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Morphology and molecules on opposite sides of the diversity gradient: Four cryptic species of the *Cliona celata* (Porifera, Demospongiae) complex in South America revealed by mitochondrial and nuclear markers

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ABSTRACT

A great number of marine organisms lack proper morphologic characters for identification and species description. This could promote a wide distributional pattern for a species morphotype, potentially generating many morphologically similar albeit evolutionarily independent worldwide lineages. This work aimed to estimate the genetic variation of South America populations of the *Cliona celata* species complex. We used COI mtDNA and ITS rDNA as molecular markers and tylostyle length and width as morphological characters to try to distinguish among species. Four distinct clades were found within the South American *C. celata* complex using both genetic markers. The genetic distances comparisons revealed that scores among those clades were comparable to distances between each clade and series of previously described clionaid species, some of which belong to different genera. Our results also suggest that one of the clades has a broad discontinuous distribution in the Atlantic Ocean, while another presents high gene flow between the southern Atlantic and Pacific coasts of South America. Conversely, spicule morphology was not able to distinguish each clade, due to the high degree of overlap among them. Therefore, we considered that each recovered clade correspond, in fact, to different species that cannot be differentiated via morphological characters, which are often used to describe species within the *C. celata* species complex.

1. Introduction

A great number of marine organisms lack proper morphologic characters for species identification, which leads to poor taxonomic descriptions. This paucity of characters for species descriptions not only can complicate the identification process in the field, but also of museum deposited samples. In the past, postulated wide distributional ranges were seldom equated with the possession of poor discriminating morphologic characters. Many of such cases were, nevertheless, subsequently shown to comprise several independent evolutionary lineages (e.g., Bucklin and Hedgecock, 1982; Solé-Cava and Thorpe, 1986; Ward, 1990; Klautau et al., 1999; Carreras-Carbonell et al., 2005; Nakano and Spencer, 2007).

This problem is further enhanced since it has been historically and mistakenly accepted that marine species have a broad geographic distribution due to the lack of gene flow barriers and large dispersal capabilities (Knowlton, 1993). In many cases, when morphologic variability among "populations" is actually found, it is often considered intraspecific variation (Knowlton, 1993; Hellberg, 2009), blurring the boundaries for delimitation of clusters of individuals with a specific range of variance in characteristics, i.e., species recognition. Thus, it is important to constantly investigate the prevalence of marine species that possess large geographic ranges. The increased use of molecular methods for systematics and ecological surveys in recent years has raised an era of discovery of cryptic species, being the marine environment an important vault of these species (Bickford et al., 2006). Even so, a large part of the divergence of some quantitative traits can be driven by factors other than genetic differentiation (Lynch et al., 1999), which is an estimator of the extent to which individuals from different samples share a common gene pool. Also, due the method applied to reveal those species, the traditional species' definitions have been improved to include phylogenetic assumptions or take into account coalescence processes (see Wheeler and Meier, 2000), sometimes in spite of their intraspecific variation.

Sponges are a well known example of the critical paucity of morphological characters on which to base species identifications and build their classification (Hooper and van Soest, 2002). The many sponge taxa with little or no spicule diversity at all, offer

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an even greater challenge for sound species delimitation in this taxon (Muricy et al., 1996b; Vacelet and Perez, 1998; Klautau et al., 1999; Carvalho and Hajdu, 2001; Klautau and Valentine, 2003; Pinheiro et al., 2007). Additionally, what has been observed is that marine sponges that present a small number of spicule categories often have postulated broader geographic distributions than sponges possessing a large variety of those skeletal elements (Klautau et al., 1999). Solé-Cava and Boury-Esnault (1999) argued that species that presented a wide distribution often had the largest genetic divergence compared to those with a more restricted distribution. Frequently, those species with postulated wide distributions also comprised polyphyletic assemblages. With the advance of population genetic studies with sponges many complexes of cryptic species have been revealed (Solé-Cava and Thorpe, 1986; Solé-Cava et al., 1992; Muricy et al., 1996b; Klautau et al., 1999; Lazoski et al., 2001; Barucca et al., 2007; Blanquer and Uriz, 2007; Xavier et al., 2010, and references therein).

The sponge *Cliona celata* Grant, 1826 is considered a complex of cryptic species, largely as a consequence of their morphologic simplicity coupled with a postulated exceedingly wide distribution (Schönberg et al., 2006). The *C. celata* complex includes excavating sponges that contain only tylostyles as megascleres, possess yellow to orange coloration when alive, and comprises many species and morphotypes around the world (Schönberg et al., 2006). The few studies conducted to date on the reproduction of *C. celata* populations revealed its oviparity, but there is a marked lack of information about the reproductive cycle and dispersal abilities of species within the complex (e.g., Piscitelli et al., 2011). This complex includes clionaids that are morphologically close to the *C. celata* described by Grant (1826) and redescribed in Rosell and Uriz (2002), whose type locality is New Heaven (Scotland) and holotype is putatively lost (Rützler, 2002). Rosell and Uriz (2002) also accepted the possibility of *Cliona* species having wide distributions, for example, *Cliona amplicavata* Rützler, 1974 and *C. celata*.

The *C. celata* species complex comprises a series of recognized taxa sharing the possession of tylostyles as their sole category of spicules, and this is where disparity has to be sought for species' recognition (Rützler, 2002; Schönberg et al., 2006). For example, Sará (1978) described *Cliona diversityla* Sará, 1978 with tylostyles in two size categories, purportedly distinguishable from other similar species by their shape and dimension. Conversely, Carballo et al. (2004) utilized primarily statistical arguments in support of the validation of *Cliona californiana* de Laubenfels 1935, originally proposed as a variety of *C. celata*. These were based on slight differences found in the dimensions of tylostyles when contrasting Californian *C. celata* var. *californiana* and Atlanto-Mediterranean populations of *C. celata*. A good example of how tylostyle's morphometric data could be used for species delimitation in the *C. celata* complex was shown by Schönberg et al. (2006). In their study, a new species was described, *Cliona minuscula* Schönberg, Grass and Heiermann, 2006, based on the minute dimensions of its tylostyles that were compared to virtually all other clionaid species belonging to the *C. celata* complex (Schönberg et al., 2006).

Up to now it has been considered that the *C. celata* complex in South America comprises four described species: *C. celata*, *Cliona chilensis* Thiele, 1905, *C. diversityla*, and *C. lisa* Cuartas, 1991. The first two are most commonly assigned for the region: *C. celata* is pointed out as occurring in the Atlantic Ocean, from the southeastern coast of the United States, and the Caribbean (Old, 1941; Hartman, 1958) through the southeastern coast of Brazil (Boury-Esnault, 1973; Prado et al., 2004; Muricy and Hajdu, 2006); *C. chilensis* is assigned for Argentina (Burton, 1940; Palermo et al., 1996, 1998), below the Atlantic distribution of *C. celata*, extending to the Pacific coast of South America all the way to the Galápagos Islands (Thiele, 1905; Desqueyroux and Moyano, 1987; Desqueyroux-Faúndez and van Soest, 1997; Pansini and Sarà, 1999; Willenz et al., 2009; E. Hajdu and G. Lobo-Hajdu, personal observation at the coast of Peru). The main distinguishing character used to differentiate *C. celata* and *C. chilensis* is their disjunctive distribution, since the original diagnostic character (i.e., alignment of the papillae) is now considered plastic, and no detailed statistical analysis has yet been performed to support the validity of *C. chilensis*, the younger of these two names. Notwithstanding, the biogeographic criterion for *C. chilensis* and *C. celata* identification is cloudy. This is because there is no clear morphologic distinction between them and no author, until now, has reported both species together for any locality. For example, some authors assign the occurrence of *C. celata* in Argentina (e.g., Cuartas, 1991), while others (e.g., Burton, 1940) have assigned *C. chilensis* for the same region (López-Gappa and Landoni, 2005). The other two species, *C. diversityla* and *Cliona lisa*, were described for the Magellanic region (Sará, 1978) and Mar del Plata (Cuartas, 1991), respectively. These two latter species have been described based on just one or two specimens and, consequently, without any population variation being recorded for their morphometric characters. This is in conformity to what is commonly done in the alpha taxonomy of sponges, a consequence of most species being apparently rare (Gaston, 1994). But, after their description, there has not been any citation of both species from faunistic surveys in these areas. Therefore, our ability to verify the status of both Argentinean species is hampered by the lack of data on morphologic plasticity at the population level.

The shortage of morphologic characters used in species diagnoses, the undertaking of identifications made solely on the basis of purportedly disjunctive distributions, and the notorious bioerosion potential of these sponges, renders the *Cliona celata* complex in South America an important target for a molecular review of these species' genetic, morphologic and geographic boundaries. The present study used the subunit I of the cytochrome *c* oxidase (COI) of mitochondrial DNA (mtDNA) and the internal transcribed spacers (ITS) of the nuclear ribosomal RNA (rRNA) genes to determine the genetic and geographic population limits of species. Additionally, we aimed at estimating the degree of morphologic variation in tylostyle dimensions and to compare these with that reported from additional species within this complex.

2. Material and methods

All samples used in this study were collected by SCUBA or snorkeling, and were preserved in 93% ethanol until DNA extraction. All vouchers were deposited at the Porifera Collection (MNRJ) at Museu Nacional (MN) of Universidade Federal do Rio de Janeiro (UFRJ). A total of 30 specimens of *C. celata* and 22 of *C. chilensis* from South America were used in this study. Fig. 1 shows sampled localities. In addition, samples *C. celata* from Strangford Lough, Ireland (the nearest locality from *Cliona celata*' type locality that we could get samples from) and from other clionaid species were also included (Supplementary Appendix A).

3. DNA extraction, PCR amplification and sequencing

DNA extraction was conducted according to Lôbo-Hajdu et al. (2004) using a lysis buffer (4 M guanidine hydrochloride, 50 mM Tris HCl pH 8.0, 0.05 M EDTA, 0.5% sodium-N′-lauroylsarcosine, 1% β-mercaptoethanol) followed by extraction with phenol:chlorophorm (1:1) and precipitation with ethanol 100%. The PCR reaction mix contained 1 U *Tth* polymerase (BIOTOOLS), 2.5 mM $MgCl_2$, 80 μM dNTP, 0.5 μM of each primer, and approximately 10–100 ng of DNA template. The whole ITS region, including part of the 18S rRNA, ITS1, the 5.8S rRNA gene, ITS2, and part of the 28S rRNA was amplified together using the primers 18S (5′-TCATTTAGAGGAAGTAAAAGTCG-3′) and 28S (5′-GTTAGTTTCTTTTCCTCCGCTT-3′) (Lôbo-Hajdu et al.,