Cyttarocylys ampulla, a Polymorphic Tintinnid Ciliate of the Marine Plankton

John R. Dolan\textsuperscript{a,1}, Richard W. Pierce\textsuperscript{b}, and Charles Bachy\textsuperscript{c}

\textsuperscript{a}Université Pierre et Marie Curie and Centre National de la Recherche Scientifique (CNRS), UMR 7093, Laboratoire d'Océanographie de Villefranche, Marine Microbial Ecology, Station Zoologique, B.P. 28, 06230 Villefranche-sur-Mer, France
\textsuperscript{b}P.O. Box 132, North Attleboro, MA 02761-0132, USA
\textsuperscript{c}Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA

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Tintinnid species are traditionally distinguished via lorica features. Recently, sequencing has revealed polymorphism, i.e., genetically identical individuals with distinct lorica morphologies. One such polymorphic species is Cyttarocylys ampulla; individuals can display lorica morphologies of formally different species of Cyttarocylys and Petalotricha, well-represented in the literature. We compiled and analysed a global database of species records to determine if there is a main form and if different morphotypes have distinct temporal or spatial distributions. The two genera show very similar widespread distributions but with some statistical evidence of spatial segregation. Examining co-occurrence among the common ‘species’ we found most were rarely found alone, only 6-14\% of the records for all species except for 2 forms: C. eucycrphalus and P. ampulla reported alone in 34\% and 43\%, respectively, of their records. We identify them as the main forms and analysed data of global distributions, spatial distribution across the Mediterranean in summer and winter and temporal distributions from a site in the Adriatic. The two main forms show frequent co-occurrence, similar lack of strong seasonality and widespread geographic distributions. We tentatively conclude that the different lorica morphologies may only reflect conditions of high temporally variability such as quantities and composition of prey. Directions for further research are suggested.

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Keywords: Choreotrichida; ITS; microzooplankton; polymorphism; SSU rDNA; Tintinnina.

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\textsuperscript{1}Corresponding author; fax +33 4 93 76 38 34
e-mail dolan@obs-vlfr.fr (J.R. Dolan).

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Introduction

Sequencing of protistan cells has revealed the existence of a variety of interesting phenomena. These range from evidence of gene flow between Arctic and Antarctic populations in the benthic ciliate *Euplotes nobilii* (Di Giuseppe et al. 2013) to the existence of truly cryptic species in the diatom genus *Pseudo-nitzschia* (Amato et al. 2007) and pseudo-cryptic species in the foraminifer *Globoccona inflata* (Morard et al. 2011). Recently a case was uncovered of genetic homogeneity among morphologically diverse and geographically separated populations of the foraminiferan *Globigerinoides sacculifer* providing clear evidence of ‘inconsistent scaling of morphological and genetic diversity’ in protists (Andre et al. 2013).

For tintinnid ciliates, features of the lorica (or shell) have been traditionally used to distinguish species and group higher level taxa. Experimental work has shown that different lorica types can be constructed by the same species, specifically those of *Favella* (Laval-Peuto 1977, 1981, 1983). Lorica characteristics alone have long and often been described as seemingly inadequate for delineating species (e.g. Boltovskoy et al. 1990; Davis 1981; Schulz and Wulf 1929; Williams et al. 1994). However, the case of *Favella*, shown to construct loricas attributed previously to the genus *Coxiella*, while calling into question the reality of the entire genus *Coxiella* (e.g. Agatha and Strüder-Kypke 2013), was the singular unequivocal case of polymorphism until recently. Now sequencing of single cells of tintinnid ciliates has unveiled the existence of both cryptic species and polymorphic species in variety of tintinnid genera.

For example, in the cosmopolitan genus *Heliocostomella*, most of the described species are difficult if not impossible to distinguish unambiguously as the morphological characteristics of the lorica, supposedly distinguishing species, actually form a continuum between species (Santoferrara and Alder 2009). In Korean waters, sampling daily over an annual cycle showed two temporally disjunct populations, those of summer and winter, and both are morphologically variable (Xu et al. 2012).

Sequencing of single cells revealed that the summer and winter populations are genetically distinct, likely different species, both apparently capable of forming loricas characteristic of a variety of *Heliocostomella* species and therefore indistinguishable using lorica morphology. Likewise, recently three species of the genus *Cymatocylis* with very different lorica morphologies were shown to be variants of a single species (Kim et al. 2013). These examples concern species within a genus but perhaps the more intriguing case is that which forms the subject of this study: several species, previously of two genera from different families, found to be genetically identical.

Bachy et al. (2012) found identical SSU-rDNA and ITS sequences for several tintinnids with loricas corresponding to those of various species of the genus *Cyttarocyclus*, family Cyttarocyclididae, and the genus *Petalotricha*, family Petalotrichidae (Fig. 1). Consequently, they proposed a new combination *Cyttarocyclus ampulla* for the forms sequenced. The species concerned can be considered as ‘flagship species’ (Foissner et al. 2009) as all are relatively large and conspicuous; furthermore some have been known for well over a century as original descriptions date back to Haeckel (1873) and Fol (1881).

In a recent classification (Agatha and Strüder-Kypke 2013), the two genera concerned are the singular genera of different families. In traditional morphological terms, the families are distinguished primarily by the structure of the lorica wall and secondarily by overall shape. Thus, according to Kofoid and Campbell (1939), the family Petalotrichidae for the genus *Petalotricha*, has as its distinguishing characteristic ‘hyaline or minutely aveolar wall’ and ‘stout bowl-shaped lorica’ while the family Cyttarocyclididae, for the genus *Cyttarocyclus*, the “distinguishing characters are in its regularly reticulated pattern of wall structure and its more conical bowl”. For well over a century (Saville Kent 1881) *Petalotricha* with relatively smooth-surfaced, bowl-shaped lorica were thought completely different from *Cyttarocyclus* showing a reticulated or sculpted lorica structure of a conical shape. Comparing the drawings which illustrated the original descriptions...
of the two type species, *Cyttarocylis cassis* and *Petalotricha ampulla*, it is not surprising that the differences were judged large enough to justify placement in different families (Fig. 2). It is thus remarkable that the forms of Figure 1 are but a single genotype. Somewhat ironically, Kofoid and Campbell (1939, p 147), did remark regarding the genus *Petalotricha*: “Somewhat isolated, resembling *Cyttarocylis* in form but the fundamental wall structure and surface pattern at once bar close genetic relationship between these two genera”. Oddly enough, the seemingly large differences mask a very similar lorica ultrastructure in *Cyttarocylis* and *Petalotricha*. Both have lorica that are “trilaminar” composed of thick inner and outer layers enclosing a tubular central layer (Laval 1972; Laval-Peuto 1994). The similarity in lorica ultrastructure led Agatha and Strüder-Kypke (2013) to suggest that there is perhaps a close relationship between the two genera belying their placement in distinct families.

With contrasting lorica wall textures, the basic morphologies of *Cyttarocylis* and *Petalotricha* spp appear distinct. However, many authors have noted that distinguishing species within either genera is difficult because morphologies intermediate between those designated as distinct species are found (e.g., Balech 1962). This has led some authors to propose synonymy, for example with regard to *Cyttarocylis brandti*, *C. cassis* and *C. eucrycephalus* (Alder 1999) and with regard to *Petalotricha ampulla* and *P. major* (Balech 1968).

![Figure 1](image)

**Figure 1.** Single cell sequencing of these 6 cells showed identical or nearly identical (1 base out of 1,500) SSU-rDNA and ITS +5.8S sequences (% in parentheses). The ‘Cell ID’ is the cell/individual identifier as found in GenBank. A new combination *Cyttarocylis ampulla* groups these forms. The lorica morphologies correspond to those reported in the literature as *Cyttarocylis cassis* (A), *Cyttarocylis brandti* (B), *Cyttarocylis eucrycephalus* (C), *Petalotricha major* (D), *Petalotricha ampulla* (E) and *Petalotricha major* (F). Figure adapted from Bachy et al. 2012 in which different names were associated with some of the lorica morphologies.
Confusion in the species designations of *Cyttarocylis* and *Petalotricha* spp. is due at least in part to the fact that the standard monograph used to identify tintinnids since the 1930’s, that of Kofoid and Campbell (1929), presents sketches of species incorrectly scaled, exaggerating the differences in sizes between species, and in conflict with the text descriptions (Fig. 3). Therefore identifications made based solely on the illustrations in Kofoid and Campbell (1929) quite likely will differ from those based on the text description and consultation of the original species description. Consequently, the species identifications are somewhat subjective. For example, the morphologies shown in Figure 1 from Bachy et al. 2012 were given different names from those employed here as the respective sets of authors disagree.

To place the sequence-based evidence concerning the polymorphism of *Cyttarocylis/Petalotricha* in perspective it may be useful to compare it to the best data available for other tintinnids. Among all tintinnid genera consistently found to be monophyletic, the most extensive data set concerns the genus *Eutintinnus*. There are long 18S rDNA sequences for seven species of *Eutintinnus* (Fig. 4). Sequence similarity among these *Eutintinnus* species is generally 95 - 97%. Intriguing exceptions are high similarities (98.9% - 99.6%) of representatives of *E. apertus*, *E. lusus undae* and *E. tenuis* suggesting a polymorphic *Eutintinnus* species. Noteworthy is the lack of any 100% identity between any two *Eutintinnus* species. This is in sharp contrast with the *Cyttarocylis/Petalotricha* sequences of both SSU-rDNA and ITS sequences.

**Figure 2.** Drawings from the original descriptions of the type species of the genera *Cyttarocylis* and *Petalotricha* showing the distinct differences in lorica structure: *Cyttarocylis cassis* from Haeckel 1873 (A) and *Petalotricha ampulla* from FoU 1881 (B).

**Figure 3.** The illustrations in Kofoid and Campbell (1929) of species of *Cyttarocylis* (A) and *Petalotricha* (B). The same drawings of the most common species but re-scaled to correspond with text descriptions given in Kofoid and Campbell; where not given the dimensions from the original species descriptions were used (C). Note that the common morphotypes appear much more similar to one another when shown at the same scale.
Figure 4. Comparison of sequence similarity among *Eutintinnus* species. We selected all the sequences from *Eutintinnus* specimens available in GenBank attributed to a specific species omitting only redundant sequences, that is multiple sequences for a species from a single study. The sequences were aligned using clustalW (Thompson et al. 1994), and then trimmed to include only the region of nucleotide positions 342-1597 (1260 positions) in *Eutintinnus pectinis* GenBank accession JN871720, enabling unbiased pairwise sequence comparisons. The GenBank accession number appears below the image of the species and in the table is given after the species abbreviation. Note that overall mean similarity is about 97%. Highly similar pairs > 99% (bold), are mostly between representatives of the same species except the high values uniting *E. apertus*, *E. lusus undae* and *E. tenuis*. Images are from the corresponding studies - Bachy et al. 2012 (*E. apertus*, *E. fraknoi* JQ408159, *E. tubulosus*), Strüder-Kypke and Lynn 2008 (*E. fraknoi* EU399534), Xu et al. 2013 (*E. lusus undae*, *E. stramentus*, *E. tubulosus* JX101856), Santoferrara et al. 2013 (*E. pectinis* JN831766), Bachvaroff et al. 2012 (*E. pectinis* JN871720, *E. tenuis*), Snoeyenbos-West et al. 2002 (*E. pectinis* AF399171), Strüder-Kypke and Lynn 2003 (*E. pectinis* AY143570). Note that images of *E. pectinis* JN871720 and *E. tenuis* JN871721 are of parasitized cells, the images are shown to document loric morphology.

(Fig. 1). We are confident that the six cells exhibiting diverse loricas sequenced by Bachy et al. (2012) do represent a single species. For the purposes of this paper we will though continue to employ the names traditionally attributed to the different forms and refer to the major sets of morphs with distinct loric wall structures as *Petalotricha* and *Cyttaroclylis*.

The discovery of multiple loria morphologies of an apparently single species raises the question as to what governs the occurrence of different loria forms. As noted previously, the forms are large and conspicuous and while often termed ‘rare’ (e.g., Abboud-Abi Saab 1989; Hada 1938; Rassoulzadegan 1979) nonetheless have been reported from a very large number of sites and over an extensive period of time. The global biogeography of individual genera of tintinnids has been reported previously (Dolan and Pierce 2013; Pierce and Turner 1993), including *Cyttaroclylis* and *Petalotricha*, and both genera were described as warm water/temperate found in nearly all marine
waters except polar systems. However, close examination of species records may reveal patterns masked within such large scale distributions. We combed the literature in an attempt to compile all the species records of *Cyttarocylis* and *Petalotricha* and so extensively updated the database exploited in Dolan and Pierce (2013). Using this database, we examined the global distribution of species records of *Cyttarocylis* and *Petalotricha* postulating that there may be distinct geographic zones of overlap or exclusivity such as coastal vs. open water sites or tropical vs. temperate zones. We analysed the records of individual species to determine frequencies of co-occurrences seeking to establish if there is a main form and perhaps ‘child forms’, developmental stages. Lastly, concerning the two most commonly reported forms of *Cyttarocylis* and *Petalotricha*, we analysed data providing an annual cycle from a single site in the Northern Adriatic and large-scale spatial distributions in distinct seasons across the entire Mediterranean Sea for evidence of environmental specificity corresponding with a particular morph. Paradoxically, we found no such evidence.

**The Database of Species Records**

The original database of Pierce and Turner’s global biogeography of tintinnids (1993) was updated in Dolan and Pierce (2013) to include 753 records of the occurrences of species of *Cyttarocylis* and *Petalotricha*. For this review, additional species records were located to yield a revised database of 944 species records from 450 sites extracted from 76 publications. The database consists of species name, location, latitude and longitude (if not given, assigned based on site name or map included in the report) and reference. For some reports sampling dates were also included. The data as an Excel file is available as Supplementary Material (File S1).

We also analysed the records to examine the frequencies of co-occurrences on a generic basis (pooling all species of a genus) as well as of the major individual forms (those in each genus cumulatively representing > 90% of the species records). Thus, we tallied the occurrences alone and in all possible pairs of *Cyttarocylis acuminata*, *Cyttarocylis brandti*, *Cyttarocylis cassis*, *Cyttarocylis eucecryphalus*, *Cyttarocylis longa*, *Petalotricha ampulla*, and *Petalotricha major*.

Two monographs provided data allowing investigation of temporal pattern of abundance at a single site over an annual cycle, that of Krsinic (2010) and large scale spatial distributions, in different season- summer and in winter, that of Jörgensen (1924). Jörgensen (1924) reported species presence for samples taken during the Thor Expedition across the Mediterranean Sea from two transects, one in December 1908 - February 1909 and again June-September 1910. Station locations were obtained from Schmidt (1912). Plankton samples of the Thor expedition were obtained using nets made with a No. 20 silk cloth (Schmidt 1912) which, according to Sverdrup et al. (1942), was of a mesh size of 76 μm, sufficient to retain the lorica of *Cyttarocylis or Petalotricha* forms. Krsinic (2010) reported in graphic form data on temporal changes in abundances of *Cyttarocylis eucecryphalus* and *Petalotricha ampulla* from sampling a single site, 50 m depth, in the Northern Adriatic, near Dubrovnik, at weekly to biweekly intervals in 1996-1997 using a 50 μm mesh net.

**Global Distribution of *Cyttarocylis* and *Petalotricha***

Figure 5 shows the species records mapped in four categories: all 451 sites with species records, the 164 sites where both *Cyttarocylis* and *Petalotricha* were found, the 197 sites where only *Cyttarocylis* was found and finally the 88 sites where only *Petalotricha* were found. It should be noted that these records are presence/absence records. The overall distributions of *Cyttarocylis* and *Petalotricha* are the “warm water” distributions which characterizes many tintinnid genera ranging from *Amplectella* to *Xystonellopsis* (Dolan and Pierce 2013). The major difference between the “warm water” distribution and a “cosmopolitan” distribution is a general absence from sub-polar and polar waters. There were no obvious differences (e.g. coastal vs. open water or tropical vs. temperate zones) in the geographic distribution of any of the different categories of sites (i.e., sites from which only *Cyttarocylis* or only *Petalotricha* were reported, and sites in which both were found). Thus, there were no obvious geographic patterns of segregation or co-occurrence evident from mapping the data. However, consideration of overall frequencies suggested some spatial segregation.

*Cyttarocylis* species were reported from 361 of the 451 sites, an overall frequency of 0.80, while *Petalotricha* was reported from 252 of the 451 sites, an overall frequency of 0.56. Based on these frequencies, the number of sites at which they occur together is predicted to be \((0.80 \times 0.56) \times 451 = 202\) sites significantly larger than 164, the actual number of sites where they were found.
Figure 5. Geographic distribution of species records. Note that there are no obvious geographic patterns distinguishing the distributions. However, *Cyttarocyulis* and *Petalotricha* were reported together less frequently than expected based on overall frequencies of occurrence of *Cyttarocyulis* and *Petalotricha*. See Results for details.

together ($\chi^2$ test). This led us to analyse the co-occurrences of the major species to determine if some forms were found only in the presence, or conversely, in the absence of others. We reasoned that if some forms were developmental stages of others, immature or 'child-forms', they would usually be found in the presence of the mature form and rarely found alone. If one form is found alone often relative to the other forms, it is perhaps a mature or main form from which the others are derived.

**Co-occurrence Among Forms**

Table 1 gives the results of the co-occurrence analysis. All the common forms are found most often with at least one other form. Most of the forms are in fact rarely found alone, having been reported in the absence of other *Cyttarocyulis* or *Petalotricha* in only 6 -14% of all of their records. These potentially 'child forms', as they almost always occur with another form, are *C. acuminata*, *C. brandti*, *C. casis*, *C. longa* and *P. major*. Two, rather than one, forms stood out as reported alone in a substantial portion of the records: *C. eucrephyalus* reported alone in 34% of its records and *P. ampulla* reported alone in 43% of its record; thus it appears there are 2 'main forms'.

**Global Distribution of the Main Forms**

Mapping the species records of the two main forms, *Cyttarocyulis eucrephyalus* and *Petalotricha ampulla* reveals interesting but difficult to explain differences between the distributions of the two forms (Fig. 6). Notably, both have reported from a very wide range of marine environments ranging from zones of upwelling, i.e., California Current for *C. eucrephyalus* and Benguela Current for *P. ampulla*, to oligotrophic tropical waters of both the Pacific and Atlantic Oceans. Intriguingly, *C. eucrephyalus* has been recorded much more extensively in the Central Pacific and the Gulf of Mexico than *P. ampulla* which appears to be more common in the Southern Atlantic and the Pacific waters of Asia than *C. eucrephyalus*. However, these broad patterns of occurrence do not
correspond with known marine biomes, typically relatable to marine provinces separated by currents and characterised by different rates of primary production, seasonality, temperature, etc. (i.e., sensu Longhurst 1998).

### Distribution of the Main Forms in the Mediterranean Sea

The Mediterranean Sea is characterised by large seasonal differences in water column stratification and primary production. Furthermore, in the summer there are marked West to East differences in water column characteristics corresponding to a well-known gradient of mesotrophic conditions in the western to marked ultra-oligotrophic conditions in the east. The differences from west to east include deepening of depth of the chlorophyll maximum layer, lower concentrations of chlorophyll, nutrient salts and concentrations of both autotrophic as well as heterotrophic prokaryotes and protists (e.g. Dolan et al. 1999, 2002).

The Thor Expedition sampled across the Mediterranean in the winter of 1908-1909 and again in the summer of 1910. The two main forms, Cyttarocylis eucecryphalus and Petalotricha ampulla were recorded extensively by Jörgensen (1924) in material from the Thor Expedition in the winter and summer samples. With regard to C. eucecryphalus, Jörgensen noted that it always occurred “singly or very scarce” except for one winter sample from the central Mediterranean, noted as ‘several specimens’. P. ampulla, on the other hand while also noted as usually ‘singly or very scarce’ was described as ‘abundant, common, predominant, numerous’ for several samples from the far Western Mediterranean in winter. Nonetheless, both of the forms were found more or less throughout the entire Mediterranean Sea from Gibraltar to Cyprus in samples collected both in the winter transect and the summer transect (Fig. 7). Despite the

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**Table 1.** Frequencies of co-occurrences among the common forms of Cyttarocylis and Petalotricha. Note that all are usually reported in the presence of at least one other form. However, of the seven common forms, five are very rarely found alone (6-14% of records for the species) while two appear exceptional as they are found alone fairly frequently, C. eucecryphalus and P. ampulla, 34% and 43%, respectively of the species records.

<table>
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<th>Species (Σ sites found), abbreviation</th>
<th>Alone % sites</th>
<th>w/Ca % sites</th>
<th>w/Cb % sites</th>
<th>w/Cc % sites</th>
<th>w/Ce % sites</th>
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<tr>
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<td>16</td>
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</table>

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**Figure 6.** Global distribution of the species records of Cyttarocylis eucecryphalus and Petalotricha ampulla. Note that there are broad zones of overlapping distribution with some differences such an apparent under-representation of P. ampulla in the Central Pacific and absence of C. eucecryphalus in the Southern Atlantic. However, the differences in distribution do not correspond with obvious qualities or characteristics of the biomes.
large differences in conditions, both seasonally and from west to east, there was little evidence of spatial segregation. For most of the stations from which either form was recorded, both were found together.

**Temporal Abundance Patterns of the Main Forms**

Quantitative data for *Cyttarocylis eucecryphalus* and *Petalotricha ampulla* were provided by Krsinic for a site sampled in the Northern Adriatic at weekly to biweekly intervals in 1996-1997 (Krsinic 2010). In agreement with most reports from around the globe, concentrations of both forms were relatively low. Typical concentrations of tintinnids in the Central and Eastern Mediterranean are usually about 10-30 cells l\(^{-1}\) for the top 100-200 m of the water column (e.g. Dolan 2000; Pitta et al. 2001). The concentrations recorded for both forms were equal to 0.01-0.1 cells l\(^{-1}\), detectable only through analysing material from 10’s to 100’s of liters. Despite the low abundances, clear population dynamics were evident with peaks in abundance about every 2 months throughout the year. Figure 8 shows the largely parallel changes in abundance of the two forms. Scatterplots of the abundance of one form against the abundance of the other yielded a significant positive relationship. The regression reflects the fact that except for an early winter peak of *C. eucecryphalus*, on average the population of *Cyttarocylis/Petalotricha* was usually dominated by the *Petalotricha* form.

**Causes and Consequences of Polymorphism**

The lack of any clear sorting among the forms corresponding to different biomes or seasons argues against the distinct morphologies representing forms with distinct ecologies. In this regard it is noteworthy that the morphotypes of *Cyttarocylis ampulla* are united in one characteristic - loria oral diameter. This is also the case the case for the forms of the other recently revealed polymorphic species, *Cymatocylis affinis/convallaria*. The forms formally known as different species of *Cymatocylis*

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**Figure 7.** The occurrence of *Cyttarocylis eucecryphalus* and *Petalotricha ampulla* in samples taken across the Mediterranean Sea during the Thor expedition of 1908-1910. Note that both forms were found across the Mediterranean in both winter and summer and most often occurred together. There was little evidence of seasonal or spatial segregation between *C. eucecryphalus* and *P. ampulla*.
vary greatly in overall shape and length but are similar in lorica oral diameter (Kim et al. 2013).

Loricais oral diameter is known to be a conserved morphological character (Laval-Peuto and Brownlee 1986) as many tintinnid species appear to have loricas which can vary greatly in overall length but are very consistent in oral diameter. Not only a conserved taxonomic character, loricais oral diameter is relatable to basic ecological characteristics of the tintinnid species as well (Dolan 2010; Montagnes 2013). It is, not surprisingly, related to the size of the prey ingested. Tintinnids are capable of ingesting prey items ranging in size from prokaryotes (Bernard and Rassoulzadegan 1993) to cells of a size almost as large as themselves (Kamiyama and Arima 2001). However, loricais oral diameter is a good predictor of the preferred prey size, considered as prey size corresponding with the maximal clearance rate. This size is about 20% of loricais oral diameter (Dolan 2010; Montagnes 2013). Loricais oral diameter is also inversely related to maximum growth rate (Dolan 2010; Montagnes 2013). These range from rates of 1.5 - 2 (r, d⁻¹) for relatively small-mouthed species with loricais oral diameters < 20 μm to maximum reported rates of about 0.5 (r, d⁻¹) for species with loricais oral diameters species (100 μm diameter), the size of that of Cyttarocylis (approx. 100 μm diameter). Rather than ecological differentiation, the similarity in loricais oral diameter among the different forms of Cyttarocylis argues in favour of an ecological similarity at least with regard to the size of preferred prey ingested (about 20 μm in diameter) and maximum growth rate (about 0.5 d⁻¹).

Predator-induced changes in morphology to forms more predation-resistant, while unknown among tintinnids, are known among other ciliates (e.g. Wiackowski et al. 2004; Wickham and Gugenberger 2008). However, while there are several different forms of Cyttarocylis, they are all similar in overall size and shape (unlike the different forms of Cymatocylis) and none feature obvious anti-predation structures such as spines or other protuberances as known in other ciliates. Consequently, it is difficult to see how polymorphism in Cyttarocylis could relate to predation pressure.

Loria formation has been examined in only a few species. However, what little is known about loria formation does suggest that different morphologies maybe the result of differences in the quantities and qualities of prey available prior to cell division. This is based on the accounts of loria formation made by Laval-Peuto (1976, 1977, 1981), summarized below, concerning Favella - the only species in which ‘lorica variability’ has been studied using cultures.

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**Figure 8.** Temporal changes in the abundances of Cyttarocylis eucryphalus and Petalotricha ampulla at a site in the Northern Adriatic near Dubrovnik based on data from Kršinić 2010. Temporal changes in abundances based on sampling in 1996-1997 and the inset plot show the relationships of the abundance of each as a function of the other form. Note that the two forms show very similar temporal patterns of abundance although Petalotricha is generally the numerically dominant form. Abundances of the two forms are significantly related (p < 0.01, r = 0.61, n = 24).
The feeding stage, the trophont, accumulates ‘lorica-forming granules’ in the posterior portion of the cell and these increase in markedly in number in the hours preceding cell division. Coinciding with the formation of a new mouth, the granules migrate to the anterior zone of the cell, the future protor cell. When cell division is complete, the opisthe has the new oral structure and keeps the lorica. The protor, with the original (parental) oral structure and the lorica-forming granules swims off to form a new lorica. The newly divided cell will form its lorica using intracellular reserves, the lorica forming granules. Globules of lorica material are extruded from the anterior portion of the cell in the zone of somatic ciliature. The first stage is formation of a ring around the cell. Material is added to the ring downwards to form a short cone. The lorica is then constructed upwards from the open anterior end by adding bands of material to the rim of the opening. The last stage is the final formation of a posterior horn and the smoothing of the exterior lorica wall, both appear to be the result of a slow flow of lorica material towards the posterior end mediated by the helical swimming of the ciliate. The entire process takes 3 - 4 h.

In Favella, distinct differences in lorica structure of protor and opisthe occur, apparently resulting from the amount and quality of lorica-forming granules carried away by the protor. The overall lorica length and size of the aboral horn depend on the quantity of lorica forming granules as construction stops when the granules are exhausted. Different lorica wall architectures, smooth-walled “favella” vs. helical sutured “decipiens” forms can be constructed. The duration of lorica formation is similar in the two cases. Laval-Peuto concluded that the differences in the quality, not quantity, of lorica forming granules likely was the cause. A more viscous substrate material produces the “decipiens” form with a rough wall, with irregular alveoli and obvious suture points. The standard ‘favella’ is from a more fluid substrate which fuses without any traces, and produces a long aboral horn and a smooth continuous lorica wall. Notably, still another lorica type is made by Favella whenever a lorica is lost or abandoned - a replacement lorica type. A “coxielliform” lorica, a lorica formed of broad bands, are made by cells of both “favella” and “decipiens” types if separated from their loricas.

Loric formation in Favella admittedly might not correspond with that of Cyttarocylis. However, it seems plausible to attribute polymorphism to differences known from work with Favella. Thus, different loricas might be produced from differences in the loriaca forming granules made by the ciliate in the hours immediately proceeding cell division. An additional possible source of polymorphism known from Favella is a difference between loricas formed directly following cell division and replacement loricas.

Kofoid himself noted that different morphologically distinguished ‘species’ of the same genus are very often found together, rather than being geographically separated (Kofoid 1930). He considered the phenomenon as evidence of sympatric evolution. The possibility that distinct morphologies do not scale with distinct genetic types was not considered by Kofoid. Recent sequence-based evidence suggests that polymorphism may be common and consequently, morphology-based estimates of biodiversity may represent inflated values compared to actual species diversity. In the particular case of Cyttarocylis the inflation is probably minor. Although different forms are usually found together (Table 1) they are also usually present in very low concentrations compared to other tintinnids. For example, the cells shown in Figure 9, (the forms C. cassis, C. eucecryptalus and C. brandti) represented only four cells in a sample containing 1070 tintinnids of either 27, counting them as single species or 29, species, if each is treated as separate species. Counting them as one or three species would produce very minor differences in most diversity metrics. The larger question is if polymorphism is common amongst tintinnids or not. Limited data available to date suggests that many of the species described based on lorica morphology may be variants of other species. Consequently, observed morphological diversity may exceed actual genetic diversity. By how much? Consider a radical yet simple scenario in which each genus of tintinnid is found to be a single species. In the example given above, the 27 species represent 18 genera. Dominant forms are rarely of the same genus (e.g. Dolan and Pierce 2013) consequently, a decline in species richness from 27 to 18 would produce lower values of many diversity metrics but the values would remain within the same order of magnitude. An exception may be communities of shallow coastal waters which are often composed largely of Tintinnopsis species. However, the genus appears to be polyphyletic so how many species and genera are presently grouped is unclear.

**Directions for Future Research**

Left unanswered by our study is the nature of the mechanism generating polymorphism. Experimental investigations of the roles of food quality and
quantity or abiotic conditions are an obvious avenue of exploration. Cultured populations may also permit examination of conjugation. To our knowledge, conjugation in *Cyttarocylis* or *Petalotricha* has not been observed. Mating type experiments would enable verification that the distinct morphotypes are indeed not reproductively isolated. However, such work with cultured populations is problematic as most tintinnids have proven resistant to culture since their discovery (i.e., Müller 1779). In particular, *Cyttarocylis* poses special challenges as natural abundances are usually quite low, just a few specimens per cubic meter. Furthermore, it appears especially resistant to culture. Laval-Peuto whose skill in culturing enabled her to document loriciform variability in *Favella* was unsuccessful not only in culturing but even in maintaining *Cyttarocylis* in the laboratory for more than a few hours (Laval-Peuto, pers. commun.). Given the apparent difficulty in pursuing experimental approaches in *Cyttarocylis* as well as other culture-resistant protists, genomic approaches appear to be a reasonable alternative.

Examining a variety of genes may be needed to untangle relationships between closely related forms. A comparative genomics study showed that within the unicellular eukaryotes, the genes coding for proteins have high divergence relative to the 18S rDNA sequence, suggesting that the ribosomal genes could be too conservative (Piganeau et al. 2011). To assess the similarity of other genes between the *Petalotricha* and *Cyttarocylis* forms, the single-cell approach based on isolation from the environment and sequencing of other markers than the 18S might provide evidence that the different morphotypes are genetically distinct. At present such analyses are difficult because of an insufficient amount of DNA material in a single-cell, but potentially an intermediate step of whole genomic amplification could be used as it has been successfully applied to some uncultivated protist groups (Heywood et al. 2010; Yoon et al. 2011).

We need to sequence numerous single cells from the same species showing polymorphism or from closely related species in order to see if polymorphism might be more common than we think. We support Andre et al.’s call, based on their work in the foraminifera, for a closer look at the scaling between morphological and genetic diversity as the two may be inconsistent (Andre et al. 2013).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.protis.2013.11.002.

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