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High genetic diversity in the hydroid *Plumularia setacea*: A multitude of cryptic species or extensive population subdivision?

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ABSTRACT

The marine hydroid *Plumularia setacea* has a near-cosmopolitan distribution. As in other sessile invertebrates with limited dispersal abilities, the wide distribution could also be a taxonomic artefact and the species might in fact be a complex of sibling species. To investigate this, a set of worldwide samples of *P. setacea* and several closely related species was examined using the mitochondrial markers 16S and COI, as well as the nuclear marker ITS. The results suggest an even higher degree of genetic diversity than expected. Almost all sampled regions had only private haplotypes and the resulting trees split into a multitude of geographically delimited lineages, this both for the mitochondrial and nuclear markers. In the framework of a genealogical species concept, these lineages would qualify as cryptic species. Using alternative species concepts, the results could be reconciled with traditional taxonomy by regarding *P. setacea* as a single species with an extensive population subdivision. A rapid molecular clock, limited dispersal abilities, and localized clonal propagation are likely the factors that explain the high but dispersed genetic diversity within this species.

1. Introduction

Due to the apparent large scale uniformity of the marine environment, it has been assumed until quite recently that marine species generally exhibit higher degree of connectivity among geographically distant populations, leading to wide, sometimes cosmopolitan, distribution patterns. Cosmopolitanism implies high dispersal, colonizing capacity, and tolerance to a wide array of environmental conditions. However, the use of genetic methods has shown that many of these "cosmopolitan species" might actually be complexes of sibling species (e.g. Palumbi, 1992, 1994; Knowlton, 1993, 2000; Sanford and Kelly, 2011). This seems particularly true for morphologically simple invertebrates with few taxonomically exploitable characters and thus with a high potential of hidden speciation (e.g. Ladner and Palumbi, 2012). Numerous studies have shown this on a regional level, but even on a global scale there are several examples, e.g. for sponges (Xavier et al., 2010), jellyfish (Dawson and Jacobs, 2001), bryozoans (Gomez et al., 2007; Nikulina et al., 2007) and chordates (Caputi et al., 2007).

Hydrozoans (Cnidaria, Hydrozoa) seem to be no exception. More than in any other marine invertebrate group, many hydrozoan species have very wide distributions. While this is understandable for holopelagic, off-shore species like siphonophores and trachyline medusae, there are also a number of sessile hydroids known to have a very wide, circumglobal distribution. One example is *Obelia geniculata*, a common sessile hydroid found in nearly all temperate to cold waters and producing a medusa with limited dispersal capacity. Govindarajan et al. (2005) could show that this nominal species is likely a complex of three geographically delimited species. Similar observations were made for other genera by Schuchert (2005), Miglietta et al. (2009), Folino Rorem et al. (2009), Martinez et al. (2010), Lindner et al. (2011), and Pontin and Cruickshank (2012). Additionally, using a barcoding approach, Moura et al. (2008–2012) found that many nominal hydroid species are composed of distinct lineages, suggesting that they could be cryptic species. However, the sole existence of distinct and deep mitochondrial lineages alone is not evidence enough that a species is composed of several biological- or phylogenetic species. Strongly divergent mitochondrial lineages can also be found within the same biological species due to persistent ancient polymorphisms or genetic introgressions and hybridisations (e.g. Ladner and Palumbi, 2012). Only when nuclear genes show concomitantly the same lineages, sufficient evidence for distinct species is given (using a genealogic species concept; Baum and Shaw, 1995; Hudson and Coyne, 2002; Mallet, 2007).

Similar to *Obelia geniculata* mentioned above, *Plumularia setacea* is a common marine hydroid found circumglobally in tropical to temperate seas, usually in very shallow depths. It lacks a medusa

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stage and it is larviparous. Its dispersal capacity via the planula larvae appears quite limited (Hughes, 1986). It can be found on seaweeds and it has rarely been found on man-made objects (Morri and Boero, 1986). Potentially the colony can therefore also disperse via floating seaweeds or on ship-hulls. Its broad distribution could thus be explained by rafting (Cornelius, 1992). Alternatively, its putative wide distribution might also be a taxonomic artefact due to overlapping phenotypes of several distinct species, either allopatric or sympatric ones (Schuchert, 2013a).

Several species closely resembling *Plumularia setacea* are also known, some needing a re-evaluation of their taxonomic status. Examples of better known nominal species are:

- *P. warreni* and *P. strictocarpa* only differ in subtle differences of the reproductive organs or strategy; both occur in sympatry with *P. setacea* and are therefore likely good biological species.
- *P. gaimardi* is rather distinct morphologically (see Schuchert, 2013a) and occurs sympatrically with *P. setacea*. *P. gaimardi* it is thus likely a distinct species.
- *P. lagenifera* is restricted to the NE Pacific and sympatric with *P. setacea*. Its taxonomic status has been re-evaluated recently (Schuchert, 2013a).

More nominal *Plumularia* species exist that are, however, mostly ill known and very rare (comp. Calder et al., 2009). Their status can only be re-evaluated correctly once it is known if *P. setacea* is really a cosmopolitan species or a species complex.

The question of this study was thus:

- Is *P. setacea* a true, cosmopolitan species (meaning reciprocally monophyletic to other nominal *Plumularia,* and with mitochondrial and nuclear markers recombining independently as in a panmictic population),
- or is *P. setacea* composed of independently evolving lineages (*P. setacea* is polyphyletic in relation to congeners, the mitochondrial and nuclear markers delimit the same sub-lineages and demonstrating thus their genealogic unity).

To answer these questions, samples of *P. setacea* and its relatives from all oceans were studied using two mitochondrial markers (parts of the 16S and COI gene) and a rapidly evolving nuclear marker (ITS, internal transcribed spacer separating the tandemly repeated 18S and 28S ribosomal RNA genes).

2. Material and methods

2.1. Collection and identification of samples

Plumularia colonies were collected at various localities as given in Table 1. Identifications were made based on morphological characters using Millard (1975), Cornelius (1995), and other studies cited in Schuchert (2013a, 2013b), therefore using a morphotype species concept. One to three plumes per sample were preserved in 95% ethanol and kept at −20 °C. *Plumularia gaimardi* has previously been identified as *P.* cf. *lagenifera* in Leclère et al. (2009; 16S sequence Genbank number FJ550491), but following Schuchert (2013a) the identification of this specimen had to be revised.

2.2. DNA extraction and voucher specimens

DNA was extracted usually from a single plume, thus representing a single genotype, as given in Schuchert (2005) and dissolved in 100 µl TE buffer. The residual skeleton of the colony was used to make permanent microscopic preparations which were deposited as voucher specimens in the Natural History Museum of Geneva (MHNG). The accession numbers of the voucher specimens are given in Table 1.

The DNA extracts were assigned unique numbers which are also used in Table 1 and Figs 1 and 2, thus allowing a comparison of individual isolates in the phylogenetic trees. Some of the ITS sequences had to be cloned. Their numbering system is composed of the DNA isolate number followed by the clone number after a period.

A few specimens and their DNA were obtained from other museums (Table 1).

2.3. PCR

All PCR reactions were performed in a final volume of 50 µl using a Taq polymerase and reagents purchased from Qiagen (final mixture: 1xPCR buffer, 1 mM dNTP, 1 pmol primers, 1.8 U Taq polymerase).

About 590 bp of the large mitochondrial ribosomal RNA (16S) was amplified from 1 µl of DNA extract as given in Schuchert (2005).

A fragment of about 660 bp of the mitochondrial Cytochrome Oxidase I (COI) was amplified using the reverse primer HCO2198 of Folmer et al. (1994) and a new forward primer CoF TAAACTTCAGGGTGACCAAAAAATCA (a modified HCO2198 primer of Folmer et al., 1994). This primer pair worked only in for about 2/3 of the samples and more specific, internal primers had to be designed (PF1 ATAAAGATATAGGAACATTATACTTAAT and PR3 TATAAAATTGGATCCCCTCCTCCTGC). However, even with these improved primers it was not possible to obtain results for all specimens. The PCR cycling conditions were 5 × (94°50 s/45°50 s/72°60 s) + 35 × (94°50 s/50°50 s/72°60 s).

The complete internal transcribed spacer 1 (ITS1), 5.8S rDNA, and ITS2 region of the nuclear ribosomal DNA was amplified using primers designed to anneal to the 18S (GTCGCTACTACCGATTGAATGG) and 28S (CGCTTCACTCGCCGTTACTAGG) ribosomal genes (shortened primers of those given in Martinez et al., 2010). The PCR cycling conditions were 28 × (94 °C 20 s/51°45 s/72°90 s).

2.4. Sequencing and alignment

The PCR products were purified by spin columns and then directly sequenced in both directions by Macrogen, Inc. (Seoul, Korea) with the same primers as used in the amplification reaction.

About half of the ITS sequences proved to be unreadable due to a mixture of at least two sequences (intragenomic variation). In these cases, the PCR product was cloned using the TOPO® TA Cloning Kit from the Invitrogen Company, following the protocol provided by the manufacturer. Each transformation yielded 10–200 or more *E. coli* colonies. Six randomly picked clones were suspended each in 100 µl TE buffer and heated to 95 °C for 5 min. 1 µl of this crude extract was then used to amplify the cloned ITS fragment using the same PCR conditions as given above. Three clones were then directly sequenced as described above.

All sequences in this study have been deposited in Gen-Bank (accession numbers from KF982071 to KF982249) (www.ncbi.nlm.nih.gov/genbank/).

The sequences were edited and aligned using Bioedit Sequence Alignment Editor (Hall, 1999) and the integrated ClustalW tool using default settings (Larkin et al., 2007). Both 16S and ITS had highly variable regions and the alignments needed introducing several gaps (insertions/deletions). Alignments obtained were therefore run through Gblocks (Castresana, 2000; Talavera and Castresana, 2007) to remove ambiguously aligned regions using the default "less stringent" settings.