groups remained unresolved. Within the Dinokaryota, the aberrant dinoflagellate *Noctiluca scintillans* (Macartney, 1810) Kofoid, 1920 and the parasite *Haplozoon* Dogiel, 1906 diverged at a basal position, although with very low support (Fig. 18).

Discussion

Systematic position of ellobiopsids within the alveolates

The few available ultrastructural studies showed that the genera *Thalassomyces* and *Parallobiopsis* differed markedly in the complex cytology of the gonomere and number of flagella (Collin, 1913; Hovasse, 1926; Galt & Whisler, 1970; Whisler, 1990). In addition to these morphological differences, differences in the types of hosts parasitised (crustaceans versus polychaete worms) led to the view that ellobiopsids constituted a heterogeneous assemblage of protists with diverse affinities (Boschma, 1949, 1959). In contrast, our results based on SSU rDNA phylogenetic analysis have shown that the most representative taxa, *Ellobiopsis* and *Thalassomyces*, form a monophyletic group. We observed two slightly different SSU rDNA sequences (0.8% divergence) in a single *Ellobiopsis* specimen. The presence of divergent SSU rDNAs in a single species, although not frequent, is not exceptional in eukaryotes (see references in Alverson &
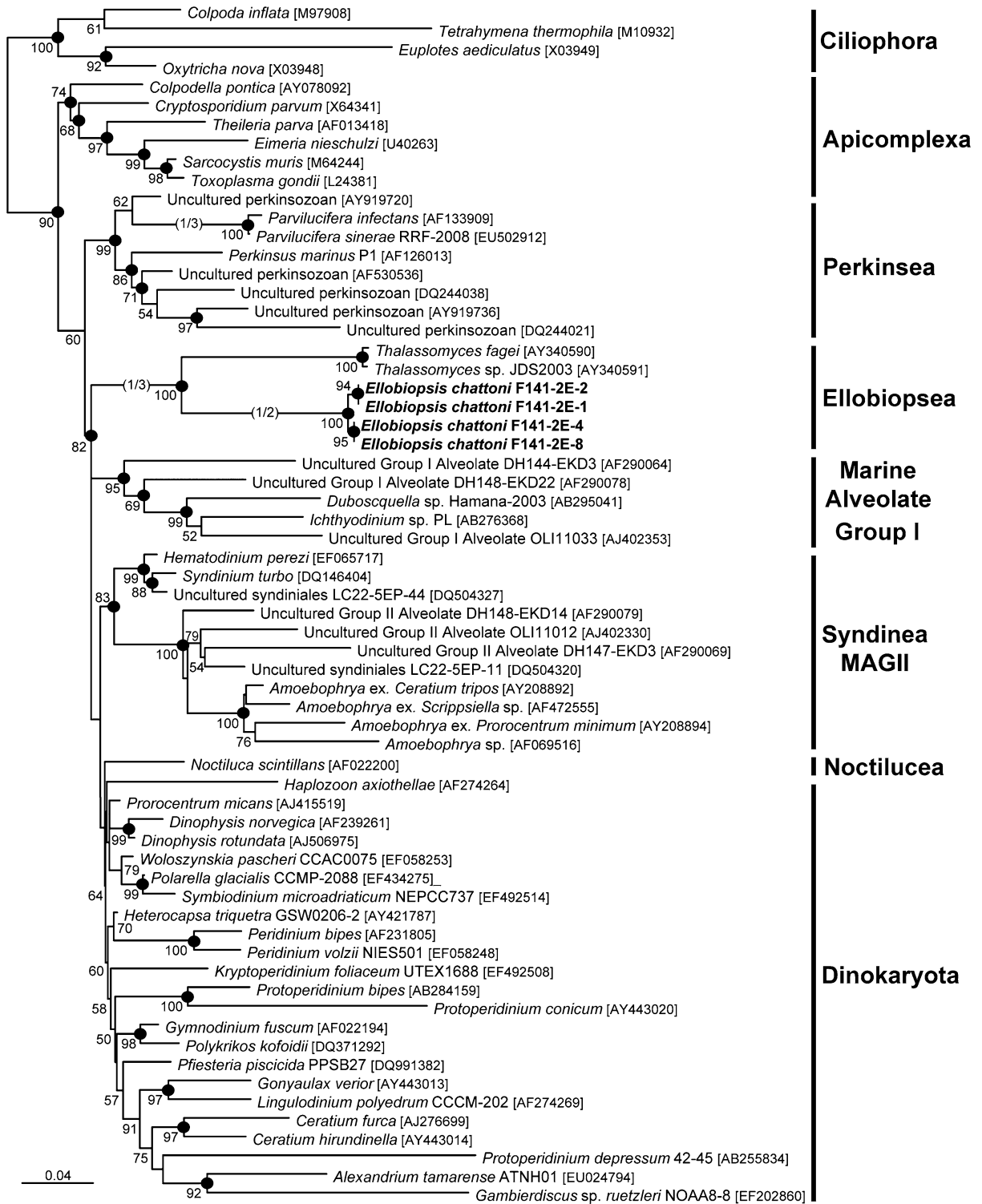
Kolnick, 2005). For example, among the alveolates, multiple polymorphic sequences have been reported from clonal cultures of perkinsid parasites (Burreson et al., 2005). They were interpreted as the result of a relatively recent hybridisation of two different species. Similarly, species of *Protoperidinium* Bergh, 1881, the most speciose dinoflagellate genus, have shown a relatively high intra-individual variability in the SSU and LSU rDNAs (Yamaguchi et al., 2006; Gribble & Anderson, 2007). The intragenomic sequence variation in *E. chattoni* should be taken into account, especially to avoid an overestimation of species diversity, in future environmental sequencing studies.

Although the monophyly of the genera *Ellobiopsis* and *Thalassomyces* was firmly demonstrated by our SSU rDNA phylogenetic analysis, the branching position of the ellobiopsids within the alveolates was much less certain. The ellobiopsids have unique ultrastructural peculiarities, such as the nuclear dimorphism and centriolar complexes (Galt & Whisler, 1970; Whisler, 1990; Schweikert & Elbrächter, 2006). Cavalier-Smith & Chao (2004) justified the ellobiopsids as sisters of dinoflagellates because they share centrioles associated with cytoplasmic channels through the nucleus and both the posterior flagellum and circumferential transverse flagellum. However, other typical dinoflagellate features, such as the dinokaryon characterised by condensed chromosomes in interphase, the two grooves (cingulum, sulcus) and the transverse ribbon-like flagellum (with mastigonemes and paraxial rod), are lacking in ellobiopsids (Galt & Whisler, 1970; Whisler, 1990; Schweikert & Elbrächter, 2006). The only described species of the Marine Alveolate Group I belong to *Duboscquella* and *Ichthyodinium*, endoparasites of tintinnid ciliates and fish eggs, respectively (Harada et al., 2007; Mori et al., 2007). They have biflagellate spores with a sulcus and cingulum that are reminiscent of the dinoflagellates (Chatton, 1952; Cachon & Cachon, 1987). However, if we restrict the dinoflagellates based on the occurrence of a dinokaryon, we must exclude *Duboscquella* and *Ichthyodinium* from them because there is no evidence of condensed interphase chromosomes in any stage of their life-cycle (Harada et al., 2007). The level of organisation of the chromosomes is also variable among the Syndinea, and the dinokaryon is missing in vegetative cells of *Noctiluca scintillans* (see Fukuda & Endoh, 2008). This character is also uneven in perkinsid parasites. For example, *Parvilucifera prorocentri* Leander &

Hoppenrath, 2008 has been described with condensed chromosomes, whereas they have not been reported in *P. infectans* Norén & Moestrup, 1999 (Norén et al., 1999; Leander & Hoppenrath, 2008). Therefore, the lack of condensed chromosomes in the highly aberrant ellobiopsids is probably a poor ultrastructural criterion for discarding the possible phylogenetic relationship with the dinoflagellates that appears to be supported by the SSU rDNA phylogeny.

Another of the apparent anomalies of the ellobiopsid parasites is the number of flagella. The spores of *Thalassomyces* are unequivocally biflagellate, whereas they are apparently uniflagellate or non-flagellate in *Parallobiopsis* and *Ellobiopsis*, respectively (Collin, 1913; Hovasse, 1926, 1952; Galt & Whisler, 1970; Whisler, 1990). However, we might expect the occurrence of biflagellate spores in all the ellobiopsids, taking into account that this is a common feature in all known relatives (Apicomplexa, Perkinsozoa, Syndinea, *Colpodella*, Dinokaryota and *Duboscquella*). Whereas, a second flagellum might have been unnoticed in the earlier and only study of *Parallobiopsis*, the production of biflagellate spores after a post-maturation process needs to be demonstrated in *Ellobiopsis*. In this study, we observed from a live *Ellobiopsis* specimen that spore formation did not apparently require the differentiation of the gonomere (Figs. 15–17). This phenomenon may be interpreted as a fast response of the parasite to the forthcoming death of the host and subsequently its own death. The mechanism of dispersion and motility of the spores remains unknown.

Ecological aspects

Copepods are the most abundant metazoans in the oceans (Mauchline, 1998) and *Ellobiopsis chattoni* has been reported infecting at least 25 copepod species and even crab larvae (Shields, 1994). Environmental sequencing surveys have revealed that the spores of parasitic alveolates, such as the Syndinea, are widely distributed throughout the oceans (Moreira & López-García, 2002; Guillou et al., 2008). In contrast, only one ellobiopsid environmental sequence is found in the GenBank database (accession EF539153). This might be explained either by the potential inefficiency of eukaryote-specific primers to amplify the highly divergent ellobiopsid sequences or, perhaps, by the

fact that the infective spores of the ellobiopsids are not abundant in the oceans or have short-lived stages.

All described *Syndinea* obligatorily kill their hosts and produce infective spores in massive numbers. In contrast, *Ellobiopsis* keeps the host alive for a long time, although it reduces its fecundity, presumably by decreasing the host reserves available for reproduction (Shields, 1994). In this study, we observed that specimens of *Ellobiopsis* rapidly form spores as a response to the host's death. It might be possible that they also have the capacity to induce the fast formation and release of spores under favourable conditions, such as high host population densities. This would limit the distribution of ellobiopsid spores to very discrete periods and locations, explaining the almost complete absence of ellobiopsid sequences in environmental surveys.

In our study, the three dominant copepod species from a single zooplankton sample were infected with specimens of *Ellobiopsis*. We assumed that a single *Ellobiopsis* species is responsible for the infection of multiple host species, although it has been reported that different copepod species appear to have different susceptibilities to the ellobiopsid infection. For example, in the North Atlantic, *Calanus helgolandicus* Claus is commonly infected by *E. chattoni*, whereas the co-occurring species, *C. carinatus* Kröyer, appears to be unaffected (Albaina & Irigoien, 2006). Several parasitic alveolates with a broad host range are well known. This is the case for the perkinsid *Parvilucifera infectans*, capable of infecting several species of dinoflagellates (Norén et al., 1999), whereas the congeneric *P. prorocentri* is only known to infect a single dinoflagellate species (Leander & Hoppenrath, 2008). A syndinian that parasitises dinoflagellates of the genus *Amoebophrya* has strains that show a high degree of host-specificity, whereas others have a relatively broad host range (Kim et al., 2008). Experimental infection studies will help elucidating whether a single strain of *Ellobiopsis* is able to infect different copepod species. In addition, further molecular and ultrastructural studies, including the survey of different seasons, hosts and geographical locations, will address the question of whether *E. chattoni* constitutes an independent species or a species complex with independent species in each host.

References

Albaina, A., & Irigoien, X. (2006). Fecundity limitation of *Calanus helgolandicus*, by the parasite *Ellobiopsis* sp. *Journal of Plankton Research, 28*, 413–418.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., et al. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389–3402.

Alverson, A. J., & Kolnick, L. (2005). Intragenomic nucleotide polymorphism among small subunit (18S) rDNA paralogs in the diatom genus *Skeletonema* (Bacillariophyta). *Journal of Phycology, 41*, 1248–1257.

Boschma, H. (1949). Ellobiopsidae. *Discovery Report, 25*, 281–314.

Boschma, H. (1959). Ellobiopsidae from the tropical West Africa. *Atlantide Report, 5*, 145–175.

Burreson, E. M., Dungan, C. F., & Reece, K. S. (2005). Molecular, morphological, and experimental evidence support the synonymy of *Perkinsus chesapeaki* and *Perkinsus andrewsi*. *Journal of Eukaryotic Microbiology, 52*, 258–270.

Cachon, J., & Cachon, M. (1987). Parasitic dinoflagellates. In F. J. R. Taylor (Ed.), *The biology of dinoflagellates* (pp. 571–610). Oxford: Blackwell.

Caullery, M. (1910). *Ellobiopsis chattoni*, n. g., n. sp., parasite de *Calanus helgolandicus* Claus, appartenant probablement aux Péridiniens. *Bulletin Scientifique de la France et la Belgique, 44*, 201–214.

Cavalier-Smith, T. (1993). Kingdom Protozoa and its 18 phyla. *Microbiological Reviews, 57*, 953–994.

Cavalier-Smith, T., & Chao, E. E. (2004). Protalveolae phylogeny and systematics and the origins of Sporozoa and dinoflagellates (phylum Myzozoa nom nov.). *European Journal of Protistology, 40*, 185–212.

Chatton, E. (1920). Les Péridiniens parasites. Morphologie, reproduction, éthologie. *Archives de Zoologie Experimentale et Générale, 59*, 1–475.

Chatton, E. (1952). Classe des Dinoflagellés ou Péridiniens. In P. Grassé (Ed.), *Traité de Zoologie* (Vol. 1, pp. 1309–1406). Paris: Masson et Cie.

Collin, B. (1913). Sur un Ellobiopside nouveau parasite des Nebalies (*Parallobiopsis coutieri* n. g., n. sp.). *Comptes Rendus de l'Academie des Sciences, 156*, 1332–1333.

Dick, M. W. (2001). *Straminipilous fungi: Systematics of the peronosporomycetes, including accounts of the marine straminipilous protists, the plasmodiophorids, and similar organisms*. Dordrecht: Kluwer, 654 pp.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research, 32*, 1792–1797.

Fukuda, Y., & Endoh, H. (2008). Phylogenetic analyses of the dinoflagellate *Noctiluca scintillans* based on β-tubulin and

Hsp90 genes. *European Journal of Protistology, 44*, 27–33.

Galt, J. H., & Whisler, H. C. (1970). Differentiation of flagellate spores in *Thalassomyces* Ellobiopsid parasite of marine Crustacea. *Archiv für Mikrobiologie, 71*, 295–303.

Grassé, P. P. (1952). Les Ellobiopsidae. In P. Grassé (Ed.), *Traité de Zoologie* (Vol. 1, pp. 1023–1030). Paris: Masson et Cie.

Gribble, K. E., & Anderson, D. M. (2007). High intraindividual, intraspecific, and interspecific variability in large-subunit ribosomal DNA in the heterotrophic dinoflagellates *Protoperidinium*, *Diplopsalis*, and *Preperidinium* (Dinophyceae). *Phycologia, 46*, 315–324.

Guillou, L., Viprey, M., Chambouvet, A., Welsh, R. M., Kirkham, A. R., Massana, R., et al. (2008). Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environmental Microbiology, 10*, 3349–3365.

Harada, A., Ohtsuka, S., & Horiguchi, T. (2007). Species of the parasitic genus *Duboscquella* are members of the enigmatic marine alveolate Group I. *Protist, 158*, 337–347.

Hovasse, R. (1926). "Parallobiopsis coutieri" Collin. Morphologie, cytologie, évolution, affinités des Ellobiopsidés. *Bulletin Scientifique de la France et la Belgique, 60*, 409–446.

Hovasse, R. (1952). *Ellobiopsis fagei* Hovasse, Ellobiopsidé parasite, en Méditerranée, de *Clausocalanus arcuicornis* Dana. *Bulletin de l'Institut Océanographique, Monaco, 1016*, 1–12.

Huelsenbeck, J. P., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*, 754–755.

Jepps, M. W. (1937). On the protozoan parasites of *Calanus finmarchicus* in the Cycle Sea area. *Quarterly Journal of Microscopical Science, 79*, 589–658.

Jobb, G., von Haeseler, A., & Strimmer, K. (2004). TREE-FINDER: A powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology, 4*, 18.

Kim, S., Park, M. G., Kim, K. Y., Kim, C. H., Yih, W., Park, J. S., et al. (2008). Genetic diversity of parasitic dinoflagellates in the genus *Amoebophrya* and its relationship to parasite biology and biogeography. *Journal of Eukaryotic Microbiology, 55*, 1–8.

Lanave, C., Preparata, G., Saccone, C., & Serio, G. (1984). A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution, 20*, 86–93.

Lartillot, N., & Philippe, H. (2004). A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution, 21*, 1095–1109.

Leander, B. S., & Hoppenrath, M. (2008). Ultrastructure of a novel tube-forming, intracellular parasite of dinoflagellates: *Parvilucifera prorocentri* sp. nov. (Alveolata, Myzozoa). *European Journal of Protistology, 44*, 55–70.

López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature, 409*, 603–607.

Mauchline, J. (1998). The biology of calanoid copepods. *Advances in Marine Biology, 33*, 1–710.

Moreira, D., & López-García, P. (2002). The molecular ecology of microbial eukaryotes unveils a hidden world. *Trends in Microbiology, 10*, 31–38.

Mori, K., Yamamoto, K., Teruya, K., Shiozawa, S., Yoseda, K., Sugaya, T., et al. (2007). Endoparasitic dinoflagellate of the genus *Ichthyodinium* infecting fertilized eggs and hatched larvae observed in the seed production of leopard coral grouper *Plectropomus leopardus*. *Fish Pathology, 42*, 49–57.

Niezabitowski, E. L. (1913). Die pfianzlichen Parasiten der Tiefsee-Decapoden-Gattung *Pasiphaea*. *Kosmos, 38*, 1563–1572.

Norén, F., Moestrup, O., & Rehnstam-Holm, A.-S. (1999). *Parvilucifera infectans* Norén et Moestrup sp. nov. (Perkinsozoa phylum nov.): A parasitic flagellate capable of killing toxic microalgae. *European Journal of Protistology, 35*, 233–254.

Philippe, H. (1993). MUST, a computer package of management utilities for sequences and trees. *Nucleic Acids Research, 21*, 5264–5272.

Reichenow, E. (1930). Parasitische Peridinea (einschließlich Ellobiopsidae). *Die Tierwelt der Nord- und Ostsee, 19*(2.d3), 85–100.

Rodríguez, F., Oliver, J. L., Marín, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology, 142*, 485–501.

Rose, M. (1933). Copépodes pélagiques. *Faune France, 26*, 1–374.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406–425.

Schweikert, M., & Elbrächter, M. (2006). First ultrastructural investigations on *Ellobiopsis* spec. (incertae sedis) a parasite of copepods. *Endocytobiosis Cell Research, 17*, 73.

Shields, J. D. (1994). The parasitic dinoflagellates of marine crustaceans. *Annual Review of Fish Diseases, 4*, 241–271.

Silberman, J. D., Collins, A. G., Gershwin, L. A., Johnson, P. J., & Roger, A. J. (2004). Ellobiopsids of the genus *Thalassomyces* are alveolates. *Journal of Eukaryotic Microbiology, 52*, 246–252.

Skovgaard, A., Massana, R., Balague, V., & Saiz, E. (2005). Phylogenetic position of the copepod-infesting parasite *Syndinium turbo* (Dinoflagellata, Syndinea). *Protist, 156*, 413–423.

Théodoridès, J. (1989). Parasitology of marine zooplankton. *Advances in Marine Biology, 25*, 117–177.

Whisler, H. C. (1990). Incertae Sedis Ellobiopsida. In L. Margulis, J. O. Corliss, M. Melkonian, & D. J. Chapman (Eds.), *Handbook of Protoctista* (pp. 715–719). Boston: Jones & Bartlett.

Yamaguchi, A., Kawamura, H., & Horiguchi, T. (2006). A further phylogenetic study of the heterotrophic dinoflagellate genus, *Protoperidinium* (Dinophyceae) based on small and large subunit ribosomal RNA gene sequences. *Phycological Research, 54*, 317–329.