1 **Morphology, genetics, and historical records support the synonymy of two**
2 **ascidian species and suggest their spread throughout areas of the Southern**
3 **Hemisphere**

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18 **Running title:** *Asterocarpa humilis* in South America

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Abstract

Taxonomic uncertainties and the lack of ecological knowledge can hinder the correct identification and the assignment of biogeographic status of marine species. The ascidian *Asterocarpa humilis* (Heller, 1878), originally described from New Zealand, has a broad distribution in shallow temperate areas of the Southern Hemisphere, having recently colonized areas of the Northern Hemisphere. A close species, *Cnemidocarpa robinsoni* Hartmeyer, 1916, has been reported in the South-Eastern Pacific and the South-Western Atlantic, and several authors considered it a junior synonym of *A. humilis*. We gathered for the first time morphological and genetic data from specimens of distant areas. We studied the morphology of specimens collected at seven locations of South America. Besides, we re-examined specimens from museum collections and revised available literature on these species. Genetic data were obtained from specimens of Argentina and compared with available sequences of *A. humilis* from Chile, New Zealand, England and France. Morphological and genetic analyses showed that all compared specimens were conspecific. Furthermore, specimens from different continents shared haplotypes and exhibited low genetic distance among them. These results, the biological characteristics of this ascidian, and its longstanding presence in different habitats from disjoint areas, allow us to question its native range. We support the idea that *A. humilis* is a cryptogenic and neocosmopolitan species that has been transported by maritime traffic through the Southern Hemisphere, revealing frequent processes of exchange through this wide area for more than a century, with presumably associated alterations in the marine biota.

Additional keywords: morphological analysis, Cytochrome Oxidase I gene, neocosmopolitan species, SW Atlantic, tunicate.

Introduction

Ascidians exhibit multiple examples of difficult species delimitation. They show great morphological variability in shape, size, and color due to genetic differentiation and local environmental conditions (Castilla *et al.* 2000, Dias *et al.* 2009, Hirose *et al.* 2010). Moreover, there is often a paucity of clear-cut diagnostic characters, and these are difficult to observe in many cases. In addition to misidentifications related to incomplete knowledge of morphology (Brunetti *et al.* 2015, Mastrototaro *et al.* 2020), the lack of traditional taxonomic reviews with formal discussions on the boundaries of taxonomic

entities can lead to the coexistence of unsupported species.

The solitary ascidian *Asterocarpa humilis* (Heller, 1878), originally described from New Zealand as *Styela humilis* (Heller, 1878), has also been reported in Tasmania (Kesteven 1909) and South Africa (Hartmeyer 1912). The species is considered introduced in Chile (Clarke and Castilla 2000; Manríquez *et al.* 2014; Turon *et al.* 2016a; Pinochet *et al.* 2017), South Africa (Monniot *et al.* 2001; Peters *et al.* 2017), Europe (England, France, and Scotland, Bishop *et al.* 2013; 2015; Alié *et al.* 2018, Nall *et al.* 2015); and cryptogenic in Argentina (Schwindt *et al.* 2020).

Cnemidocarpa robinsoni Hartmeyer, 1916, described from the Juan Fernández Archipelago, South-Eastern (SE) Pacific, is a morphologically similar species whose taxonomic entity has been largely discussed. Van Name (1945) proposed that *C. robinsoni sensu* Hartmeyer corresponded to *S. humilis sensu* Heller, based on the high individual variation in gonad shape. Later on, Brewin (1946) established the genus *Asterocarpa* for species with branched *Cnemidocarpa* type gonads (elongated and encapsulated), so *S. humilis* (= *C. robinsoni*) moved to *Asterocarpa* based on this feature. Afterwards, several authors have accepted the synonymy of the two species. In that case, the correct binomial is *Asterocarpa humilis* (Heller, 1878) (e.g. Kott 1985; Clarke and Castilla 2000; Turon *et al.* 2016b). Although morphology and genetics were used for identification of specimens (Turon *et al.* 2016b), the *A. humilis* / *C. robinsoni* problem has been never addressed comparing samples from a wide area, including the South-Western (SW) Atlantic, from this multidisciplinary perspective. In the SW Atlantic, *A. humilis* has been reported near Mar del Plata and in the San Matías Gulf (Argentina; Varela 2007); recently, the species was included in a survey of marine bioinvasions in the SW Atlantic (Argentina, Schwindt *et al.* 2020). Reports of *C. robinsoni* came from offshore the San Matías Gulf (Diehl 1977), and from the Argentinian harbors of San Antonio Este, Puerto Madryn, Puerto Deseado and Ushuaia, where it was considered cryptogenic (Schwindt *et al.* 2014). An uncertain citation of the species by Monniot and Monniot (1983) described: “*C. robinsoni*... lives on the southern coasts of Chile (surveys of the University of Concepción) and Tierra del Fuego. Specimens of this species have been found in Franklin Bay, fixed on *Pyura legumen* in 1882 by the Cape Horn Expedition on *La Romanche* (unpublished results)”. Therefore, in the SW Atlantic, there is either one species (as several taxonomists support) or there are two similar species coexisting.

During centuries, multiple species have been transported across biogeographical barriers, but in the last century the pace has significantly accelerated. Historical

information about species occurrences (including absence data) and abundances allows geographically explicit descriptions of changes in their distribution ranges. This would inform about temporal and spatial distribution patterns and, in the case of introduced species, their establishment and expansion (Carlton 2009; Tatián *et al.* 2010; Zhan *et al.* 2015). In this sense, reliable species identification is crucial for understanding the biogeographic status of the species, *i.e.* native, introduced (Essl *et al.* 2018), or cryptogenic when the available evidence is insufficient to determine the species origin (Carlton 1996). Furthermore, the continuous introduction and reintroduction of species mediated by maritime traffic make it difficult to determine the origin of many species (Darling and Carlton 2018). Even the molecular evidence could be tarnished owing to a genetic homogenization, resulting from frequent human transport and successive introductions, whereby admixture between lineages not in contact in the native range can occur (Zhan *et al.* 2015; Rius *et al.* 2015).

In this study, we aimed to revise the taxonomic status of *Asterocarpa humilis* and *Cnemidocarpa robinsoni* in southern South America using an integrative approach. A detailed morphological analysis was conducted on samples from Argentina and Chile that included recently collected specimens, already published material (Schwindt *et al.* 2014), and specimens from museum collections. Partial Cytochrome Oxidase I (COI) gene sequences of specimens from Argentina were obtained and compared with available sequences of *A. humilis* deposited in databases from other areas of the Southern and Northern Hemispheres. We reviewed the habitat (artificial and natural areas) and the historical distribution of the species. We discuss its biogeographic status in a vast interoceanic area, including South America, considering the possibilities of natural dispersal and human-mediated transport.

Material and Methods

Sampling

Specimens were collected from shallow areas of Argentina (SW Atlantic) and Southern Chile (SE Pacific) (Fig. 1, Table 1). Samplings were performed by trawl net during a campaign of the RV “BO Puerto Deseado” (Buenos Aires shelf), by direct collection in the intertidal (Playas Doradas), and by SCUBA (the rest of the localities) (Table 1). After collection, the specimens were relaxed for two hours using menthol crystals (Lambert 2009). A small piece of the muscular mantle of each specimen was preserved in 96% ethanol for genetic analyses and stored at -20 °C until processing. The

rest of the specimen was fixed in 4% buffered formalin in seawater solution for morphological studies. The material examined was deposited in the collection of the Museo de Zoología (MZUC, Universidad Nacional de Córdoba, Argentina).

Morphological analyses

We examined a total of 40 specimens; 29 were obtained from samplings performed in both, natural and artificial habitats. Besides, six were unidentified specimens from the oldest available Ascidiacea collection (years 1916-1921 catalog numbers 10996-10997, Puerto Madryn, collected in 1920) belonging to the Museo Argentino de Ciencias Naturales (MACN, Buenos Aires, Argentina). All these were considered as “unidentified/putative specimens” (U/PS). Five specimens attributed to *C. robinsoni* came from the collection of Schwindt *et al.* (2014) and were considered as “identified as *C. robinsoni*”, (ICR) (Table 1). Specimens were dissected, analyzed and photographed using a microscope camera (OMAX A35140U) attached to a stereomicroscope (Labomed CZM4 and CZM6). We inspected general features, especially the following characters: number of oral tentacles; the number of longitudinal branchial vessels; number and form of gonads; place and orientation of the gonoducts; presence/absence of pyloric caecum; presence/absence of brooding larvae. We compiled and compared the information on these features with descriptions available in the literature. These are for *A. humilis*: Heller 1878; Kesteven 1909; Brewin 1946; Kott 1952; 1985; Millar 1982; Clarke and Castilla 2000; Monniot *et al.* 2001; Varela 2007; Bishop *et al.* 2013; Turon *et al.* 2016a; Pinochet *et al.* 2017; and for *C. robinsoni*: Hartmeyer 1916; Van Name 1945; Diehl 1977.

Genetic analyses

Total DNA was extracted using standard protocols for the DNeasy Blood and Tissues Kit (QIAGEN, Dusseldorf, Germany). We used two sets of tunicate-specific primers to amplify a fragment of the Cytochrome Oxidase gene subunit I (COI) (~ 630 bp, Stefaniak *et al.* 2009; ~540 bp, Ruiz *et al.* 2020). Amplification was performed with 2 µl of each primer (10 µM), 10 µl (0.5 units) of MyTaq™ HS Red Mix DNA polymerase (Bioline), 2 µl of DNA (10 to 20 µg/ml) and PCR water to a total reaction volume of 20 µl. The PCR program included an initial denaturation at 94 °C for 2 min, followed by 32 cycles of amplification at 94°C for 1 min, at an annealing temperature of 42 °C for 1 min, and extension at 72 °C for 1 min 30 sec. After 32 cycles of amplification, a final extension

was carried out at 72 °C for 7 min. PCR products were verified by electrophoresis on 1% agarose gel with RedGel and quantified in BioTeck. Laboratory work was carried out in the Centre for Advanced Studies of Blanes (CEAB-CSIC, Spain) and the Cátedra de Genética de Poblaciones y Evolución (UNC, Argentina). Amplification products were sent to MacroGen Inc. (Seoul, Korea) to be sequenced (forward and reverse strands).

Sequences were assembled using CHROMAS v. 2.6.2 (Technelysium Pty Ltd, Tewantin Qld, Australia) and low-quality regions were deleted. All new sequences were uploaded to GenBank (accession numbers: MK495822-MK495830).

The COI sequences obtained were pooled with sequences of *A. humilis* from England and France (GenBank accession number: JX312278, Bishop *et al.* 2013), France (GenBank: MH011450, Alié *et al.* 2018), Chile (GenBank: KU299758 and KU299759, Turon *et al.* 2016b) and New Zealand (GenBank: JX312279, Bishop *et al.* 2013). No sequence labeled *C. robinsoni* was available in the databases. The sequences were aligned using MUSCLE (Edgar 2004) and collapsed into haplotypes with DnaSP v. 6 (Rozas *et al.* 2017). The number of polymorphic sites was calculated using DnaSP. A median-joining network was constructed with Network software (Bandelt *et al.* 1999) to determine the relationships among haplotypes. The overall mean distance and pairwise distances between haplotypes were calculated (p-distance) using Mega v. 7 (Kumar *et al.* 2016).

For phylogenetic reconstruction, four stolidobranch sequences obtained from GenBank database were included in the analyses for comparison: *Cnemidocarpa verrucosa* (GenBank accession number: KU299764, Turon *et al.* 2016b); *Styela paessleri* (GenBank: KU299779, Turon *et al.* 2016b); *Pyura legumen* (GenBank: KU299778, Turon *et al.* 2016b); and *Paramolgula gregaria* (GenBank: KU299769, Turon *et al.* 2016a). We also included two phlebobranch ascidian sequences as an outgroup, corresponding to the species *Corella eumyota* and *Corella antarctica* (GenBank: MG877558 and GenBank: MG877561 respectively, Alurralde *et al.* 2018; the alignment is available in the supplementary file S1).

We used three different criteria: Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). The best-fit model of nucleotide substitution for the data was selected by statistical comparison of 24 models of evolution, using the Akaike Information Criterion (AIC), with the software jModelTest version 2.1.9 (Darriba and Posada 2014). The selected evolutionary model was GTR+G, and the values of these substitution models were applied to BI and ML analysis. The BI approach was carried

out using MrBayes v. 3.2.5 (Huelsenbeck *et al.* 2015); four chains were run (with default parameters), and the starting tree was random. The analysis was run for 200,000 generations, with a sampling frequency of 100. The first 25% of the trees were discarded as 'burn-in'. The posterior probabilities supporting the nodes were calculated. MEGA v. 7 software was used for ML analysis. Node confidence was estimated using bootstrap with 1,000 replicates. For MP analyses, the TNT v.1.5 program (Goloboff and Catalano 2016) was used. A heuristic search was performed with 300 random stepwise additions of taxa and tree bisection reconnection branch swapping. Standard bootstrap (1,000 replicates) was used to assess the stability of each node.

Results

Morphological analyses

All the collected specimens were found in clumps (Fig. 2a, b). The color of living specimens was orange (Fig. 2a) and the preserved ones were pale-brown. The siphons were quadrangular; in its inner side, over a reddish background, there were four thick pale yellow or white lines interspersed with four thinner lines of the same color (Fig. 2b). The individuals analysed spanned a range of sizes. The smallest specimens were 1.2 cm wide and 2.4 cm high (U/PS) and 2 cm wide and 4 cm high (ICR); the largest were 3.2 cm wide and 4.7 cm high (U/PS) and 3.5 cm wide, 6 cm high (ICR). The tunic was thick and, in the area close to the siphons, rough. The mantle was firm and muscular. The branchial sac showed four marked folds, with eight-12 longitudinal vessels in each fold and from none to three between folds. The number of stigmata between vessels varied between eighth-16 and eighth-15 in U/PS and ICR individuals respectively. Parastigmatic vessels were also present (Fig. 2c) but can be absent in larger specimens. The number of oral tentacles varied between 43-65 in U/PS and 38-64 in ICR, of which some were longer than the rest. There were also several very small tentacles. The intestine had a primary loop and was bent to form a secondary loop including endocarps. The stomach was spindle-shaped with 19-20 folds. No pyloric caecum was found (Fig. 2d). The number of gonads was higher on the right side of the body (three-five in U/PS; four-six in ICR) than in the left side (one-two in U/PS; two in ICR). Gonads were situated above the first loop of the intestine (left side) and, in all cases, close to the endostyle. The gonads, including female and male follicles, were sausage-shaped to irregular, single or branched. Most of the gonads from the right side had between two to six branches. On the left side, most gonads were ramified, commonly in two parts, but they

can have up to five branches. Each gonad had several short gonoducts (Fig. 2e,f), pointing to several directions, mostly to the endostyle but never directed to the atrial siphon. Oviduct and sperm ducts opened separately but close together (Fig. 2g). Brooding larvae showing a single pigment spot were found in specimens collected in Puerto Madryn (Fig. 2h, i).

Genetic analyses

Nine sequences of specimens from the SW Atlantic were obtained (four from Buenos Aires shelf, two from Playas Doradas and three from Puerto Madryn) (Table 1). These nine COI sequences along with a further seven corresponding to *A. humilis* obtained from databases (from Chile, New Zealand, England and France) revealed four haplotypes with eight variable sites (seven parsimony informative sites). The most frequent haplotype (H2) was shared among specimens from Argentina (SW Atlantic, five out of nine specimens), Chile, England and France; the second most abundant haplotype (H1) was observed in Argentina (three out of nine specimens) and Chile. Haplotypes H3 and H4 were singletons found in Argentina and New Zealand, respectively. The overall mean distance (p-distance) between haplotypes was 0.011 (SE: 0.004), and the maximal distance between haplotypes was 0.015 (Table 2).

The haplotype network (Fig. 3) showed the four haplotypes separated by three to ten mutations. Haplotypes H3 and H4 occupied an intermediate position between the two more abundant haplotypes, H1 and H2.

Phylogenetic trees (BI, ML, MP) grouped all sequences of *A. humilis* and the specimens of Argentina into one well supported monophyletic clade, the "*Asterocarpa humilis* group" (posterior probability of 1, bootstrap values of 94 and 100%; Fig. 4). However, the internal topology of this clade varied slightly in the different tree reconstruction methods (Fig. 4, supplementary file Fig. S3, Fig. S4).

Discussion

We confirm the existence of a single taxonomic entity (*A. humilis*) for the SW Atlantic. We did not find relevant differences in the morphological characteristics among the unidentified specimens (those sampled in this study and specimens of the MACN collection) and individuals previously identified as *C. robinsoni* by Schwindt *et al.* (2014). We analyzed morphological features, including the number of oral tentacles, number of longitudinal branchial vessels, number and shape of gonads in each side of the body and orientation of gonoducts. They covered the range previously described for *A. humilis*

(Heller 1878; Kesteven 1909; Bovien 1921; Hartmeyer 1922; Hartmeyer and Michaelsen, 1927; Brewin 1946; Kott 1952; 1985; Millar 1982; Clarke and Castilla 2000; Monniot *et al.* 2001; Varela 2007; Bishop *et al.* 2013; Turon *et al.* 2016b; Pinochet *et al.* 2017) and *C. robinsoni* (Hartmeyer 1916; Van Name 1945; Diehl 1977) (Table 3). Our analysis of specimens did not reveal a pyloric caecum, a character reported for *A. humilis* (Clarke and Castilla 2000). The pyloric caecum is an useful morphological character for styelid taxonomy. However, the drawing by Clarke and Castilla (2000) did not show such a caecum, nor has it been mentioned in other descriptions of *A. humilis* (or *C. robinsoni*, for that matter) (e.g., Van Name 1945, Brewin 1946, Kott 1945, Turon *et al.* 2016b). All this makes the observation by Clarke and Castilla doubtful. On the other hand, in all analyzed specimens we observed the following diagnostic characters attributed to *A. humilis* (Kott 1985): branched gonads, gonoducts oriented in several directions but not towards the atrial siphon, and the presence of brooding larvae in the atrium. Few specimens had unbranched gonads, either on the left or on the right side. These characteristics have been reported in several descriptions and, according to Brewin (1946), the form of the gonads and their duct is variable. Such variation in the number of ramifications may be attributable to intraspecific variability, since they were found in small and large individuals, or to initial development stages, as previously stated (Kott 1985; Van Name 1945).

The genetic analyses based on COI sequences revealed that the specimens from Argentina share haplotypes with those identified as *A. humilis* from Chile, England and France. Likewise, the phylogenetic trees showed that sequences of the specimens from Argentina cluster together with all the available sequences of *A. humilis* (disregarding the geographic origin) in a well-supported monophyletic clade. The low number of variable sites in the COI sequences and the few mutational changes among haplotypes that separate specimens from distant areas strongly suggest that they are all conspecific. In some invertebrate groups, approximately 3% of the genetic distance at the COI locus represents the expected limit of intraspecific variation (Hebert *et al.* 2003; Meyer and Paulay 2005). The specimens in the present study showed lower genetic distances (0.5-1.5%) among haplotypes. Haplotype 1, present in Argentina and Chile, showed the highest genetic distance compared to the other haplotypes (1-1.5%). These values fall within the range of intraspecific genetic distances of COI in other ascidians (0.88-3%, e.g. Lambert *et al.* 2010; Alurralde *et al.* 2018; Rocha *et al.* 2019; Viard *et al.* 2019). In a study within the *Pyura stolonifera* species complex, after a morphological and

mitonuclear genetic analysis, a genetic distance of 10% for COI was found to differentiate the sibling species *Pyura praeputialis* and *P. doppelgangera* (Rius and Teske 2013). Using the same genetic marker, Nydam and Harrison (2007) obtained a divergence value of 12.3% between *Ciona intestinalis* type A and *C. intestinalis* type B (now *C. robusta* and *C. intestinalis*, respectively; Brunetti *et al.* 2015), both of them now treated as valid species with partial reproductive isolation (Bouchemousse *et al.* 2016). Hence, the distance values observed in our samples lie within the limits of intraspecific genetic distance reported for other ascidians and are much lower than interspecies distances reported for pairs of closely related species.

We, therefore, found no evidence of a second species or genetic lineage in SW Atlantic. Further, the samples identified as *C. robinsoni* in the collection of Schwindt *et al.* (2014) were morphologically indistinguishable from *A. humilis* specimens. In the absence of a clear morphological and genetic distinction, we corroborate the statement by Kott (1985) and later authors (Turon *et al.* 2016b), who considered both species synonyms (see synonymy in supplementary file S2), with *A. humilis* having precedence.

Assuming the synonymy of *C. robinsoni* with *A. humilis*, the species could be considered native from the Southern Hemisphere, since it has its oldest records in distant areas such as New Zealand, Tasmania, Argentina, South Africa and, more recently, Australia and continental Chile (see references in Table 3 and 4). However, this finding is at odds with the idea that benthic species with short-living lecithotrophic larvae, like ascidians (hours to few days of the free-swimming period) are unable to naturally spread over large geographical distances (Tarjuelo and Turon 2004; Stolfi and Brown 2015). This period is even shorter for brooding ascidians (Lambert *et al.* 1995; Lambert 2004; 2005), as is the case of *A. humilis*. Alternatively, wide distribution in the Southern Hemisphere can represent a Gondwanan relict, as it was suggested for the *Pyura stolonifera* complex (Kott 1985; 2006). Nevertheless, such ancient origin resulted in the speciation of each of the alleged isolated populations of this complex species (Rius and Teske 2011), while we found a marked genetic homogeneity in samples from very different areas.

The presence of *A. humilis* has been reported in the Southern Hemisphere in both, artificial (Kesteven 1909; Clarke and Castilla 2000; Monniot *et al.* 2001; Schwindt *et al.* 2014; Turon *et al.* 2016b; Page and Kelly 2016; Pinochet *et al.* 2017; this study) and natural habitats (Varela 2007; Page and Kelly 2016; this study). In the Northern Hemisphere, the species is introduced and has been mainly found in harbors (Bishop *et*

al. 2013; 2015; Kenworthy *et al.* 2018). For the SW Atlantic, *A. humilis* (reported as *C. robinsoni*) has been found in natural (Diehl 1977) and in artificial habitats, where it was considered cryptogenic (Schwindt *et al.* 2014; 2020; Table 4). Monniot and Monniot (1983) reported the presence of *C. robinsoni* in the Magellanic region of Argentina (Franklin Bay), in a presumably natural habitat (F. Monniot personal communication, specimens attached to *Pyura legumen* from MNHN collection S1 CNE 09). In the SE Pacific, we report for the first time the presence of *A. humilis* in natural habitats (Table 1), while all the previous records corresponded to artificial settings (Clarke and Castilla 2000; Manríquez *et al.* 2014; Turon *et al.* 2016a, b; Pinochet *et al.* 2017; Leclerc *et al.* 2018).

The association of a given species with artificial habitats is an indicator of an exotic status (Chapman and Carlton 1991; Oricchio *et al.* 2019). Harbors constitute sites for the introduction of marine species via ship traffic (Carlton and Geller 1993; Ruiz and Carlton 2003; Schwindt *et al.* 2014). These areas offer new available habitats (Glasby and Connell 1999, Glasby *et al.* 2007) for colonization and spread of non-native species. That, in turn, affects coastal marine biodiversity (Ruiz *et al.* 1997; Carlton 1999; Carlton 2001; Wonham and Carlton 2005). Later, recreational and fishing vessels would be involved in the local or regional dispersal of exotic species (Leclerc *et al.* 2018) connecting harbors areas with adjacent natural environments (Peters *et al.* 2017; Castro *et al.* 2020) allowing the establishment of these species in natural habitats (Zabin *et al.* 2014; López-Legentil *et al.* 2015; Ulman *et al.* 2017). *Asterocarpa humilis* has been inhabiting both artificial and natural habitats for more than a century, which hinders the assessment of its native range, and so far, we have no accurate evidence of the transoceanic transport of this species through the Southern Hemisphere. However, South American harbors are key components of the main world shipping corridors (Schwindt *et al.* 2020) since the crossing of Cabo de Hornos in the 16th century. This route has promoted interoceanic traffic and has enhanced the regular maritime traffic between Australia and South America since 1840, when the Pacific Ocean trade intensified (Rius and Teske 2011) and many marine species, such as ascidians, might have been transported.

Mechanisms that cause introductions are unlikely to affect only individual species, as transport rarely involves the movement of a single species (Chapman and Carlton 1994). Insights could be therefore gleaned from other species with similar distribution patterns to *A. humilis*. Such is the case of *Corella eumyota* (Monniot 2013

and references therein), whose range of distribution overlapped with that of *A. humilis* for at least a century. *Corella eumyota*'s range of distribution covers Australia (Kott 1952), New Zealand (Michaelsen 1922), Tasmania (Kesteven 1909), Chile (Traustedt 1882; Hartmeyer 1920), Argentina (Michaelsen 1898) and South Africa (Sluiter 1898). Then it was reported in the NE Atlantic (Lambert 2004). Both species were not recorded in Australia until Kott (1952) reported them. Neither *A. humilis* nor *C. eumyota* were included in Herdman's 1899 catalog of Australian Ascidiacea, supporting the idea of a posterior spreading in that area. Besides this similar distribution pattern, both species share reproductive characteristics such as brooding larvae that are advantageous for colonizing new habitats (Lambert 2004). These features suggest that both, *A. humilis* and *C. eumyota* could have been transported and distributed simultaneously in the Southern Hemisphere and more recently, even in the Northern Hemisphere.

The current distribution in multiple oceans of *A. humilis*, including shallow environments and different substrate types, seems to be an example of neocosmopolitanism (Darling and Carlton 2018). Neocosmopolitan species could have undergone a process of anthropogenic historical dispersion through disjunct areas facilitated by maritime traffic, helped by its reproductive characteristics (self-fertilization, brooding larvae and short lecithotrophic larvae). Besides, the absence of previous reports in many areas and the existence of species that share these historical distributions, like *C. eumyota*, could be considered evidence of its non-native status and distribution mediated by human transport.

Historical record analyses are valuable to assess both biological invasions with a long-term perspective and the invasibility of marine ecosystems, when reliable species identification and the biogeographic status of them are available (Zhan *et al.* 2015). The negligible morphological and genetic differences found between specimens from distant areas support the idea of the same specific entity, *Asterocarpa humilis* (Heller, 1878) having precedence, in the Southern and Northern Hemisphere. The records of *A. humilis* in artificial and natural habitats suggest that transport and spread of this species have been occurring for a long historical time in the Southern Hemisphere, and allow us to consider this species as neocosmopolitan. Early registers in the SE Pacific (1916) and in the SW Atlantic (1882), confirm that the species has been present for, at least, one century in a wide area of the Southern Hemisphere, being its native area still unresolved. Therefore, until further evidence is available (e.g. phylogeographic studies), we suggest to assign a cryptogenic status to *A. humilis* in its distributional range in the Southern

Hemisphere (including the SW Atlantic).

This study stresses the usefulness of traditional morphology coupled with DNA analysis for species identification, because they strengthen the taxonomic diagnoses, particularly in cases of cryptic complexes or in species with a high morphological variation (see, e.g., Nishikawa *et al.* 2014; Rocha *et al.* 2019; Turon *et al.* 2020; Viard *et al.* 2019). Also, we emphasize the need to make detailed diagnoses and to generate species barcodes to avoid confusion or misidentification in the future. The joint analysis of the biological information, of the historical records of the distribution in different habitats and localities, and the comparison with similar information from other species is fundamental to analyze the biogeographic status and the probable spread patterns of marine invertebrates, particularly in the case of cosmopolitan species.

Conflicts of interest

The authors declare no conflicts of interest.

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703 Figure captions

704

705 Fig. 1. Map of sites sampled in this study for morphological and genetic analyses. Red
 706 circles indicate new samplings for this study; yellow circles show localities with
 707 sequenced specimens included in our genetic analyses. Black ovals show information of
 708 localities, GenBank accession numbers, and the corresponding haplotype number.
 709 Specimens from Punta Colorada (AR), Albatros Shipwreck (AR), Punta Este (AR) and
 710 Piedra Lile (CH) were only used for morphological analyses. AR: Argentina, CH: Chile,
 711 NZ: New Zealand, FR: France, EN: England.

712 Fig. 2. a) clumps of specimens attached to a rock (basal face), intertidal of Playas
 713 Doradas (Argentina); b) living specimens showing colour pattern of the oral siphons; c)
 714 part of the branchial sac, showing stigmata and parastigmatic vessels; d) dissected
 715 specimen (branchial sac removed), showing gonads; e), f) details of gonoducts (arrows)
 716 opening in several directions (e: endostyle); g) details of oviduct and sperm duct; h)
 717 brooding larvae found in the atrial cavity; i) larva.

718 Fig. 3. Haplotype network of COI gene sequences. Circle sizes are proportional to the
 719 number of total sequences with each haplotype. The colors within circles indicate the
 720 proportion of specimens of each site per haplotype. Dashes along the connecting lines
 721 represent mutational steps. mv1: median vector representing a hypothesized sequence
 722 which is missing and is required to connect existing sequences within the network with
 723 maximum parsimony.

724 Fig. 4. Consensus phylogenetic tree using BI from COI partial sequences. Support
 725 values corresponded to the three methods used (BI, ML and MP). Only values > 0.5 for
 726 BI, > 50% for ML and MP are shown. Sequences of *Corella eumyota* and *C. antarctica*
 727 were used as outgroup. Hyphen (-) denotes bootstrap value < 50% and posterior
 728 probabilities < 0.5; underscore (_) indicates that the clade did not appear in the
 729 corresponding tree.

730

iv. Tables (each table complete with title and footnotes)

Table 1. Sampling sites in SW Atlantic, Argentina (1), and SE Pacific, Chile (2). *Specimens not sequenced, ^a campaign in oceanographic vessel R/V Puerto Deseado (Buenos Aires shelf).

Sampling sites	Origin of samples	Sampling method	N° Specimens	Depth	Habitat	GPS position	N° sequences	Haplotype	GenBank accession number
Buenos Aires shelf (1)	This study	Trawl net ^a	4	50 m	Natural	-38.69028, -57.91805	4	H2	MK495824 MK495826 MK495827
Playas Doradas (1)	This study	Direct collection	6	Intertidal	Natural	-41.635705, -65.01893	2	H3	MK495828
Punta Colorada (1)	This study	SCUBA diving	4	1 m	Artificial (dock)	-41.696800, -65.015497	-	H1	MK495829 MK495830
Puerto Madryn-a. Muelle “Almirante Storni” (1)	This study	SCUBA diving	7	1 m	Artificial (dock)	-42.736143, -65.03174	3	-	*
								H1	MK495822
								H2	MK495823 MK495825

Puerto Madryn-a. Muelle "Almirante Storni" (1)	Schwindt et al. 2014	SCUBA diving	5	1 m	Artificial (dock)	-42.736143, -65.03174	-	-	*
Puerto Madryn-b. "Albatros" shipwreck (1)	This study	SCUBA diving	3	5 m	Artificial	-42.764863, -65.003748	-	-	*
Puerto Madryn-c. Punta Este (1)	This study	SCUBA diving	4	10 m	Natural	- 42.7741667, -64.95333	-	-	*
Puerto Madryn	MACN Collection	-	6	-	-	-	-	-	*
Piedra Lile (2)	This study	SCUBA diving	1	5 m	Natural	-43.182333, -73.639667	-	-	*

Table 2. Pairwise genetic distances between haplotypes. The p-distance values are shown. H: haplotype. H1: shared between Argentina and Chile, H2: shared among Argentina, Chile, England and France, H3: present in Argentina, H4: present in New Zealand.

	H1	H2	H3
H2	0.015	-	-
H3	0.015	0.010	-
H4	0.010	0.010	0.005

Table 3. Main morphological characters of *Asterocarpa humilis* observed in this study and from the literature. E: endostyle, DL: dorsal lamina, R: right, L: left, -: without data.

Country/ Sampling locality	Maximum Size (cm)	Number of oral tentacles	Number longitudinal vessels	Piloric caecum	Shape of gonads	Number of gonads	Orientation of gonoducts	Brood Larvae	Species name	Taxonomic references
Southern Hemisphere										
New Zealand/ (type locality)	1.4	15-20	9 folds; 2 between folds	-	-	1 branched (R); 1(L)	-	-	<i>Styela humilis</i>	Heller 1878; Hartmeyer and Michaelsen 1927
New Zealand/ D'Urville Island	2.5	60 alternating big and small	RE O (10) 2 (11) 3 (11) 3 (10) 2 LD O (10) 2 (11) 2 (12) 2 (9) 2 LE	-	-	2 branched (R); 1(L)	-	-	<i>Styela cerea</i>	Sluiter 1900; Hartmeyer and Michaelsen 1927
New Zealand/ Carnley Harbour- Auckland Island	4	30-50 (15- 23 longer and shorter between them)	E 4 (11) 3 (13) 3 (13) 3 (10) DL (10) 3 (14) 3 (13) 4 (11) 5 E	No	Elongate, branched masses. cavity.	1 branched (L); 2 sausage- shaped (R)	Several apertures leading into the atrial.	-	<i>Cnemidocarpa aucklandica</i>	Bovien 1921

New Zealand/ Queen Charlotte Sound and Stewart Island	4.5	60	D 0 (10 3 (11) 3 (9) 4 (8) 3 E.	No	-	1 gonad U-form (L); 2 irregular and branched gonad (R)	-		<i>Cnemidocarpa cerea</i>	Michaelson 1922
New Zealand/ Otago Harbor	3.5	27-60 3 orders	E 1-2 (8-10) 2-3 (9-10) 3 (9-12) 3 (8-12) 0 DL.	No	In clusters, large and lobed	2-9(R);1-2(L)	Small, open in periphery of clusters	Yes	<i>Asterocarpa cerea</i>	Brewin 1946
New Zealand/ Foveaux Strait	1.8	16	RE O (8) 2 (8) 2 (7) 2 (7) 2 LD O (8) 2 (8) 2 (7) 2 (7) 2 LE	No	Individual gonads fused at their blind ends	5 (R); 1(L)	Ducts at the opposite ends of the fused gonads	Yes	<i>Asterocarpa cerea</i>	Millar 1982
Tasmania/ Hobart Harbor	2	20+40 2 orders 60+20	LD (9) 3 (11) 3 (11) 3 (9) 3 ER; LD (10) 3 (12) 2 (11) 2 (8) 2 EL	No	compact mass formed by several fused gonads.	2- Several gonads fused; 2 fused (L)	-	-	<i>Dendrodoa gregaria</i>	Kesteven 1909; Hartmeyer and Michaelson 1927

Australia (South)/ Malayan Archipelago Indo-Pacific area	3	15+15+ minute interspersed	E 3 (8) 3 (8) 2 (10) 2 (7) 0 DL.	-	Armed, stellate	4-5(R);1- 2(L)	Directed ventrally	-	<i>Asterocarpa cerea</i>	Kott 1952
Australia/ Albany; Spring Bay; Hobson Bay	4	45 3 orders	Up to 3 (up to 12) Example: E3(8)3(8)2(10)2(7)0 DL	-	Branched	3-4 (D); 2-3 (I)	Directed ventrally	-	<i>Asterocarpa humilis</i>	Kott 1985
Chile/ Juan Fernández Islands	1.8	64 4 orders	RE 3 (9) 2 (11) 2 (11) 2(9) 0 LD 0 (9) 3 (11) 2 (10) 2 (9) 1 LE	No	Compound irregular, branched	2-3(R); 1 (L)	Into the endostyle	-	<i>Cnemidocarpa robinsoni</i>	Hartmeyer 1916
Chile/ Antofagasta	3	40 16 large	E: 2 (7-8) 2 (9-10) 2 (9-10) 2 (8-9) 0 LD	Yes	Ramified	3-4(R); 2 (L)	Into the endostyle	Yes	<i>Asterocarpa humilis</i>	Clarke and Castilla 2000
Chile/ Muelles UCN and Tongoy Bay	4	-	E: 2-4 (8-11) 2-4 (8- 11) 2-4 (8-11) 2-4 (8-11) 0 LD	-	Ramified	-	Into different directions	-	<i>Asterocarpa humilis</i>	Turon <i>et al.</i> 2016b

Chile/ Talcahuano	3.5	-	2-3 (9-11)	-	In clumps	-	Into different directions	-	<i>Asterocarpa humilis</i>	Pinochet <i>et al.</i> 2017
Argentina /Chubut	3.2	38-50 several sizes	E 3 (9-10) 3 (10-11) 3 (12) 3 (11-12) 0 DL.	-	Branched horseshoe	1-5(R); 1(L)	-		<i>Cnemidocarpa robinsoni</i>	Diehl 1977
Argentina / Mar del Plata and San Matías Gulf	-	40 3 orders	E 0 (9) 2 (10-11) 2 (11) 2-3 (9) 0 DL	-	More or less stellated, branched curvated	2-3(R); 1 (L)	-		<i>Asterocarpa humilis</i>	Varela 2007
Argentina and Chile/several locations	6	38-65 Two orders of big ones and several minute ones	0-3 (8-113)	No	Irregular, single, branched	3-6(R); 1-2(L)	Pointing in different directions, especially to the endostyle	Yes	<i>Asterocarpa humilis</i>	This study
South Africa/ Ciudad del Cabo	3.5	20+20; 2 orders	LD 0 (9) 3 (10) 3 (9) 3 (8) 3 RE ; LD 0 (10) 2 (9) 3 (9) 2 (8) 2 LE	-	Several fused gondas	3-4(R); 2-3 (L)	Short gonoduct	-	<i>Cnemidocarpa asymmetra</i>	Hartmeyer and Michaelson 1927

South Africa/ Hout Bay Harbor	6	40; 4 orders	E 0-4 (5-7) 3 (6-8) 3 (8) 3 (8-9) 0 DL.	No	Ramified forming a mass	4-6(R); 1-2(L)	-		<i>Cnemidocarpa humilis</i>	Monniot <i>et al.</i> 2001
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Northern Hemisphere

England and France/ several locations	4	-	E 1 (9) 3 (10) 3 (11) 3 (10) 0 DL 0 (10) 3 (11) 3 (10) 3 (9) 2 E	-	Branched gonads parallel to and partially overlying the endostyle	Several (R); 1-2 (L)	Remote from the atrial siphon in different directions	Yes	<i>Asterocarpa humilis</i>	Bishop <i>et al.</i> 2013
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Table 4. Available reports of *Asterocarpa humilis* and *Cnemidocarpa robinsoni* in natural and artificial habitats. The descriptions of Heller 1878, Hartmeyer 1912, 1916 and Monniot and Monniot (1983) did not mention the type of habitat where the specimens were collected.

	Natural Habitats	Artificial Habitats
<i>Asterocarpa humilis</i>		
New Zealand	Brewin 1946; Brewin 1948; Millar 1982; Page and Kelly 2016.	Brewin 1946; Page and Kelly 2016.
Australia	Kott 1985.	Kott 1985.
Tasmania	Kott 1985.	Kesteven 1909.
South Africa	Millar 1962.	Peters <i>et al.</i> 2017.
Chile	this study.	Clarke and Castilla 2000; Manríquez <i>et al.</i> 2014; Turon <i>et al.</i> 2016b; Pinochet <i>et al.</i> 2017; Leclerc <i>et al.</i> 2018.
England	-	Bishop <i>et al.</i> 2013; Bishop <i>et al.</i> 2015.
France	-	Bishop <i>et al.</i> 2013, Bishop <i>et al.</i> 2015; Alié <i>et al.</i> 2018; Kenworthy <i>et al.</i> 2018.
Scotland	-	Nall <i>et al.</i> 2015; Kakkonen <i>et al.</i> 2019.
SW Atlantic	Varela 2007; this study.	Varela 2007; this study.
<i>Cnemidocarpa robinsoni</i>		
SW Atlantic	Diehl 1977.	Schwindt <i>et al.</i> 2014.

Fig. 1. Map of sites sampled in this study for morphological and genetic analyses. Red circles indicate new samplings for this study; yellow circles show localities with sequenced specimens included in our genetic analyses. Black ovals show information of localities, GenBank accession numbers, and the corresponding haplotype number. Specimens from Punta Colorada (AR), Albatros Shipwreck (AR), Punta Este (AR) and Piedra Lile (CH) were only used for morphological analyses. AR: Argentina, CH: Chile, NZ: New Zealand, FR: France, EN: England.

Fig. 2. a) clumps of specimens attached to a rock (basal face), intertidal of Playas Doradas (Argentina); b) living specimens showing colour pattern of the oral siphons; c) part of the branchial sac, showing stigmata and parastigmatic vessels; d) dissected specimen (branchial sac removed), showing gonads; e), f) details of gonoducts (arrows) opening in several directions (e: endostyle); g) details of oviduct and sperm duct; h) brooding larvae found in the atrial cavity; i) larva.

Fig. 3. Haplotype network of COI gene sequences. Circle sizes are proportional to the number of total sequences with each haplotype. The colors within circles indicate the proportion of specimens of each site per haplotype. Dashes along the connecting lines represent mutational steps. mv1: median vector representing a hypothesized sequence which is missing and is required to connect existing sequences within the network with maximum parsimony.

Fig. 4. Consensus phylogenetic tree using BI from COI partial sequences. Support values corresponded to the three methods used (BI, ML and MP). Only values > 0.5 for BI, > 50% for ML and MP are shown. Sequences of *Corella eumyota* and *C. antarctica* were used as outgroup. Hyphen (-) denotes bootstrap value < 50% and posterior probabilities < 0.5; underscore (_) indicates that the clade did not appear in the corresponding tree.

Table S1. Multiple sequence alignment fasta matrix of *COI* sequences used in this study

> Haplotype 1

GATATTAGAACTTTATACTTTATCTTAAGAGTCTGAGCTAGATTTATTAGATCAGGAATAAGTGTCTAATTCGTAAGTCTGAGCTTTCTCAGGTAGGTCAGTTAATTAG
AGATGGGCAGCTATATAATGTAGTAGTTACCGCCCATGCCTTTGTTATAATCTTTTTTTTTGTAATACCTATTATAATTAGGAGATTTAGAAATTGGTTGTTACCTCT
TATGTTAGGTAGTCCGGATATAGCTTTTCCACGAATAAATAATATAAGCTTTTGGTTGCTACCTCCGGCGTTATTTTTATTGTTAATCAGCTCGGTTGTTGAGAGTG
GGGCAGGTACTAGGTGGACTGTTTATCCACCACTCTCTGGTAATTTAGCACATTCAGGAGCTTCAGTAGATTGTGCTATTTTTCTTTGCATTTAGCAAGAGTTTCT
AGTATTTTAAGGTCTTTAAATTTTTTAACGACTATGTTTAAACATAAAAAACAAAAGATGGGATATATTTTCTATTCCCTTGTTTTGTTGGACGGTATTAGTAAGTAC
TGTATTGTTATTACTGTCTTTACCTGTATTAGCAGCAGCTATTACAATATTATTATTGATCGTAATTA

> Haplotype 2

GATATTAGAACTTTATACTTTATCTTAAGAGTTTGAGCTAGATTTATTAGATCAGGAATAAGTGTCTAATTCGTAAGTCTGAGCTTTCTCAGGTAGGTCAGTTAATTAG
AGATGGGCAGCTATATAATGTAGTAGTTACCGCCCATGCCTTTGTTATAATCTTTTTTTTTGTAATACCTATTATAATTAGGAGATTTAGAAATTGGTTGTTACCGCT
TATGTTAGGTAGCCCGGATATAGCTTTTCCACGAATAAATAATATAAGCTTTTGGTTGTTACCTCCGGCGTTATTTTTATTGTTAATCAGCTCGGTTGTTGAGAGTG
GGGCAGGTACTAGGTGGACCGTTTATCCACCACTCTCTGGTAATTTAGCACATTCAGGAGCTTCAGTAGATTGTGCCATTTTTCTTTGCATTTAGCAAGAGTTTCT
AGTATTTTAAGGTCTTTAAATTTTTTAACGACTATGTTTAAACATAAAAAACAAAAGATGGGATATATTTTCTATTCCCTTGTTTTGTTGGACGGTATTAGTAAGTAC
TGTATTGTTATTACTGTCTTTACCTGTATTAGCAGCAGCTATAACAATATTATTATTGATCGTATTA

> Haplotype 3

GATATTAGAACTTTATACTTTATCTTAAGAGTTTGAGCTAGATTTATTAGATCAGGAATAAGTGTCTAATTCGTAAGTCTGAGCTTTCTCAGGTAGGTCAGTTAATTAG
AGATGGGCAGCTATATAATGTAGTAGTTACCGCCCATGCCTTTGTTATAATCTTTTTTTTTGTAATACCTATTATAATTAGGAGATTTAGAAATTGGTTGTTACCGCT
TATGTTAGGTAGCCCGGATATAGCTTTTCCACGAATAAATAATATAAGCTTTTGGTTGTTACCTCCGGCATTATTTTTATTGTTAATCAGCTCGGTTGTTGAGAGTG
GGGCAGGTACTAGGTGGACCGTTTATCCACCACTCTCTGGTAATTTAGCACATTCAGGAGCTTCAGTAGATTGTGCTATTTTTCTTTGCATTTAGCAAGAGTTTCT
AGTATTTTAAGGTCTTTAAATTTTTTAACGACTATTTTAAACATAAAAAACAAAAGATGGGATATATTTTCTATTCCCTTGTTTTGTTGGACGGTATTAGTAAGTACT
GTATTGTTATTACTGTCTTTACCTGTATTAGCAGCAGCTATTACAATATTATTATTGATCGTAA

> Haplotype 4

GATATTAGAACTTTATACTTTATCTTAAGAGTTTGAGCTAGATTTATTAGATCAGGAATAAGTGTCTAATTCGTAAGTCTGAGCTTTCTCAGGTAGGTCAGTTAATTAG
AGATGGGCAGCTATATAATGTAGTAGTTACCGCCCATGCCTTTGTTATAATCTTTTTTTTTGTAATACCTATTATAATTAGGAGATTTAGAAATTGGTTGTTACCGCTTATGTTAGGTAGCCCGGATATA
GCTTTTCCACGAATAAATAATATAAGCTTTTGGTTGCTACCTCCGGCATTATTTTTATTGTTAATCAGCTCGGTTGTTGAGAGTGGGGCAGGTACTAGGTGGACCGT
TTATCCACCACTCTCTGGTAATTTAGCACATTCAGGAGCTTCAGTAGATTGTGCTATTTTTCTTTGCATTTAGCAAGAGTTTCTAGTATTTTAAGGTCTTTAAATTT
TTTAAACGACTATGTTTAAACATAAAAAACAAAAGATGGGATATATTTTCTATTCCCTTGTTTTGTTGGACGG

> *Cnemidocarpa verrucosa* (KU299764)

AGATATTAGAACTTTATATTTTATTTTAAGAGTTTGGGCCAGATTTATTAGGTGAGGAATAAGTGTTTTAATTCGAACAGAATTATCTCAGGTTGGTCAGCTCATTA
GAGATGGTCAGTTATATAATGTAATTGTTACAGCTCATGCAATTTGTAATAATCTTTTTTTTTGTTATACCTATTATAATTAGAAGATTTGGTAATTGGTTGTTACCTT

TAATAATTAGGAGTCCAGATATGGCTTTCCCTCGTATAAATAACATAAGTTTTTGATTATTACCTCCAGCTCTATTTTTGTTGATTATTAGTTCTGTTGTTGAGAGCA
GAGCAAGAAGTGGTGAACAGTTTATCCACCTCTATCAAGAAATTTAGCACACTCAAGAGCTTCAGTAGACTGTGCAATTTTTCTTTACATTTGGCCGGAGTTTCT
AGTATCTTAAGATCTTTAAATTTTTAACTACTATGTTTAATATAAAAACAAAAAGATGGAATATATTTTCAATCCCATTGTTTTGTTGAACAGTGTTAATTACTAC
TATTCTATTGCTACTGTCTTACCCGTATTAGCTGCTGCTATTACTATGCTTTTATTTGATCGT_____

> *Pyura legumen* (KU299778)

GGTTTATTAGAACAGGAATAAGTGTTTTGATTCTGAATAGAGTTGTCTCAAGTTGGACAGGTTATTAGGGATAGGCAGTTGTACAATGTAATTGTAACAGCTCATGC
TTTTGTTATAATTTTTTTTTTCGTGATGCCGATTATAATCGGTGGGTTTAGAAATTGGTTGTTACCTCTTATGATTGGGAGTCCTGATATAGCTTTCCACGATTAAA
TAATATAAGTTTTTGATTACTTCCTCCAGCATTGTTTTGTTGTTAATTAGTTCGATGATCGAGAGTGGTGTAAGGACAAGATGAACAGTTTATCCTCCATTGGCAA
GAAATCTTGACATTCTAGGCCAGCCTTAGATTGTGCTATTTTTCTTTGCATTTAGCTAGGGTATCTAGTATTTTAAGGTCTTTAAATTTTTAACTACTATGTTTA
ATATAAAAGGAAAGAGATGAAATCTCTTTAGTTTACCTTTATTTTGTTGGACTGTTTAATTACTACTATTTTGTTGTTATTATCTTTGCCTGTTTTGGCTGCGGCTA
TCACTATGCTTCTTTTTGATCGTAATTTAATACTTCTTTTTTCGATC_____

> *Styela paessleri* (KU299779)

AAGATATTGGGACATTATATTTTATTTTTAGAAATTTTTTCAGGCTTAATTAGGTCTAGAATAAGTGTATTAATTCGATTGGAGTTATCTCAGTCAAGAATAGTGATA
AGAGATAGGCAATTGTATAATGTTATTGTAAGTCTCATGCTTTTCATTATAATTTTTTTCTTTGTTATACCAATAATAATTAGGAGGTTTAGAAATTGATTATTACCA
TTAATATTAAGTCTCTCTGATATAGCATTTTCCTCGACTTAATAACATAAGTTTTTGATTATTACCCCTTCATTATTTTATTATTAATTAGTGCATTTATTGAGAGTA
GAGTAAGGACAAGATGGACAGTTTATCCACCATTATCTAGTAATTTGTCTCATTCAGAGCAGCAGTTGACTGTGCAATTTTTCTTTACATTTGGCAAGAATTAGT
AGTATTTTAAGATCCTTAAATTTTTTGACAATATTTAATATAAAAAGAAAAGGTATAAGTCTATTTAATATAACCATTATTTTGTTGAACATTTTAGTTACAAC
TATTTTATTATTATTAAGTCTTCCAGTATTGGCAGCTGCCATTACAATACTTTTGTTGATCGTAATATAAATACTACTTTTTTTGATC_____

> *Corella eumyota* (MG877558)

TTAGAGCTTTCTCAAAGTAGGAGAGTTATTTTCAGATAGACAACTTATAACGCTATTGTTACTGCTCATGCTTTTGTAATAATTTTTTTTTTTGTTATACCAGTTATA
ATTAGAGGTTTTAGAAATTGATTATTGCCTTTAATAATTAGAGCTCCTGATATAGCTTTTCCACGAATAAATAATATAAGCTTTTGACTTTTACCACCAGCTTTTTTT
TTATTAATTTCTTCTCTTATAAGAGTGAGGGCTAGAAGTATGATGAACAGTATATCCTCCTTTATCTAATAATATAACCCATTCTAGATCATCTGTTGATTTTGCT
ATTTTTCACTTCATTTAGCTAGAGTGTCATCAATTTTAAGATCTATTAATTTTTTAGTAAGTATATTTAATATAAAGTCTAATAGATTTAATTTTATTAATATGAGT
TTATTTTGTTGATCAGTTATAAATTACAATATTTTATTAGTATTATCTTTACCTGTATTAGCAGCTGCTATTTCTATACTTTTATTTGACCGTAATTTAAACACTTCTT
TTTTTGACCCAAGAAG_____

> *Corella antarctica* (MG877561)

TTTTTAGAACTTGAGCAGCATTAGTTAGAACTAGAATAAGTGTAAATTATTCGTTTAGAACTTTCTCAAACAAGAGGAGTAATTTCCGGATAGACAGCTTTATAATAC
TATTGTTACTGCTCATGCTTTTCGTAATGATTTTTTTTTTTGTTATACCCATTATAATCAGGGGTTTTAGAAATTGACTTTTACCCCTAATAGTTAGGGCCCTGATAT
GGCTTTTCCTCGAATAAATAATATGAGCTTTTGGTTATTACCCCTGCTTTTTTTTTACTTATTTCTTCTCTAATAGGTATTAAGCTAGAACAGGTTGAAGTGT
TTACCCCTCTATCTAATAACACAATCATTACAGGGCCTTCTGTTGATTCTGCTATTTTTCTCTACATTTGGCTAGGGTTTCTTCTATTTTAAGATCTATTAATTTT
TTAGTAAGTATGTTTAATATAAAAACAAAAGGTTTTCTTTTTTTGAATAAAAAGCTTATTTTGTTGATCTGTTATTATTACTACTATTTTATTAGTACTATCACTTCCTG

TTTTAGCAGCAGCTATTACTATGTTATTATTTGATCGAAATTTAAACACTTCTTTTTTTGACCCTAGAAAAAGGAGAGATCCTATTCTCTACCAACATTTATTTTGAT
TTTTTGGTCACCCTG
> *Paramolgula gregaria* (KU299769)

TTACATAGTGTTTGGCCTTATGGCATCACTTTTCAGGTCTACTTTAAGTTTAATAATGCGATTAGAGCTTAATTCTCCTTTGAATATTATATTTAATGGTAATATTTA
TAACTCCATTATTACTTCTCATGCTTTAATTATAATCTTTTTTTTCATTATACCCATTACTATGGGGAGATTTGGTAATTGGTTGATTCCTCTTTTTATTGGATCTTCT
GATATAGCATTTTCCTCGTCTTAATAATTTTTCATTTTGGCTTATACCATTTTCATTGATCATACTTCTTTCTCAATGATAATTAGAACAGGAATTAGGACAAGGTGG
ACTATATATCCTCCTCTCTCTACTATTAGAA—
ATACTAGCAATAGTGTTAATTTTAAATTTTCTGTTTCATATTGCTGGTTTGTTCATCCATTATTAGTAGCTTTAATTTTATAACTACAATTTTATTATAAGTATTTA
TAAGT—
CTGTATTTAATTTCCCTATAATGGTTTGAAGTTTTTTGATTACTTCTCTTTTATTGCTTCTTTTCATTGCCAGTTTTAGCTAGAGCTATTACATTGCTTTTATTTGATCGT
AATTTCAATACCTCATTTTTTTGATC—

Table S2. Synonymy based in WoRMS (<http://www.marinespecies.org/>)

Asterocarpa humilis (Heller, 1878) Taxonomic status: accepted
Asterocarpa cerea (Sluiter, 1900) (new combination)
Cnemidocarpa asymmetra (Hartmeyer, 1912) (new combination)
Cnemidocarpa aucklandica Bovien, 1921 (original combination)
Cnemidocarpa cerea (Sluiter, 1900) (new combination)
Cnemidocarpa gregaria (Kesteven, 1909) (new combination)
Cnemidocarpa robinsoni Hartmeyer, 1916 (new combination)
Dendrodoa gregaria Kesteven, 1909 (original combination)
Pandocia gregaria (Kesteven, 1909) (new combination)
Styela asymmetra (Hartmeyer, 1912) (new combination)
Styela cerea Sluiter, 1900 (original combination)
Styela humilis Heller, 1878 (original combination)
Tethyum asymmetron Hartmeyer, 1912 (original combination)
Cnemidocarpa humilis (Heller, 1878) (new combination)

Fig. S1. Consensus phylogenetic tree using Maximum Likelihood (ML) from *COI* partial sequences. Numbers correspond to bootstrap support. Only values >50% for are shown. Sequences of *Corella eumyota* and *C. antarctica* were used as outgroup.

Fig. S2. Topology of consensus phylogenetic tree using Maximum Parsimony (MP) from *COI* partial sequences. Numbers correspond to standard bootstrap. Sequence of *Corella antarctica* was used as outgroup.