## ORIGINAL PAPER

# A re-evaluation of morphological characters of the invasive ascidian *Corella eumyota* reveals two different species at the tip of South America and in the South Shetland Islands, Antarctica

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**Abstract** The native solitary ascidian *Corella eumyota* Traustedt, 1882) is commonly found in the Southern Hemisphere in the cold-temperate waters of the Subantarctic and Antarctic regions. Its recent spread into the Northern Hemisphere throughout the NE Atlantic gave the species the status of invasive. Together with its widespread distribution, reports on its wide variability (several distinct morphological characters, genetic discontinuities and also possible misidentifications) cast doubt on the taxonomic status of different populations of this species. This work, based on the observation, quantification and analysis of specific morphological characters in specimens collected at five different localities of South America and Antarctica, strongly indicates that there are two different species: *C. eumyota* from South America and *Corella antarctica* 

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Núcleo Milenio de Conservación Marina, F. C. Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile Sluiter, 1905) from Antarctica, which has been till now considered a junior synonym of the former. The species clearly differ in the arrangement of the gonadal ducts, the size of the larvae and the shape of the anus, among other characters. Morphological variation displays a defined, discrete grouping supporting a clear differentiation into two species. This result shows the need for careful inspection of specimens to avoid wrong interpretations in a context of changes of marine biota due to biological invasions.

**Keywords** Ascidian · *Corella eumyota* · *Corella antarctica* · Morphology · Multivariate analysis · South America · Antarctica · Invasions

#### Introduction

On a microgeographic scale, in harbours, lagoons or even along rocky shorelines, many marine invertebrate species that are recognized to have a wide distribution as well as great intraspecific variability have been proved genetically and morphologically to be distinct species (Tarjuelo et al. 2001; Dias et al. 2009; Rius and Teske 2011). New evidence contradicts the concept of demographically open marine populations and stresses the existence of a finescale structure in dispersal patterns among locations, suggesting that marine populations are more closed than previously thought (Bradbury et al. 2008; Weersing and Toonen 2009; Cowen and Sponaugle 2009).

In the Antarctic Ocean, to understand the distribution of marine species, it is necessary to consider the role of the Polar Front as a barrier, particularly in ascidians, whose larvae are lecithotrophic and short-lived. The Polar Front (Antarctic Convergence) is an irregular but permanent

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barrier (Hedgpeth 1969; Thornhill et al. 2008), contributing to the faunal isolation of the Antarctic Ocean, limiting migration from and into northern areas, or comprising at least a transitional area for pelagic fauna (Clarke and Crame 1989). For ascidians, affinities between the tip of South America and Antarctica are low (Monniot and Monniot 1983; Primo and Vázquez 2007). However, a gradient along the Scotia Arc between the Magellan Province and the Antarctic Peninsula suggests that it may act as a bridge between these two areas, in spite of the geographical distance, the great depths, the currents and the presence of the Polar Front (Tatián et al. 2005; Primo and Vázquez 2009). A higher relationship was reported between South America and the Scotia Arc islands than between the latter and the Antarctic Peninsula (Primo and Vázquez 2009). The percentage of endemic ascidian species in Antarctica ranges between 25 and 51 %, in spite of a low percentage of sector endemism; this is possibly due to the vast geographical distances from adjacent regions, as well as to the relative constancy of the hydrographic conditions and the dispersal of organisms through circumpolar currents (Primo and Vázquez 2009).

The solitary ascidian Corella eumyota (Order Phlebobranchia, Family Corellidae) was originally described from Valparaiso, Chile, and it is considered native to the Southern Hemisphere. This species shows a circumpolar distribution within the Antarctic and Subantarctic regions (Van Name 1945; Millar 1960; Kott 1969), but includes also Namibia (Turón 1988), South Africa, Australia and New Zealand (Ärnbäck-Christie-Linde 1938; Van Name 1945; Brewin 1946; Monniot and Monniot 1983; Lambert 2004). The species was encountered in France for the first time in 2002, probably introduced by anthropogenic transport (Lambert 2004). Since then, it has been detected in England (Arenas et al. 2006; Collin et al. 2010), Ireland (Minchin 2007), Spain (Varela et al. 2007; Nagar et al. 2010) and Portugal (Nagar et al. 2010). Recently, it has been included in a model used to predict tunicate invasions in Canada (Locke 2009).

The original description of *Corella eumyota* was based on several characters: body dimensions, number of oral tentacles, number of longitudinal vessels in the branchial sac, general position of the gut and qualities such as colour, general shape and textures. However, many of the features are shared with other congeners, leading to imprecision in correct identification, as suggested by the number of synonyms attributed to this species (Van Name 1945). Although the existence of three Antarctic *Corella* species was proposed by different authors: *C. antarctica* (Sluiter 1905), *C. dohrni* and *C. benedeni* (Van Beneden and de Selys Longchamps 1913), all of them were subsequently synonymized with *C. eumyota*, under the assumption of a high intraspecific variability in the latter species (Herdman 1910;

Ärnbäck-Christie-Linde 1929, 1938; Van Name 1945). In South America and Antarctica, C. eumyota is the only species known for the genus, and until now, the validity of the taxonomic status for Antarctic specimens has not been questioned. Ascidians tend to show great morphological variability in shape, size and colour due to genetic characteristics and local environmental conditions (Dias et al. 2009), and such variability complicates species identification. Van Name (1945) discussed the difficulty in finding reliable distinguishing characteristics, with much uniformity in main morphological characteristics of the body structure but great variability in minor traits, such as the exact position of the siphons or the number of oral tentacles. Even when such variability was usually attributed to environmental conditions (Monniot and Monniot 1983), a more than 10 % discontinuity in mitochondrial DNA sequences has been found between C. eumyota populations from Europe and Antarctica (Monniot et al. 2011).

This background, added to the degree of morphological variability and the wide distribution of the species, suggests population differentiation or the possible existence of different species under the same taxonomic entity. The aim of this study was to clarify the taxonomic status of *C. eumyota* by re-examining morphological and morphometric features in specimens collected in southern South America and Antarctica, applying quantitative analysis of morphological variation.

#### Materials and methods

A total of 63 specimens assumed to be *Corella eumyota* from five different localities covering a wide latitudinal range (Fig. 1) were analysed. Samples from Valparaíso (V, n = 2) and Concepción (C, n = 3) were randomly collected in 2005 by scratching areas of  $25 \times 25$  cm from pilings at 14 m depth. Samples from Puerto Deseado (PD, n = 22) and Potter Cove (PC, n = 25) are a subset from the samplings described by Tatián et al. (2010) and Tatián et al. (1998), respectively. All these samples were collected by SCUBA from 10 to 30 m depth. Samples from Las Grutas (LG, n = 11) were collected in 2011 from the lower intertidal zone. In all cases, the specimens were preserved in 5 % formaldehyde in sea water (Monniot and Monniot 1972) and then deposited in the Museo de Zoología, Universidad Nacional de Córdoba, Argentina.

#### External characters

Five body dimensions were measured: (1) total body length (lgh); (2) total body width (wth); (3) distance between the centre of both siphons (d); (4) oral siphon length (osl) and (5) cloacal siphon length (csl). Two variables (proportions)

**Fig. 1** Geographical distribution of sampling sites included in this study. Biogeographical division according to Briggs (1995): *SAW* South America West region, *SAE* South America East region, *SAS* South America South region, *ANT* Antarctic region. The Polar Front is indicated in *dashed line* 



were also generated by combining lgh/wdh (l/w) and lgh/d (l/d) to obtain, respectively, an approximation to the body shape and a comparative measure of the location of the siphons with respect to the body. At the same time the number of siphonal lobes was recorded. All measurements were made with a calliper ( $\pm 0.01$  mm).

#### Internal characters

Dissections were performed under a trinocular stereomicroscope (Labomed CZM4) provided with a digital camera. After dissection (removal of the tunic and cutting along the ventral line), the mantle musculature was described followed by: (1) number of oral tentacles (ot); (2) number of languets (sickle-shaped processes) that constitute the dorsal lamina (lgd); (3) languet sizes (lgds); (4) total number of longitudinal vessels in the branchial sac (lv); and (5) length and location of male and female ducts and openings (gop). The shape of the stigmata and anus was also recorded. When brooded larvae were present, the morphology was described and their size (tailless length) was measured.

### Data analyses

To assess differences between three sampling localities, LG, PD and PC, a nonparametric multivariate analysis of variance (PERMANOVA) was used. This analysis is based on correlation distance measures, followed by pairwise PERMANOVA between all pairs of groups as a post hoc test (*p* values corrected with the Bonferroni method), both with 10,000 permutations, to test for significant differences ( $\alpha = 0.05$ ). To determine the main variables that separate sampling localities, ANCOVA, one-way ANOVA or the

Kruskal–Wallis test was subsequently performed for each character alone. Variables that showed significant differences were used to perform cluster and discriminant analyses. Discrete or categorical variables were not used in PERMANOVA or multivariate analyses, because they render the multivariate analyses inefficient (James and McCulloch 1990). Larvae features were not used because not all the specimens showed incubating larvae and the analyses do not accept missing data. Larval size was compared between sampling localities by ANOVA.

The morphological characters of an organism generally vary with the overall body size, and this in turn may vary between different populations of a species due to environmental differences (McCoy et al. 2006; Berner 2011). As a result, the observed differences in these morphological characters can arise because specimens or populations differ in body size. Therefore, to quantify differences between the morphological characteristics of analysed localities, a correction of size (lgh) was carried out by ANCOVA, because Antarctic specimens present larger sizes. Unlike other methods, the use of ANCOVA including body size (lgh) as a covariate produces unbiased results (Berner 2011). When significant differences ( $\alpha = 0.05$ ) were observed, they were tested through the a posteriori Tukey test. Size correction was performed on those variables that were significantly associated with body size after regression analysis (i.e. wth, lgd, osl and ot). Statistical and multivariate analyses were restricted to specimens whose body length fell within the same range (1.3–5.3 cm): PD, n = 22; LG, n = 11; PC, n = 10; V, n = 2; C, n = 3. This was because, when the full set of Antarctic specimens (PC) in the population (n = 25) was considered, the differences between these specimens and the others from the

Southwestern Atlantic (PD and LG) were determined by large-sized specimens. Specimens from the Pacific Ocean (V and C) were excluded from the analysis, except for cluster analysis, because these were few.

The unweighted pair group method average (UPGMA) algorithm, based on Pearson correlation distance, was used to demonstrate overall similarity between specimens. Canonical variate analysis (CVA) was used to determine the existence of different morphologically defined groups and to identify the combination of quantitative variables that better separate the groups under study. This analysis produces a scatter plot of specimens along the first two canonical axes, producing maximal separation between all groups (multigroup discriminant analysis). The axes are linear combinations of the original variables as in PCA, and *eigen* values indicate the variation explained by these axes (Hamer et al. 2001). To avoid the effect associated with the covariation of characters, the relative importance of each variable in group discrimination was evaluated from discriminant function standardized by common variance (Balzarini et al. 2008). Length and width of ascidians can vary widely, as they do not have a rigid body. Because the usefulness of these characters to discriminate groups can be questioned, we excluded them from the CVA. All analyses were performed using the software PAST 2.1 (Hamer et al. 2001), except for the discriminant, performed with Infostat (Di Rienzo et al. 2011).

## Results

#### External and internal general appearance

All specimens showed a flattened ovate body, with the tunic delicate, slightly translucent, off-white or ivory, and cartilaginous. Specimens from South America varied

Table 1	Different	characters	analy	vsed
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between 1.4 and 4.7 cm lgh, while the Antarctic specimens varied between 2 and 15.2 lgh. The oral siphon was terminal; the cloacal siphon was situated halfway down the body. The length of the siphons was variable: they were short or long in specimens from South America and were short in Antarctic specimens. In all specimens analysed, the oral siphon had six lobes; the cloacal siphon had six, seven or up to eight lobes in some cases. Musculature was quite developed, distributed over the whole left side and around the base of both siphons. Oral tentacles varied from 50 to 150, at least the half of them twice as long as the others. The opening of the dorsal tubercle was very variable: U-shaped, C-shaped or completely irregular. Dorsal lamina showed a row of languets (triangular-shaped processes). The branchial sac, crossed by longitudinal and transverse vessels, showed coiled stigmata. The stomach was located on the right side of the body, with longitudinal folds. The male and female gonads were in grape-like clusters over the intestinal loop and part of the stomach.

Characters that differed between populations

Differences were recognized in some characters (Table 1), which made it possible to define the existence of two morphologically distinct groups. The first was of Antarctic specimens (PC), and the second was of South American specimens (PD, LG, V and C). These groups were defined by *gop* and *lgd*, since their variability did not overlap between the sampling localities. There was constancy in *ot*, *osl* and *csl* within each group. PERMANOVA revealed significant differences between all sampling localities (LG, PD and PC) (Wilks'  $\lambda = 0.02151$ , F = 726.7; p < 0.001), and, except for *d*, *l/d* and *csl*, all characters were found to have significant differences when considered individually (p < 0.001). Antarctic specimens had a wider body and

Characters	Population						
	LG	PD	V	С	РС		
Siphons	Short	Long	Short	Short	Short		
Number of oral tentacles	130–174	104–162	53–97	80–90	41–58		
Languet sizes	One	One	One	One	Two		
Gonoducts	Short. Open on the left surface of the ovotestis; far from the base of the atrial siphon				Long. Follow the rectum. Open above the anus, at the base of the atrial siphon		
Anal aperture	Round, with its margin almost smooth to slightly lobulated				Folded, with its margin sharply lobulated (finger-like lobes)		
Larval size	192.98 µm (±4.77 SE)	nd	nd	nd	298.25 µm (±9.1 SE)		

LG Las Grutas (Argentina), PD Puerto Deseado (Argentina), V Valparaiso (Chile), C Concepción (Chile), PC Potter Cove (Antarctica). SE standard error, nd no data

more languets in the dorsal lamina than South American ones. The latter showed more longitudinal vessels, more oral tentacles and longer siphons than the Antarctic specimens.

In PC specimens, the number of oral tentacles varied between 50 and 80. The languets of the dorsal lamina had two different sizes: the shorter about half the length of the longer ones. Male and female ducts were long and followed the trajectory of the intestine above these gonadal ducts. The male duct ran above the female duct. Both gonadal ducts opened above the anus, at the base of the atrial siphon (Fig. 2a). The hook-shaped opening of the male duct was located slightly above the female duct, tapering towards the tip, while the opening of the female duct was oval in shape; both showed lobed margins. The anus showed a ventral fold, and the margin had numerous finger-like lobes (Fig. 3a).

For the South American specimens, oral tentacles varied between 80 and 150. The languets in the dorsal lamina were all of the same size. The female and the male ducts were short, less than 1 mm in length. Both opened into the atrium, very close to each other, near the ventro-lateral margin and away from the base of the atrial siphon (Fig. 2b). The opening of the female duct was U-shaped, the male duct opened into a small slit, and both had finely lobed margins. While the location of these openings was constant over the surface of the gonads, the orientation of the openings varied; the male duct was always located at the base of the female duct. The anus was round and its margin varied from almost smooth to slightly lobulated, but never as marked as in PC specimens (Fig. 3b).

Brooded larvae (Fig. 4) were present in most specimens from LG and in two specimens from PC. Both groups of 961

larvae were slightly translucent and off-white in colour. LG larvae had the tail coiled around the trunk with the end lying behind the trunk (Fig. 4a). In specimens from PC, the larvae (Fig. 4b) had always the tail right and different stages of development were recognized. Immature stages carried three cone-shaped papillae in a triangular disposition, while mature larvae showed early siphons. In all cases only the ocellus was observed. Significant differences (p < 0.001) were registered in average body length (without considering the tail): while the mean of LG larvae reached 192.98  $\mu$ m (±4.77 SE, n = 15), PC larvae reached a mean of 298.25  $\mu$ m (±9.1 SE, n = 15). The larvae were agglutinated by vitelline coats, but in different body parts: in LG specimens, larvae were close to the very short gonadal ducts at the posterior end of the atrium as reported by Lambert (2004), while in PC specimens they were located at the base of the atrium, far from the gonadal ducts.

Cluster analysis displayed two separate groups with a 95 % similarity between specimens. Group *a* included Antarctic specimens, while group *b* included South American specimens (Fig. 5). The cophenetic correlation coefficient was 0.91. Canonical variate analysis (CVA), based on *osl*, *l/w*, *ot*, *lv* and *lgd* characters, showed that 90.61 % of the variation between localities (LG, PD and PC) was explained by the first canonical axis. A plot of the first and second canonical axes clearly identified differences among Antarctic and South American specimens (Fig. 6). Discriminant function standardized by common variance indicated that *ot* contributed most to differentiate the groups, followed by *lv*, *lgd* and *osl*, while the *l/w* contribution was very low. The discriminant function was able to classify correctly 100 % of PC specimens, 86.36 %

Fig. 2 Diagrammatic representation of gonadal ducts disposition in the body. a Arrangement in specimens from Antarctic population. b Arrangement in specimens from South American populations. 1 gonads (dotted line), 2 stomach, 3 oesophagus, 4 intestine, 5 gonadal ducts: sperm duct (dashed line) and oviduct (black)





Fig. 4 Detail of larvae. a Larvae from LG specimens. b Larvae from PC specimens

of PD specimens (three of these were classified as LG) and 90.91 % of LG specimens (one was allocated to PD).

#### Discussion

The re-evaluation of the morphological characters makes it possible to distinguish two different species of Corella, which have so far been considered under the same entity, Corella eumyota Traustedt, 1882. The morphology of all the analysed South American specimens matches the statements made for C. eumyota, and there is no doubt about the validity of that species. Conversely, all the characters observed in the Antarctic specimens match the original description of Corella antarctica Sluiter, 1905, which has long been considered a junior synonym of the former. The species C. antarctica proposed by Sluiter (1905) was based on the observations made primarily of small specimens, though of the 27 specimens examined, the two largest reached 7 and 13 cm. Nonetheless, Herdman (1910) considered that C. antarctica was just a larger polar form belonging to the same variable entity described by Traustedt (1882). Certainly, difference in body size (length) was not enough evidence to create a new division in the genus (Herdman 1910), nor to propose the synonymy of both *Corella* species. Imprecise decisions regarding what constitutes a reliable morphological character have failed to adequately delimit both species and have led to the taxonomic uncertainty described here. Similarly, qualitative or semi-quantitative descriptions of diagnostic morphological characters have disregarded the distribution of morphological variation in this ascidian group.

The sharpest differences observed here between the species refer to the length and disposition of the male and female ducts and openings, the size of the larvae, the shape and margin of the anus and the size of the languets that constitute the dorsal lamina. Despite the extensive literature regarding *Corella eumyota* (Traustedt 1882; Herdman 1910; Ärnbäck-Christie-Linde 1929, 1938; Van Name 1945; Brewin 1946; Millar 1960; Kott 1969; Monniot and Monniot 1983; Lambert et al. 1995; Lambert 2004; Varela et al. 2007; Lagger et al. 2009), little attention has been paid to the arrangement of the gonadal ducts (Sluiter 1905;

**Fig. 5** Unweighted pair group method average (UPGMA) clustering of 48 specimens, using Pearson correlation distance. Groups are: *a* Antarctic specimens (*PC* Potter Cove); *b* South American specimens. *PD* Puerto Deseado (Argentina), *LG* Las Grutas (Argentina), *V* Valparaiso (Chile) and *C* Concepción (Chile)





Fig. 6 Two-dimensional plot of morphological traits analysed by canonical discriminant analysis (CVA). Traits are: osl oral siphon length, l/w length/width (body shape), ot number of oral tentacles, lv total number of longitudinal vessels in the branchial sac, lgd number of languets in the dorsal lamina

Brewin 1946) until the Lambert (2004) analysis. Sluiter's description (1905) for *C. antarctica* mentioned that "... the oviduct and the deferent duct are long, follow the curvature of the intestine and end little beyond the anus...". In the present study, this character was observed in the Antarctic specimens (Fig. 2a). However, Traustedt's description (1882) lacks such a characterization of reproductive structures against which Sluiter's observations could be

compared. Later, Brewin (1946) described the arrangement of the gonadal ducts for C. eumyota, but even the match with Sluiter's description seems to have been dismissed or at least gone unnoticed. It is possible that Brewin may not have considered Sluiter's description, since by that time C. antarctica had already been synonymized with C. eumyota. Recently, Lambert (2004) argued that Brewin has incorrectly described the gonadal ducts, because the intestine and associated membranes are whitish and could be easily mistaken as gonadal ducts, if the branchial sac was not removed during dissection. In addition, Lambert (2004) warned that "... gonoducts are unusually short; both open into the atrium very close to each other on the left surface of the ovotestis near its ventro-lateral edge, far from the base of the atrial siphon..." (Fig. 2b). Both the Sluiter's (1905) and Lambert's (2004) statements have been confirmed in this study, endorsing the hypothesis of two different species. Similarly, based on morphological adaptations to brooding and the length of the male and female ducts, the north temperate C. inflata, long considered a junior synonym of C. willmeriana, was reinstated and separated from the latter (Lambert et al. 1981).

Examination of larvae size, considering just the trunk, has disclosed differences between PC and LG specimens: the mean size of the latter (192.98  $\mu$ m) is coincident with the 197.86  $\mu$ m mean reported by Lambert et al. (1995) for *C. eumyota*. Significant differences in larval size have also been found between other *Corella* species (Lambert et al.

1981), which is a further point to consider for distinction between the present species. Regardless of the disposition of the male and female duct openings, the finding of embryos and larvae in two Antarctic (PC) specimens suggests a similar reproductive strategy to that described for *C. eumyota*. The latter is an incubator species that retains the embryos near the opening of the short oviduct, which is less than 1 mm in length (Lambert 2004). After hatching, the larvae remain in the atrium until they are competent to swim out through the atrial siphon (Lambert et al. 1995). Thus, *C. antarctica* is another example of a solitary ascidian with ovoviviparous reproduction, something uncommon among solitary ascidians.

The difference in the shape of the anus, with the presence of a ventral fold, has not been reported before. In contrast, the anal margin of C. eumyota has been described as wavy and irregularly toothed (Traustedt 1882) for specimens from Chile, but also smooth for those found in Spain (Varela et al. 2007). Such descriptions fit the observations made in South American specimens, suggesting intraspecific variability in this morphological character. All the Antarctic specimens exhibited a different arrangement: the opening of the anus has numerous fingerlike lobes. Although this is in agreement with other authors who found lobes in the margin of the anus after examination of Antarctic specimens (Monniot et al. 2011), Sluiter (1905) described the anal margin in C. antarctica as smooth. The reliability of this character should be therefore considered with caution, since it may be variable as in C. eumvota. A similar case occurs with the different size of the languets of the dorsal lamina. According to Sluiter (1905), the dorsal lamina of C. antarctica is represented by numerous languets of different sizes (small and large); Ärnbäck-Christie-Linde (1938) found the same in Antarctic specimens. All specimens from Antarctica exhibited this pattern, but in the case of South American ones, all processes of the dorsal lamina were the same size. This character, however, may also be questionable, considering that in samples from Auckland Islands, New Zealand, the existence of different lengths in these processes was mentioned as "occasional" (Herdman 1910). However, a possible explanation for the unclear pattern of this morphological character might be that both species occur in sympatry in New Zealand, and this has been ignored so far. This situation was recently reported in Australia for the Pyura stolonifera species complex (Rius and Teske 2011), whose taxonomic uncertainties bear a resemblance to C. eumyota.

Taxonomic knowledge and assessment of existing biodiversity in ascidians may be hampered either by a lack of rigorous morphological analyses or by the hasty consideration of high phenotypic variation. Characters traditionally used in the taxonomy of *Corella* species seemed to be continuous among the populations. The oral tentacles (*ot*) were described as filiform and of two lengths, in number from 50 to just over 100 (Traustedt 1882; Sluiter 1905; Herdman 1910; Ärnbäck-Christie-Linde 1929; Van Name 1945; Brewin 1946; Turón 1988). The languets of the dorsal lamina (*ldl*) have been referred as triangular processes without specifying the number and size of these processes. Reports of the length of siphons (*osl* and *csl*) were contradictory: some referred to short siphons (Traustedt 1882; Sluiter 1905; Van Name 1945; Kott 1969), while others mentioned long siphons (Herdman 1910; Ärnbäck-Christie-Linde 1929).

However, when all those characters were subjected to statistical analysis, it was possible to separate two main groups, significantly defined by these morphological characters, as well as being distant geographically. Based on the CVA scatter plot and the high percentage of accuracy in discriminant function for both South American populations (86.36 % for PD and 90.91 % for LG), it seems that two morphotypes of *C. eumyota* may exist. This apparent differentiation responds mainly to a longer size in the oral siphon (*osl*) for LG specimens; the full remaining set of characters does not follow this trend. While this could be attributed to intraspecific variation, such a character may be subject to an environmental condition, and more specimens should be analysed to evaluate this relationship.

The degree of separation between siphons and the location of the cloacal siphon along the dorsal line of the body were mentioned in the previous literature as typical characters. Thus, some descriptions placed the cloacal siphon about halfway along the left side of the body (Traustedt 1882; Herdman 1910). Other authors mentioned a separation distance of one-third of total body length (Ärnbäck-Christie-Linde 1929) or warned that it can be variable (Van Name 1945; Brewin 1946). The present results show that the distance between siphons (d) and the ratio *l/d* are not informative. Clearly, this distance is somewhat vague, and while it has been proposed as an additional benchmark in species identification, it should be noted that this feature has also been proposed as a diagnostic character for other congeners, such as Corella japonica Herdman, 1880 (Herdman 1882).

Morphological characters traditionally used in *Corella eumyota* diagnosis have been considered to be vague, failing in an accurate delimitation of the species. The subjective interpretation of such characters may have been misleading in the approach to Antarctic specimens. However, in this work, two different species were differentiable by univariate and multivariate analyses of the same diagnostic characters when used as quantitative morphological data. Although this sort of procedure for the construction of classifications is not often used today, numerical techniques are useful at the level of microtaxonomy, allowing

taxonomic decisions regarding species and intraspecific variation (Lanteri et al. 2006). These approaches have been useful to resolve many outstanding issues regarding the taxonomy of corals (Miller 1994) and even in soft-body marine invertebrates such as jellyfish (Bolton and Graham 2004). Furthermore, their integration with molecular data has led to the same taxonomic conclusions in medusae (Dawson 2003) and ascidians (Dias et al. 2009). Quantitative descriptions and statistical assessment of morphological variation in Corella species proved to be more objective and enabled comparisons of morphological variation between populations. Thus, comparative univariate statistics and multivariate techniques are likely to be useful for defining species boundaries in highly variable groups of ascidians and similarly for understanding ranges of presumed intraspecific morphological variation.

The separation between Antarctic and South American populations due to the constancy of some morphological differences and the results obtained by multivariate analyses support the existence of an Antarctic species that corresponds to *C. antarctica* Sluiter, 1905. Therefore, the name *C. eumyota* should be retained for South American specimens.

The currently accepted distribution range for C. eumyota is wide, passing the sharp biogeographical barrier of the Polar Front. However, the dispersal ability and the environmental conditions in which C. eumyota and C. antarc*tica* live indicate that these species should not be sympatric in the area comprised in this study. Water temperature and currents play a significant role in the distribution of benthic marine species, from their influence on larval dispersion, recruitment and population success (Cowen and Sponaugle 2009). Sessile benthic fauna often exhibit certain provincialism (Lomolino et al. 2010), resulting in small areas or well-defined biogeographic regions, with high probabilities of reproductive isolation (Boltovskoy et al. 2005). This is true for ascidians (Primo and Vázquez 2007, 2009), as they form associations of species in patches, responding to small-scale changes in the type of substrate, depth and currents. Among these, substrate type has been proposed as the more restrictive condition for the settlement and distribution of ascidians in Antarctic shallow areas (Sahade et al. 1998; Tatián et al. 2005). The dispersal ability of gametes, eggs or larvae is itself a limiting factor for the spread of these organisms and influences the degree of genetic differentiation among populations (Bradbury et al. 2008). The larval stage is usually short in ascidians, especially in brooding species, which generally swim for a few minutes or a few hours before settling and undergoing metamorphosis (Lambert 1968; Lambert et al. 1995; Lambert 2005). In C. eumyota, larvae are not released until competent to settle; many larvae settle on adults, resulting in large clumps of specimens of that species (Van Name 1945; Lambert et al. 1995; Lambert 2004). At Potter Cove, large clusters of *C. antarctica* have been observed, associated with other ascidians such as *Molgula pedunculata*, *Cnemidocarpa verrucosa* and *Ascidia challengeri* (Sahade et al. 1998; Tatián et al. 1998).

Allopatry can also be considered the result of physiological constraints, since the particular climatic conditions and relative constancy of the hydrographical conditions in Antarctica have modelled a highly stenothermal biota and a fauna with a strong temperature-dependent biogeographical pattern (Peck 2002). The specialization to permanently low temperatures implies a reduced tolerance of high temperatures as a trade-off (Pörtner et al. 2007). C. antarctica could not withstand the temperatures prevailing in temperate areas, while C. eumyota could not tolerate low polar temperatures, although it appears to have a wide range of environmental tolerance. In a recent study (Tatián et al. 2010), the latter species was found attached to plates deployed at Puerto Deseado (47° 45'S) but was not recruited on plates deployed at Ushuaia harbour (54° 49'S). The annual mean water temperatures at these two harbour areas are 9.3 and 7 °C, respectively (Tatián et al. 2010), the former probably being the lower limit that the species could tolerate. Similarly, the southernmost distribution of C. eumyota along the Pacific coast of South America is 49°S (Tatián and Lagger 2009).

There are at least two records of C. antarctica, both on the Antarctic Peninsula. The first one was the type locality, Booth (Wandel) Island (65.08°S 64.0°W) in the north-east of Wilhelm Archipelago (Sluiter 1905), west of the Antarctic Peninsula. The second was Potter Cove (South Shetland Islands) from which come the specimens analysed in the present study. Other descriptions (Ärnbäck-Christie-Linde 1938; Monniot and Monniot 1974; Monniot et al. 2011) of *Corella* specimens from Antarctica (Paulet Island and Deception Island, respectively) match our observations made for C. antarctica. That species is recognized by the arrangement of male and female ducts, to which are added several morphological traits varying in a characteristic range. Hence, the species described by Brewin (1946) as C. eumyota at Portobello Peninsula, New Zealand, match C. antarctica, even though she did not detail the characteristics of the dorsal languets. Nevertheless, Lambert (2004) described short ducts in specimens from the same locality as Brewin's specimens. Thus, it may be that the species occur in sympatry in New Zealand, in which case crosses between them may happen and, therefore, there is a possibility of finding specimens with an intermediate morphology.

Recently, because of its introduction and rapid spread in Europe, *Corella eumyota* has been included in a model conducted to predict invasions by nonindigenous tunicates into Canada, as one of the species most likely candidates to do it (Locke 2009). Niche requirement modelling studies on invasive species are based on the assumption that the native range is known, especially for understanding the potential risks posed by an invader, including its potential distribution (Therriault and Herborg 2008). Based on genetic evidence, Dupont et al. (2007) mentioned the possibility that populations from South Africa and New Zealand, both considered under the native range of the species, have been introduced from other source populations. This highlights the fact that the native range of many species that are widely distributed and suspected of being cryptogenic is unresolved and suggests data limitations that may compromise modelling approaches (Therriault and Herborg 2008; Locke 2009). Therefore, uncertainty about which species are native or otherwise, as well as about the true identity of the species under consideration, is one of the largest obstacles to understanding broader patterns of invasion and highlights the importance of more rigorous and integrative taxonomic studies. In the case of Corella species, the close inspection of specimens from more localities, particularly Antarctic ones, is necessary to avoid wrong determinations in a context of changes of the marine biota and biological invasions. All this evidence provides incentives for examining both species more closely, especially considering a genetic and biogeographical approach.

## Conclusions

The observation and quantification of morphological characters and the application of statistical analysis coupled with multivariate methods enabled two different ascidian species to be distinguished. The variability observed among specimens was not randomly distributed, but defined discrete clusters that were also separated geographically, corresponding to two species of the same genus: Corella eumyota from South America and C. antarctica from Antarctica. The latter was currently considered as a junior synonym of the former; however, our results show that both species should be considered as separate taxa. The informative value of the characters considered diagnostic has been evaluated in specimens collected at 5 localities from South America and Antarctica. Characters such as siphon length, number of oral tentacles, number of languets in the dorsal lamina and number of longitudinal vessels turned out to be, despite their variability, characters with high information value. While the arrangement of the gonadal ducts, the larval size and the shape of the anus are the most important differences, the validity of the size of languets of the dorsal lamina and the presence of lobes at the anal margin as diagnostic characters should be corroborated through the examination of specimens from other localities. The present results have implications in biogeography, since the Polar Front would be a barrier for the dispersion of *Corella* species.

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