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Short Communication

Molecular phylogeny supports division of the ‘cosmopolitan’ taxon *Celleporella* (Bryozoa; Cheilostomata) into four major clades

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1. Introduction

The bryozoan family Hippothoidae is represented throughout the oceans by mm-scale vine-like or discoid colonies that encrust firm substrata such as macroalgae, colonial invertebrates, living serolian isopods, shells of living molluscs, dead shells, rocks, glass and plastics (Hastings, 1979; Morris, 1980; Moyano, 1987; Barnes, 2002). Classical taxonomy (Gordon and Hastings, 1979) recognizes three extant hippothoid genera: *Hippothoa*, *Plesiotothoa* and *Celleporella*, of which the last is by far the most speciose. As with a number of other bryozoan taxa (Davidson and Haygood, 1999; Mackie et al., 2001, 2006; McGovern and Hellberg, 2003; Schwaninger, 1999), there has been a tendency to assign geographically distant representatives to ‘cosmopolitan’ species. For example, *Celleporella hyalina* (L.) is reported ubiquitously from temperate and cold seas in the Northern Hemisphere and widely, though less frequently, from similar environments in the Southern Hemisphere (Hincks, 1880; Kluge, 1975; Morris, 1980; Moyano, 1987). Molecular phylogenetics verified by mating trials, however, has revealed prolific cryptic speciation within the *C. hyalina* complex (Gómez et al., 2007a).

Cryptic speciation notwithstanding, differentiation within the genus *Celleporella* is sufficient for the recognition of a number of morphospecies (Ryland and Gordon, 1977; Morris, 1980; Gordon, 1984). Form of the ancestrula (founder zooid), initial budding pattern (single latero-distal or mid-distal first bud, or two symmetrical latero-distal buds) and colonial growth form (vine-like or mat-like;

unilaminar or bilaminar) allow grouping of known morphospecies into four categories, which Moyano (1987) defined as the subgenera *Celleporella*, *Antarctothoa*, *Austrothoa* and *Neothoa*. A sparsely documented fossil record, however, impedes phylogenetic assessment of the proposed taxonomy. Here we use mtDNA sequence data and phylogenetic analysis to verify the morphologically defined taxonomy, assess the contribution of cryptic species to diversity, and provide a phylogenetic framework for further studies of these Bryozoa, especially in the southern oceans.

2. Material and methods

2.1. Sample collection and DNA preparation

Colonies representing 16 of 30 recognized species (Supplementary Tables 1 and 2) were collected from macroalgae or occasionally from mussel shells and fixed in ethanol. Localities represented temperate and cold seas from around the globe (Supplementary Fig. 1, Supplementary Table 1). To avoid contamination from epibionts and gut contents, embryos were obtained from brood chambers using a suction micro-pipette and DNA extracted using Chelex resin (Instagene, Biorad). Occasional lack of embryos necessitated extraction from ancestrulae or from small colonial fragments (5–10 zooids). PCR conditions and CO1 primers were as listed in Gómez et al. (2007a). DNA sequences (592 bp) are available in GenBank (Accession Nos. EU168335–EU168398). Colonies used for the DNA sampling were examined by SEM or light microscopy and identified as far as possible from taxonomic descriptions based largely on shape of the zooidal orifice, zooidal sculpturing and initial budding pattern from the

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ancestrula (Hayward, 1993; Moyano, 1987; Moyano and Gordon, 1980; Ryland and Gordon, 1977).

2.2. Phylogenetic analysis

Maximum likelihood phylogenies were estimated using TREEFINDER (Jobb et al., 2004) with a General Time Reversible (GTR + G) model (chosen by MODELGENERATOR, AIC1, Keane et al., 2006), data partitioned by codon position, with 1000 bootstrap pseudoreplicates. Bayesian inference was carried out using MrBayes v3.1.1 (Ronquist and Huelsenbeck, 2003) with the run comprising 2×10^6 generations (burnin = 12,000, samplefreq = 100) with a general time reversible model where MrBayes was allowed to optimize model parameters according to a codon-based data partition.

3. Results and discussion

3.1. Molecular phylogeny

The CO1 trees display four strongly supported major clades (Figs. 1 and 2) that correspond to the morphologically defined taxa *Celleporella*, *Antarctothoa*, *Austrothoa* and *Neothoa* recognized by Moyano (1987). The diversity of lineages found in *Antarctothoa* is comparable to that of *Celleporella*, presented in more detail by Gómez et al. (2007a), and comprises many mitochondrial lineages, some of which correspond to previously known species but others of which are new to science (Wright et al., 2007). Bayesian inference and maximum likelihood both suggest a basal split between *Antarctothoa* and the remaining groups, while the branching order of *Austrothoa*, *Neothoa* and *Celleporella* is not well resolved. This problem does not appear to be caused by saturation, since accumulation of transitions and third-codon-position substitutions is approximately linear throughout (Supplementary Fig. 2A and B). Instead, it may be that the four major groups diverged within a short period of time and that there is little phylogenetic signal for the branching order in the studied region of mtDNA. More data will be required to resolve this issue.

The *Celleporella* group is divided into four clusters representing (1) the *C. hyalina* species complex (Gómez et al., 2007a), (2) *C. angusta*, (3) a sister lineage to *C. angusta* and isolated from South Africa (sp. 1), and (4) sp. 2 isolated from three closely neighbouring sites in the NW Atlantic (Supplementary Table 1).

The *Antarctothoa* group shows many deep lineages (Figs. 1 and 2) corresponding to known morphospecies and to three morphologically distinct groups newly described as *A. cancinoidi*, *A. polystachia* and the *A. annea* species complex (Wright et al., 2007).

3.2. Mapping morphology onto molecular phylogeny

Poor resolution of basal nodes frustrates the mapping of diagnostic morphological characters (Moyano, 1987) onto

the molecular phylogeny (Figs. 1 and 2). It is reasonable to infer, however, that symmetrical budding of the ancestrula and unilaminar colonial structure shared by *Antarctothoa*, *Austrothoa* and *Neothoa* are plesiomorphic, and hence that, the spiral astogeny resulting from asymmetrical budding of the ancestrula and the bilaminar colonial structure due to frontal budding of *Celleporella* are apomorphic. We argue that the unreduced condition of gonozooids (female zooids) is plesiomorphic since it represents lesser modification of the basic autozooidal plan. We can adduce no evidence for deciding whether the schizoporelloid or tatifform ancestrula is plesiomorphic.

3.3. Elevation of subgenera to genera

From the above distinctions in molecular phylogeny and morphology, we propose that three of the four subgenera of *Celleporella* Gray 1848 erected by Moyano (1987) should be elevated to full generic status. The formal taxonomy is as follows.

- Subgenus *Antarctothoa* Moyano, 1987...Genus *Antarctothoa* Moyano, 1987 n. st.
- Type species *Escharina bougainvillei* d'Orbigny 1847
- Subgenus *Neothoa* Moyano, 1987...Genus *Neothoa* Moyano, 1987 n. st.
- Type species *Hippothoa patagonica* Busk 1859
- Subgenus *Austrothoa* Moyano, 1987...Genus *Austrothoa* Moyano, 1987 n. st.
- Type species *Celleporella yagana* Moyano and Gordon, 1980
- Subgenus *Celleporella* Moyano, 1987...Genus *Celleporella* Gray 1848
- Type species *Cellepora hyalina* Linné 1767

3.4. An identification key to genera

Using the diagnostic morphological characters proposed by Moyano (1987, p. 98), we offer a dichotomous key for identifying *Celleporella*, *Antarctothoa*, *Neothoa* and *Austrothoa* (Table 1). Application of the key to known species is summarized in Supplementary Table 2.

3.5. Taxonomic ambiguities

Classification of *Celleporella carolinensis* remains problematical, since this species has symmetrical initial budding typical of *Antarctothoa* (Table 1; Ryland, 1979; Wright and Hughes, 2001), but differs from other known species of *Antarctothoa* in having a warm temperate-sub tropical distribution (Ryland, 1979). Our analysis of *C. carolinensis* alone was confounded by an apparent CO1 pseudogene and so the phylogenetic placement of this species must await the application of alternative molecular markers.

Within the genus *Neothoa* we have sampled representatives of *N. patagonica* and *N. chiloensis*. Although the two

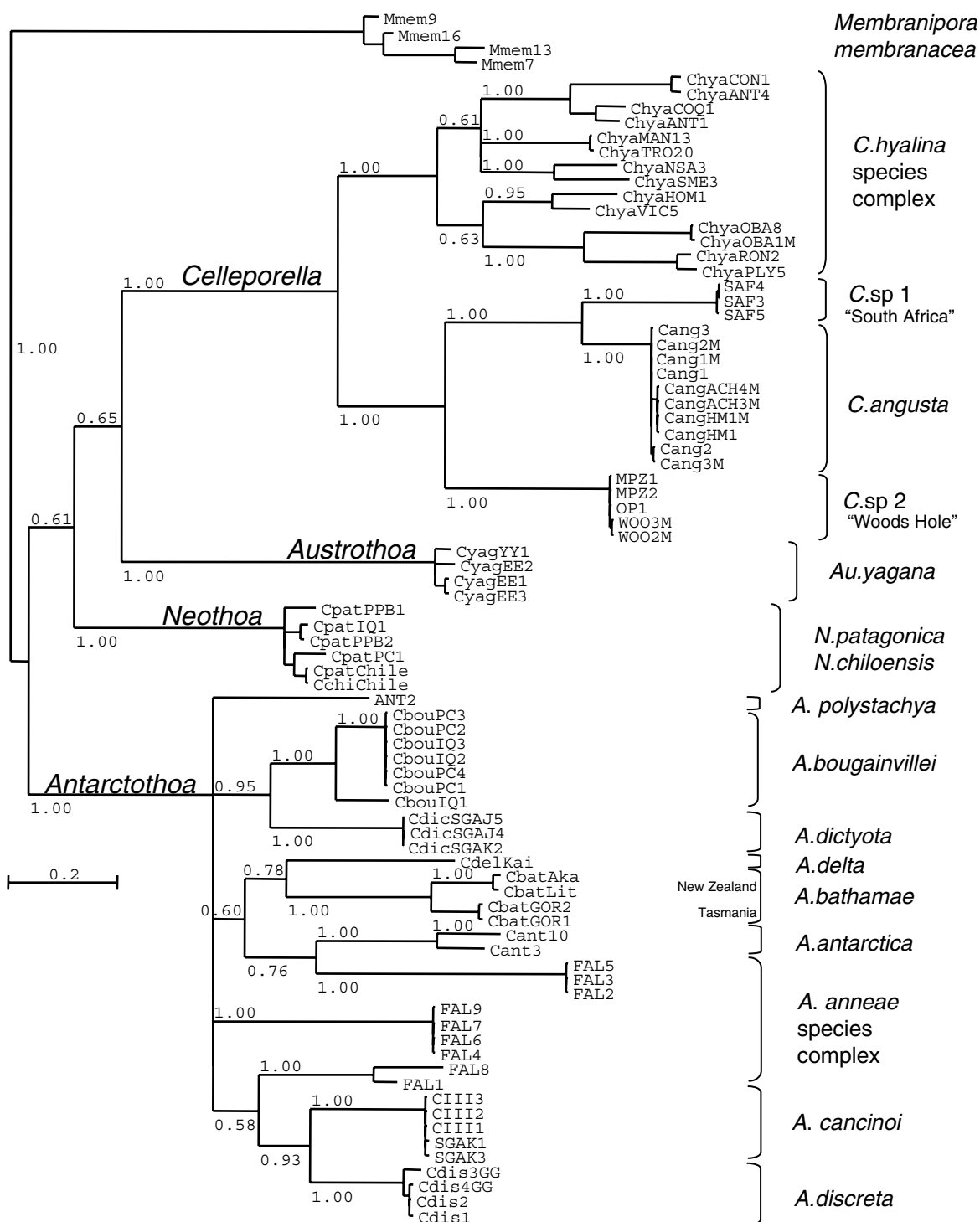


Fig. 1. Bayesian inference tree of CO1 sequence data for the former hippothoan genus *Celleporella*. Names on the tree are the subgenera proposed by Moyano (1987) but which are elevated to genera in the present study. Numbers represent posterior probability values after 2×10^6 generations with nodes collapsed below 0.5 support. Scale bar indicates substitutions per site.

species are morphologically distinct (Moyano, 1987), our sequence data for *N. chiloensis* is nested within the diversity of *N. patagonica* with whom it shares a haplotype. Much more extensive sampling and phylogeographic analysis will be required however to resolve the true relationship between these species.

3.6. Species diversity within *Celleporella*

The genus *Celleporella* as shown in Figs. 1 and 2 illustrates the species complex previously named *C. hyalina* and described in more detail by Gómez et al. (2007a). The taxa included in our trees represent 7 of the 10

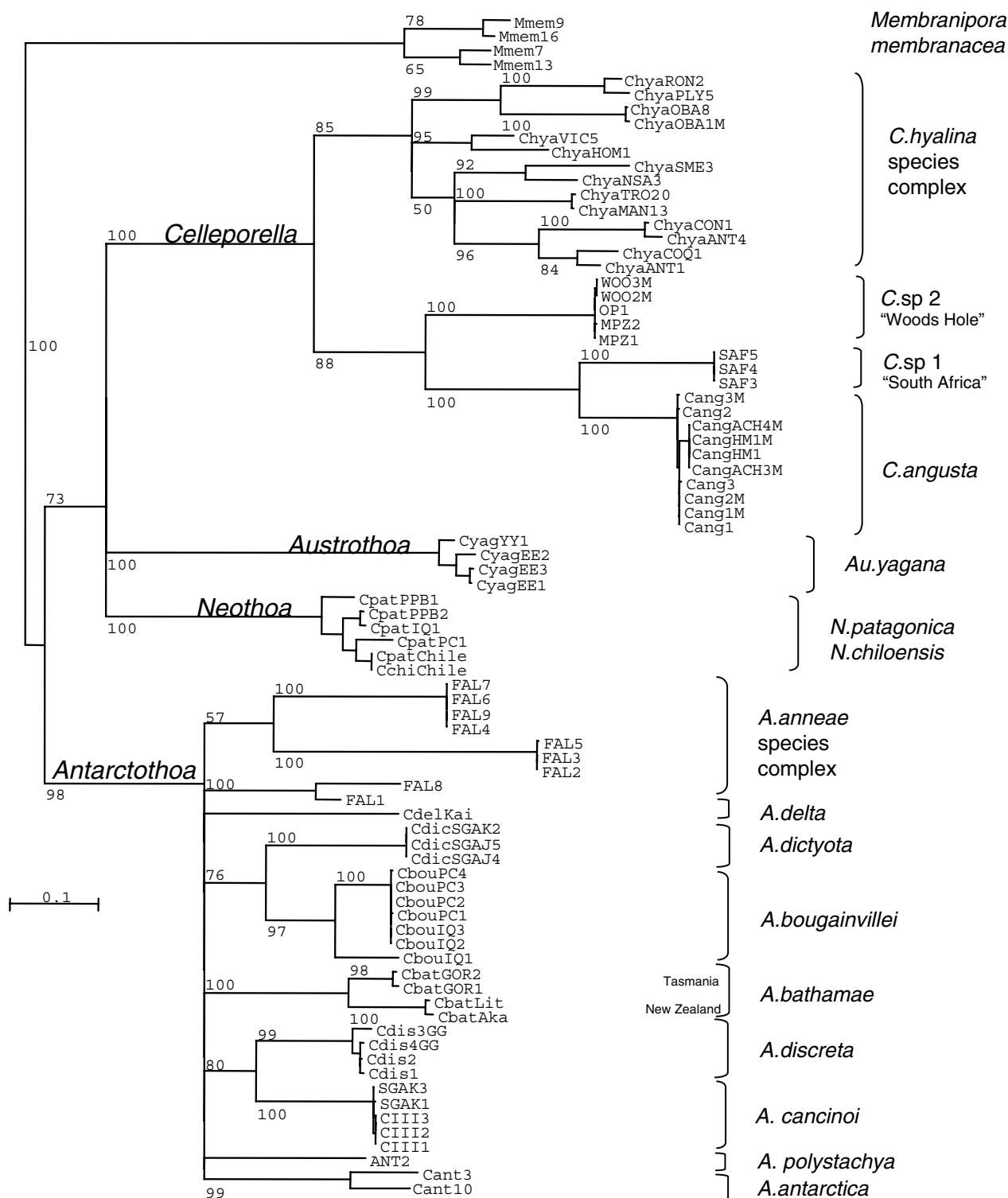


Fig. 2. Maximum likelihood tree of CO1 sequence data for the former hippothoan genus *Celleporella*. Numbers represent percentage bootstrap support and nodes with less than 50% support collapsed. Other details are as for Fig. 1.

geographical lineages described by Gómez et al. (2007a). Many of these have been shown to be reproductively incompatible, and may themselves contain phylogeographic variation that equates to reproductive incompatibility and hence to biological species (Gómez et al.,

2007a, b). At similar levels of CO1 divergence to that observed in the *C. hyalina* species complex we see a cluster of three other lineages that include *C. angusta* and two undescribed species. It is likely then that this also comprises a species complex.

Table 1

Key to the genera *Celleporella*, *Antarctothoa*, *Austrothoa* and *Neothoa*

Ancestrula schizoporelloid (orifice set within a calcified frontal wall as in autozooids) ...	1
Ancestrula tatiform (orifice set within a membranous frontal wall) and bears 6 'spines' ...	2
1 Initial budding symmetrical (ancestrula produces two disto-lateral buds and/or one medio-distal bud); female zooids relatively unreduced (half to equal in size and similar in form to autozooids) ...	<i>Antarctothoa</i>
Female zooids reduced to a triangular form less than a third the size of autozooids; initial budding asymmetrical (ancestrula produces one disto-lateral bud, generating spiral budding pattern) ...	<i>Celleporella</i>
2 Female zooids unreduced; colony pluriserial (mat-like) ...	<i>Austrothoa</i>
Female zooids reduced; colony uniserial or loosely pluriserial (vine-like or forming an open network) ...	<i>Neothoa</i>

3.7. Species diversity within *Antarctothoa*

The genus *Antarctothoa* contains a number of genetically distinct clades displaying well supported subdivision (Figs. 1 and 2) at depths comparable to those of biological species verified within the *C. hyalina* complex, discussed above. Thus, *A. bathamae* shows a geographical split between isolates from Tasmania and those from New Zealand, and the Argentinean sample of *A. bougainvillei* comprises two sympatric clades, while the Falkland sample of *A. annea* comprises three. Our investigations of the *C. hyalina* species group reveal that many isolates with such a level of genetic divergence (>2%) are reproductively incompatible as tested by production of viable outcrossed offspring (Gómez et al., 2007a). We therefore suggest that many of these groups are likely to be good biological species. It is likely that more detailed sampling of *Antarctothoa* would reveal further cases of 'cryptic' speciation. Such an investigation would not only assist in the cataloguing of biodiversity but would also importantly help us to understand the biogeography of the southern seas and the processes that may have generated such biodiversity.

3.8. Phylogeography

Apart from the outlying *A. galaica* (César-Aldariz et al., 1999) which may have reached Spain via shipping, and *C. carolinensis* which has uncertain taxonomic affinity (above), *Antarctothoa* has been recorded only from the Southern Hemisphere (Ryland and Gordon, 1977; Moyano and Gordon, 1980; Moyano, 1987; Branch and Hayward, 2005). Apparently endemic to Antarctica and Sub-Antarctic islands are *A. antarctica*, *A. discreta*, *A. dictyota*, *A. polystachya*, *A. alia* and *A. marionensis*, of which the first three are represented in Figs. 1 and 2. Endemic to New Zealand and/or Australia are *A. delta*, *A. bathamae*, *A. cancer*, *A. tongima* (Ryland and Gordon, 1977) and *A. tuberculata* (Hincks, 1880), of which the first two are represented in Figs. 1 and 2. *Antarctothoa muricata* is possibly a relict, known only from the widely separated Kerguelen and Juan Fernández archipelagos (Moyano, 1987), but was not accessible to us. *Antarctothoa annularis* is recorded from coast of South Africa influenced by the Benguela current (Morris, 1980), but again was not

sampled. *Antarctothoa bougainvillei* is recorded from Antarctica and Sub-Antarctic islands, extending to the Magellanic province of South America (Moyano, 1987; López-Gappa, 2000), but as discussed above this taxon may comprise a cryptic species complex. *Antarctothoa cancinoi* and the *A. annea* species complex may previously have been recorded as *A. bougainvillei*, with which they co-occur in the Magellanic province.

Celleporella and *Antarctothoa* may be regarded as Northern and Southern Hemisphere equivalents, each comprised of predominantly cool temperate-polar species. *Celleporella*, however, also contains a minority of warm-temperate clades, including *C. angusta* and *Celleporella* sp. 1 and 2 (Figs. 1 and 2), while *Antarctothoa* possibly includes the warm-temperate species *C. carolinensis*.

3.9. Deeper phylogeny

Although the phylogenies presented here validate the subdivision proposed by Moyano (1987), our analysis does not resolve well the deeper relationships. Nevertheless, cosmopolitan distribution and sampling accessibility make Hippothoidae a promising model for assessing global patterns of marine biodiversity in terms of biogeographical history, anthropogenic dispersal and climate change. In order to realize such potential, it will be necessary to produce a deep phylogeny of the family, a goal Moyano (1987) found impossible to achieve using morphological characters alone. Phylogenetic analysis of multiple gene sequences, including conserved nuclear loci, may be a fruitful approach in order to tackle this problem in future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2007.08.014](https://doi.org/10.1016/j.ympev.2007.08.014).

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