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Molecular and morphological evidence that *Phymactis papillosa* from Argentina is, in fact, a new species of the genus *Bunodosoma* (Cnidaria: Actiniidae)

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Phymactis papillosa *is a rocky shore sea anemone that is commonly found in the Pacific Ocean, from the Gulf of California to Tierra del Fuego, and in the Mar del Plata region, Argentina. The genus* Phymactis *is closely related to* Bunodosoma *and, due to character plasticity, a number of misidentifications have occurred. Therefore, the presence of* P. papillosa *in Argentina has been doubted but the matter had not been investigated in detail. Here we analyse* P. papillosa *specimens from Argentina and compare them, using molecular and morphological markers, to specimens from the species' type locality. In a phylogenetic analysis using 19 allozyme markers and ribosomal internal transcribed spacers sequences of different sea anemone genera, including all West Atlantic* Bunodosoma *species, we have found that the specimens from Argentina were genetically divergent from* P. papillosa *from Chile and closely related to West Atlantic* Bunodosoma *species. The genetic and morphological analyses indicate that those specimens belong to a new species of the genus* Bunodosoma*, described here as* B. zamponii *sp. nov.*

Keywords: allozymes, ribosomal, internal transcribed spacer, phylogeny, specific status, Actiniaria, new species

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INTRODUCTION

Members of the Actiniidae family, one of the largest in the order Actiniaria, are among the best-known sea anemones. Nevertheless, distinguishing genera and species in Actiniidae is often very difficult due to great character variability (Daly, 2003, 2004) and because species are often defined by the absence of characteristics (Daly *et al.*, 2008). Two genera in this family, *Bunodosoma* Verrill, 1899 and *Phymactis* Milne-Edwards, 1857, are closely related and clearly differ from other actinids by the presence of typical non-adhesive vesicles on the column and acrorhagi with holotrichs in the fosse (Carlgren, 1899, 1924, 1949; Belém, 1988; Haussermänn, 2004).

The genus *Bunodosoma* has 13 described species, mostly in tropical and subtropical waters, occurring on both coasts of the Atlantic Ocean and on the Pacific coast of the Americas. On the Atlantic coast of South America, only three *Bunodosoma* species are found: *B. cangicum* Corrêa, 1973, occurs along the Brazilian coast (Belém & Monteiro, 1981; Gomes *et al.*, 1998) and in Uruguay (Zamponi *et al.*, 1998a); *B. caissarum* Corrêa in Belém, 1988, is an endemic Brazilian species that occurs in the south-east and south Brazil and some oceanic islands (Zamponi *et al.*, 1998a); *B. granuliferum* (Le Sueur, 1817), has been recorded in the Caribbean and Brazilian north-east (Paranhos *et al.*, 1999) and south-east (Grohmann, 1998) regions.

In a recent revision of the genus *Phymactis* Haussermänn (2004) recognized two valid species: *P. papillosa* (Lesson, 1830) and *P. sanctahelenae* (Lesson, 1830). Other species of the genus were considered with unknown status and *P. polydactyla* (Hutton, 1879), recorded only from New Zealand (Fautin, 2011), was mentioned as belonging to the genus *Bunodosoma. Phymactis sanctaehelenae* has only been recorded in St Helena Island and it does not seem to be found in the Pacific Ocean (Carlgren, 1949). *Phymactis papillosa* (=*P. clematis*), the genus type species, was originally described from the Chilean coast (Valparaíso region). Later, the species was recorded on other localities in the Pacific Ocean, from the Gulf of California to Tierra del Fuego (Patagonia), including Juan Fernandez Archipelago, Pascua Island and the Galapagos Islands (Carlgren, 1922, 1951, 1959; Carter-Verdeilhan, 1965; Sebens & Paine, 1978; Brattstrom & Johanssen, 1983; Rivadeneira & Oliva, 2001; Haussermänn, 2004; Fautin *et al.*, 2007; Garese *et al.*, 2009).

Phymactis papillosa has also been recorded in Argentina (as *P. clematis*), where it is a very common sea anemone species distributed along the coast of Mar del Plata (Zamponi, 1977; Acuña & Zamponi, 1996; Zamponi & Perez, 1996; Oliveira *et al.*, 2009). However, the specific status of these populations has been questioned (Haussermänn, 2004).

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Molecular markers have been widely used to investigate taxonomic problems in the Actiniaria because of their independence from morphological characters (Carter & Thorpe, 1981; Bucklin & Hedgecock, 1982; Solé-Cava *et al.*, 1985, 1994; Billingham & Ayre, 1996; McFadden *et al.*, 1997; McManus *et al.*, 1997; Monteiro *et al.*, 1997; Manchenko *et al.*, 2000; Schama *et al.*, 2005; Stoletzki & Schierwater, 2005; Acuña *et al.*, 2007; Gusmão, 2010). Therefore, they are particularly interesting when trying to resolve disputes regarding species limits. In many marine invertebrates it has been demonstrated that much of the assumed intraspecific morphological variability in fact represents differences between species (Knowlton, 2000).

The aim of the present work is to analyse the relationship among the different *Bunodosoma* species occurring in South America and to determine the specific status of *P. papillosa* from Argentina. We used molecular (allozyme electrophoresis and DNA sequencing of the ribosomal internal transcribed spacers (ITS)) and morphological data to compare *P. papillosa* (identified as *P. clematis*) from Argentina with *P. papillosa* from Chile and with other South American *Bunodosoma* species. Actiniid species *Anthopleura cascaia* Corrêa in Dube, 1977 and *Actinia bermudensis* (McMurrich, 1889) were used as outgroups.

MATERIALS AND METHODS

Sample collection

Bunodosoma cangicum, *B. caissarum* and *Anthopleura cascaia* specimens were collected in Búzios (south-east Brazil, 22°57′S 43°10′W). *Bunodosoma cangicum* specimens were also collected in Tamandaré (north-east Brazil, 03°45′S 38°36′W). *Bunodosoma granuliferum*, the genus type species, was collected in Curaçao (Boca Sami, 12°06′N 68°55′W). *Phymactis papillosa* specimens from Argentina were collected at Santa Clara del Mar (37°50′S 57°29′W) and Mar del Plata (38°05′S 57°32′W) in three localities (Punta Cantera Beach, Acantilados and Escollera Norte). Samples of *P. papillosa* var. *rubra-viridis* Haussermänn, 2004 from the Chilean coast were collected at Coquimbo (29°57′S 71°19′W). *Actinia bermudensis* samples were collected in Florianopolis (27°26′S 48°34′W), south of Brazil and in Bermuda (32°18′N 64°44′W). To minimize the possible collection of clone mates, all individuals were sampled at least 1 m apart. The anemones were wrapped in damp paper towels and transported to the laboratory, where they were processed for each analysis. For the molecular work (both allozymes and DNA extraction) samples were stored in liquid nitrogen until required for analysis. Figure 1 shows the collecting sites.

Morphological analysis

Specimens of *P. papillosa*, *B. cangicum* and *B. caissarum* were observed *in situ* and also in aquaria. Collected specimens were anaesthetized in a 7% magnesium chloride solution and then fixed and preserved in 4% formaldehyde. For the new species described, type and voucher specimens have been deposited at the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN), in the Actiniarian Collection of Universidad Nacional de Mar del Plata (UNMdP), Argentina and in the Museu Nacional do Rio de Janeiro (MNRJ), Brazil. Some specimens were also deposited at the

Fig. 1. Collection sites for all species analysed in the study.

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Cnidarian Collection of Anthozoan Research Group (GPA) at the Universidade Federal Rural de Pernambuco (UFRPE), Brazil. For purposes of comparison, several specimens collected and identified by Zamponi and deposited in the Cnidaria Collection of UNMdP were also examined.

Measurements of pedal disc width and column height were made on preserved material. Longitudinal and transverse sections, 8–10 μm thick, were made from paraffin embedded specimens. Sections were stained with haematoxylin–eosin and Mallory triple stain methods (Kiernan, 1990). Cnidae measurements were taken from undischarged capsules in squash preparations mounted with fresh water and without stain at 1000X magnification. Relative frequencies of nematocyst types are subjective estimates based on all the cnidae observed on slides. The nomenclature of cnidae follows that of Schmidt (1969, 1972, 1974).

Allozyme analysis

Frozen tissue samples were homogenized with 100 μl of distilled water and analysed by horizontal 12.5% starch gel electrophoresis, using a Tris Citrate pH 8.0 buffer (Ward & Beardmore, 1977). A total of 15 enzymes, coding for 19 loci were used: acid phosphatase (*ACP*, # E.C. 3.1.3.2); catalase (*CAT*, # E.C. 1.11.1.6); α-esterase (*α-EST*, # E.C. 3.1.1.1); glucose-6-phosphate isomerase (*GPI*, # E.C. 5.3.1.9); glutamate dehydrogenase (*GDH*, # E.C. 1.4.1.3); glutamate-oxaloacetate transaminase (*GOT*, # E.C. 2.6.1.1); hexokinase (HK, # E.C. 2.7.1.1); isocitrate dehydrogenase (*IDH*, # E.C. 1.1.1.42); malate dehydrogenase (*MDH*, # E.C. 1.1.1.37); malate dehydrogenase NADP (ME, # E.C. 1.1.1.40); mannose-6-phosphate isomerase (*MPI*, # E.C. 5.3.1.8); octopine dehydrogenase (*ONDH*, # E.C. 1.5.1.11); α-prolyl-phenylalanine peptidase (*PEP*, # E.C. 3.4.11); phosphoglucomutase (*PGM*, # E.C. 5.4.2.2); and phosphogluconate dehydrogenase (*PGD*, # E.C. 1.1.1.4.4). Enzymes were stained according to Manchenko (1994).

DNA extraction and amplification

For the DNA extractions, 10 mg of frozen tissue was homogenized in a microcentrifuge tube in 500 μl of CTAB extraction buffer (CTAB 2%, EDTA 20 mM, 2-mercaptoethanol 0.2% v/v, Tris 100 mM, NaCl 1.4M, proteinase K 30 μg) following the protocol of Damato & Corach (1996) modified with a precipitation with 3M sodium acetate and 100% ethanol at −20°C. The pellet was re-suspended in ultrapure water. The two ribosomal internal transcribed spacers (ITS1 and ITS2), together with the 5.8S rDNA were directly amplified using the polymerase chain reaction (PCR). Each 20 μl PCR reaction consisted of 20 ng of DNA template, 1 unit of Taq DNA polymerase, 0.8 μM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$ and 1 mg/ml BSA, in 1X PCR buffer. The primers used were the 18SF (5′TCA TTT AGA GGA AGT AAA AGT CG 3′) and 28SR (5′GTT AGT TTC TTT TCC TCC GCT T 3′) designed by Lôbo-Hajdu *et al.* (2004). Cycling conditions were 4 minutes at 94°C, followed by 35 cycles of 1 minute at 92°C, 1 minute at 42°C and 1 minute at 72°C, with a final extension step of 5 minutes at 72°C. PCR products were treated with 0.5 units of exonuclease and 2 units of shrimp alkaline phosphatase and sequenced by the dideoxy termination method on an ABI3500 automatic sequencer. Although multiple copies of the ribosomal genes are usually present in the genome, it has been shown that concerted evolution is a major force maintaining the uniformity of paralogues and therefore direct sequencing of PCR products usually does not interfere with phylogenetic analyses (Hillis *et al.*, 1991).

Data analyses

Allozyme data were analysed using the program BIOSYS–2 (Swofford & Selander, 1981). Allele frequencies were calculated and all loci were tested for Hardy–Weinberg equilibrium with an exact test (Haldane, 1954) with Bonferroni correction for multiple tests (Lessios, 1992). Unbiased estimates of heterozygosity and pairwise genetic identity and distance (Nei, 1978) were estimated for all populations analysed. The genetic distances were then used to build an Unweighted Pair Group Method with Arithmetic Mean dendrogram (Sneath & Sokal, 1973) with 2000 bootstrap replicates using the program TFPGA v1.3 (Miller, 1997; http://www.marksgeneticsoftware.net/tfpga.htm). An exact test for population differentiation as described in Raymond & Rousset (1995) was performed also using TFPGA v1.3. A factorial correspondence analysis (FCA) was performed with the program GENETIX 4.05 (Belkhir *et al.*, 2002). This type of analysis is especially useful for estimating associations between multiple independent qualitative variables, where no *a priori* hypothesis is present (Valentin, 2000).

Sequences of the two internal transcribed spacers (ITS1 and ITS2) were aligned using Clustal W (Thompson *et al.*, 1994), followed by eye inspection. Neighbour-joining (NJ) and maximum likelihood (ML) methods were used for reconstructing the group's phylogeny (Felsenstein, 1981; Saitou & Nei, 1987). In this analysis only the species *Actinia bermudensis* was used as outgroup.

The model of evolution was estimated using the log-likelihood score as implemented in the programs Modeltest version 3.5 (Posada & Crandall, 1998). The program PAUP* 4.0b10 (Swofford, 2000) was used for the ML analysis and the program MEGA 3 (Kumar *et al.*, 2004) was used for the NJ analysis.

For the ML approach a full heuristic search with tree bisection reconnection (TBR) and starting tree obtained via NJ was used. For the NJ analyses the Jukes–Cantor distance (Jukes & Cantor, 1969) was used. Bootstrap analyses were carried out for both NJ and ML methods, using 1000 replicates. The resulting trees were drawn using the program Figtree v1.3.1 (Rambaut, 2006–2009; http://tree.bio.ed.ac.uk/).

RESULTS

Allozymes

Gene frequencies and sample sizes for all studied loci are given in Table 1. Significant deviations from Hardy–Weinberg expectations (heterozygote deficiencies) were only found for the PGD locus in the Punta Cantera population. Heterozygote deficiencies are common in marine invertebrates and could be due to a variety of different factors such as gel scoring errors, null alleles, aneuploidy, effects of selection or the Wahlund effect (Zouros & Foltz, 1984; Hare *et al.*, 1996). Heterozygosity levels (H) were high in most populations (Table 1) but were well within the range of

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Table 1. *Bunodosoma* spp., *Phymactis papillosa* and *Anthopleura cascaia*. Gene frequencies of the populations studied. N, number of individuals analysed; H_{obs} and H_{exp}, direct count and Hardy–Weinberg expected mean heterozygosities per locus, respectively; PC, Punta Cantera Beach; SC, Santa Clara del Mar; Tam, Tamandaré.

	P. papillosa	*B. zamponii*		*B. cangicum*		*B. granuliferum*	*B. caissarum*	*A. cascaia*
Loci	**Chile**	**PC**	**SC**	**Tam**	**Búzios**	**Curaçao**	**Búzios**	**Búzios**
PGI-2								
N	13	18	17	14	16	16	5	5
A	0	0	0	0	0	0.37	0	0
B	0	0	0	0	0	0.63	1.00	0
C	0	0.08	0.21	0	0	0	0	0
D	0	0	0	0.93	1.00	0	0	0
E	1.00	0.86	0.79	0.07	0	0	0	1.00
F	0	0.06	0	0	0	0	0	0
PGD								
N	13	18	18	14	16	16	7	5
A	0	0	0	0	0	0.28	0	0
B	1.00	0	0	0.14	0.38	0.69	0	0.10
C	0	0	0	0	0	0	0	0.90
D	0	0.17	0.06	0.79	0.59	0.03	0.79	0
E	0	0	0	0.07	0.03	0	0.21	0
F	0	0.83	0.94	0	0	0	0	0
MPI								
N	16	15	11	11	14	14	6	5
A	0	0.07	0.04	0.04	0	0	0	0
B	0	0.63	0.64	0.46	0.61	0	0	0.50
C	0	0.30	0.32	0.50	0.39	0.46	0.75	0.50
D	1.00	0	0	0	0	0.54	0.25	0
GOT								
N	2	18	18	13	14	16	5	5
A	1.00	0	0	0	0	0	0	0
B	0	0.19	0.08	0	0	0	0.30	0
C	0	0.72	0.81	0.62	0.54	0	0	0
D	0	0	0	0	0	0	0.70	0
E	0	0.08	0.11	0.38	0.46	0	0	0.90
F	0	0	0	0	0	1.00	0	0.10
ACP								
N	5	18	18	14	13	15	7	5
A	0	0.06	0.06	0.04	0.04	0	0	0
B	0	0.88	0.94	0.92	0.96	0	0	0
C	0	0.06	0	0.04	0	0	1.00	0
D	1.00	0	0	0	0	1.00	0	1.00
CAT								
N	13	18	15	14	16	16	7	5
A	0.12	0	0	0	0	0	0	0
B	0.88	0.50	0.17	0.07	0	0	0	0
C	0	0.31	0.60	0.29	0.22	0	0	0.10
D	0	0.19	0.23	0.64	0.78	0	1.00	0.90
E	0	0	0	0	0	1.00	0	0
EST								
N	6	18	17	13	13	14	7	5
A	0	0	0.03	0.08	0	0.18	0	0
B	0	0.34	0.41	0.08	0.19	0.32	0	0.20
C	0	0.58	0.53	0.73	0.64	0.33	0.50	0.80
D	1.00	0	0	0	0	0	0	0
E	0	0.08	0.03	0.11	0.15	0.07	0.50	0
GDH								
N	12	18	18	14	16	15	7	5
A	0	0.03	0.08	0	0	0	0	0
B	0	0.72	0.81	1.00	0.97	0	0	0
C	0	0.25	0.11	0	0.03	0.03	0	0
D	0	0	0	0	0	0	1.00	0.10
E	0	0	0	0	0	0.97	0	0.90
F	1.00	0	0	0	0	0	0	0
HK								
N	13	18	16	14	13	16	7	4

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Table 1. Continued

	P. papillosa	*B. zamponii*		*B. cangicum*		*B. granuliferum*	*B. caissarum*	*A. cascaia*
Loci	**Chile**	**PC**	**SC**	**Tam**	**Búzios**	**Curaçao**	**Búzios**	**Búzios**
A	1.00	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	1.00
C	0	0.64	0.28	0.29	0.15	0	0	0
D	0	0.36	0.53	0.57	0.57	0	0.50	0
E	0	0	0.19	0.14	0.26	0.06	0.50	0
F	0	0	0	0	0	0.69	0	0
G	0	0	0	0	0	0.25	0	0
IDH-1								
N	6	7	10	10	6	3	5	2
A	0	0	0.15	0.15	0	0.17	0.40	0
B	1.00	1.00	0.85	0.85	0.83	0.83	0.60	1.00
C	0	0	0	0	0.17	0	0	0
IDH-2								
N	8	15	15	14	11	7	5	5
A	1.00	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	1.00
C	0	0.07	0	0.18	0	0.21	0	0
D	0	0.63	0.80	0.057	0.86	0.79	0.90	0
E	0	0.27	0.20	0.25	0.14	0	0.10	0
F	0	0.03	0	0	0	0	0	0
MDH-1								
N	1	18	18	14	11	16	7	5
A	0	1.00	1.00	1.00	1.00	1.00	0	0.40
B	0	0	0	0	0	0	1.00	0.60
C	1.00	0	0	0	0	0	0	0
MDH-2								
N	1	16	18	14	14	16	7	5
A	1.00	0	0	0	0	0	0	1.00
B	0	0	0	0	0	0.59	0.57	0
C	0	0	0	0	0	0.41	0	0
D	0	0.47	0.72	0.10	0	0	0.36	0
E	0	0	0	0.04	0.15	0	0.07	0
F	0	0.53	0.28	0	0	0	0	0
G	0	0	0	0.32	0.36	0	0	0
H	0	0	0	0.54	0.39	0	0	0
ME-1								
N	6	15	17	14	9	7	5	5
A	0	0	0	0	0	0	0	0.70
B	1.00	0	0	0	0	0	0	0.30
C	0	1.00	1.00	1.00	1.00	0	0	0
D	0	0	0	0	0	1.00	1.00	0
ME-2								
N	6	18	17	14	14	16	5	5
A	0	0	0	0	0	0	0	1.00
B	0	0.92	0.97	0.93	1.00	0	0	0
C	0	0	0	0	0	0	1.00	0
D	0	0.08	0.03	0.07	0	0	0	0
E	1.00	0	0	0	0	1.00	0	0
ODH								
N	3	13	13	11	14	9	5	5
A	1.00	0	0	0	0	0	0	1.00
B	0	0	0.15	0	0	0	0.20	0
C	0	0	0	0.95	0.93	0	0	0
D	0	0	0	0.05	0.07	0.28	0	0
E	0	0	0	0	0	0.72	0	0
F	0	1.00	0.85	0	0	0	0.80	0
PEP-1								
N	3	13	7	9	11	16	4	5
A	1.00	0.12	0.14	0.22	0.27	0	0	0.10
B	0	0.73	0.57	0.44	0.68	0	0.37	0.90
C	0	0	0	0	0	0.03	0.26	0
D	0	0.15	0.29	0.34	0.05	0	0.37	0

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Table 1. Continued

	P. papillosa	*B. zamponii*		*B. cangicum*		*B. granuliferum*	*B. caissarum*	*A. cascaia*
Loci	**Chile**	**PC**	**SC**	**Tam**	**Búzios**	**Curaçao**	**Búzios**	**Búzios**
E	0	0	0	0	0	0.81	0	0
F	0	0	0	0	0	0.16	0	0
PGI-1								
N	13	18	18	14	16	16	7	5
A	0	0	0.14	0.04	0	0.44	0	0
B	0	0	0	0	0	0.28	0	0
C	0	1.00	0.86	0	0	0.28	0	0
D	1.00	0	0	0.96	1.00	0	0.93	0.60
E	0	0	0	0	0	0	0.07	0.40
PGM								
N	4	18	18	14	14	16	7	5
A	1.00	0	0	0	0	0	0	0
B	0	0	0	0	0	0	1.00	0
C	0	0.12	0.14	0.11	0.07	0	0	0
D	0	0.44	0.44	0.50	0.68	0.12	0	0.80
E	0	0.44	0.42	0.39	0.25	0	0	0.20
F	0	0	0	0	0	0.82	0	0
G	0	0	0	0	0	0.06	0	0
Ho	0.01	0.33	0.30	0.28	0.28	0.30	0.22	0.20
He	0.01	0.32	0.33	0.33	0.30	0.30	0.26	0.20

those usually observed in sea anemone species (Solé-Cava & Thorpe, 1989; Russo *et al.*, 1994).

Genetic distance found between *Phymactis papillosa* from Chile and the putative *P. papillosa* from Argentina was very large, at a level typically found between different genera (Thorpe & Solé-Cava, 1994; Vianna *et al.*, 2003). The smallest gene divergence was found between the two *Bunodosoma cangicum* populations (D = 0.009; Table 2). The Santa Clara and Punta Cantera *P.* cf. *papillosa* populations also presented little gene divergence (D= 0.017). Within the *Bunodosoma* genus, *B. granuliferum* was found to be the most divergent species (D ranges from 1.024 to 1.363). The samples of the putative *P. papillosa* from Argentina clustered with the *Bunodosoma* species analysed clearly indicating that *P.* cf. *papillosa* from Argentina is not conspecific with *P. papillosa* from Chile (Figures 2 & 3). The FCA graphically shows the differentiation found among the species studied, indicating a close relationship between *B. cangicum* and *P.* cf. *papillosa* from Argentina (Figure 3). However, the genetic distance values between those two species (D ranging from 0.345 to 0.399) were within the range usually found between different species of the same genus (Thorpe & Solé-Cava, 1994; Vianna *et al.*, 2003). Furthermore, those two species showed a high degree of gene frequency differentiation across loci (Fisher's exact test; $P < 0.0001$), confirming their genetic distinctiveness.

DNA sequencing analysis

The ITS1/5.8S/ITS2 region was amplified in all samples and ranged in length from 665 to 740 base pairs. The alignment was made with the three regions together. The two ITS regions were used as different data sets in order to avoid heterogeneity among sites, although the results of a preliminary NJ combined analysis were exactly the same (i.e. same tree topology and bootstrap confidence, results not shown). Average nucleotide composition was 20.4% T, 29.2% C, 24.0% A and 26.5% G. Base composition did not vary significantly between species. The complete ITS1, 5.8S and ITS2 sequences were deposited in GenBank with accession numbers JN118557–JN118569.

A partition homogeneity test (Farris *et al.*, 1995) as implemented in PAUP* was performed to verify if the two ITS regions would give significantly different results. The test result indicates no evidence that the two regions were incongruent, so the analyses were subsequently done with the combined data sets. The evolutionary model chosen by

Table 2. *Bunodosoma* spp., *Anthopleura cascaia* and *Phymactis papillosa* unbiased genetic identities (above diagonal) and distances (below diagonal) between pairwise populations (Nei, 1978). PC, Punta Cantera Beach; SC, Santa Clara del Mar; Tam, Tamandaré.

	Species	**Population**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	*B. zamponii*	PC	–	0.686	0.671	0.983	0.243	0.272	0.332	0.155
2	*B. cangicum*	Tam	0.377	–	0.991	0.708	0.232	0.359	0.349	0.150
3	*B. cangicum*	Búzios	0.399	0.009	–	0.697	0.245	0.354	0.370	0.156
4	*B. zamponii*	SC	0.017	0.345	0.360	–	0.250	0.283	0.315	0.125
5	*B. granuliferum*	Curaçao	1.415	1.462	1.406	1.387	–	0.293	0.280	0.256
6	*B. caissarum*	Búzios	1.302	1.023	1.039	1.264	1.229	–	0.266	0.109
7	*A. cascaia*	Búzios	1.102	1.051	0.993	1.156	1.272	1.324	–	0.361
8	*P. papillosa*	Chile	1.863	1.896	1.860	2.076	1.362	2.215	1.019	–

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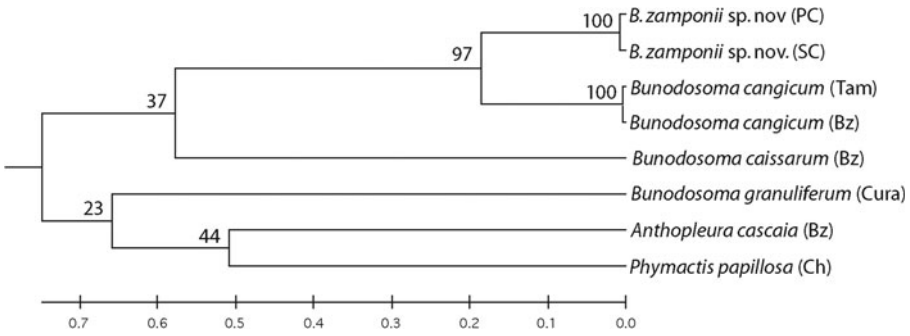

Fig. 2. Unweighted Pair Group Method with Arithmetic Mean dendrogram of allozyme unbiased genetic distances (Nei, 1978) between the populations studied. Bootstrap values for 2000 replicates on branches.

Modeltest was the Jukes–Cantor model with a gamma distribution of among site variation (JC + G).

Both phylogenetic methods gave similar results (same tree topology) and the relationships found between the studied species were the same as those found in the analyses of allozyme loci. Once again, the putative *Phymactis papillosa* samples from Argentina were genetically more similar to *Bunodosoma* than to *P. papillosa* from Chile (Figure 4). High divergence levels (16–20%) were observed among the genera studied further emphasizing the separation of the two closely related genera *Bunodosoma* and *Phymactis* (18% mean Jukes–Cantor distance). The mean intraspecific variability levels observed for *Bunodosoma* ITS sequences (0.25%), although expected for Anthozoans (Forsman *et al.*, 2005, 2009; Fukami *et al.*, 2008), were smaller than those observed in other Actiniaria populations (Stoletzki & Schierwater, 2005; Acuña *et al.*, 2007; Gusmão, 2010). This lower evolutionary rate may explain the lack of significant differences between *B. cangicum* and the putative *P. papillosa* from Argentina, which could be clearly separated by allozyme data.

The joint results of the allozyme and DNA sequence data clearly show that the common intertidal anemone from Argentina identified as *P. clematis* by Zamponi (1977) does not belong to that species but, instead, is a species of the genus *Bunodosoma*. The high differentiation observed in the allozyme analyses, together with the morphological diagnostic characteristics observed lead us to conclude that the anemones formerly named *P. clematis* in Argentina belong to a new species of *Bunodosoma*, that we describe below.

SYSTEMATICS

Order ACTINIARIA

Family ACTINIIDAE Rafinesque, 1815

Genus *Bunodosoma* Verrill, 1899

Bunodosoma zamponii sp. nov.

(Figures 5–8)

Phymactis clematis; Zamponi, 1977: 139,141 (Argentina); Pollero, 1983; Patronelli *et al.*, 1987; Zamponi, 1989, 1993, 2000, 2005; Excoffon & Zamponi, 1991; Acuña & Zamponi, 1995, 1996, 1997; Acuña *et al.*, 1996; Zamponi & Perez, 1996, Genzano *et al.*, 1996; Acuña, 1997; Zamponi *et al.*, 1998a, b; Gomes *et al.*, 1998; Excoffon *et al.*, 1999; Patronelli *et al.*, 2005, 2008; Olivera *et al.*, 2009; not *Phymactis clematis* (Drayton in Dana, 1846: 130).

TYPE MATERIAL

Holotype: MACN (In-35365), Atlantic Ocean, Mar del Plata (38°05′S 57°32′W), Punta Cantera, intertidal, Coll. P.B. Gomes, 16 September 1999, preserved in formalin.

Paratypes: UNMdP (C.A. 27), three specimens, MNRJ (6274), one specimen, both samples collected at the same time and place as the holotype.

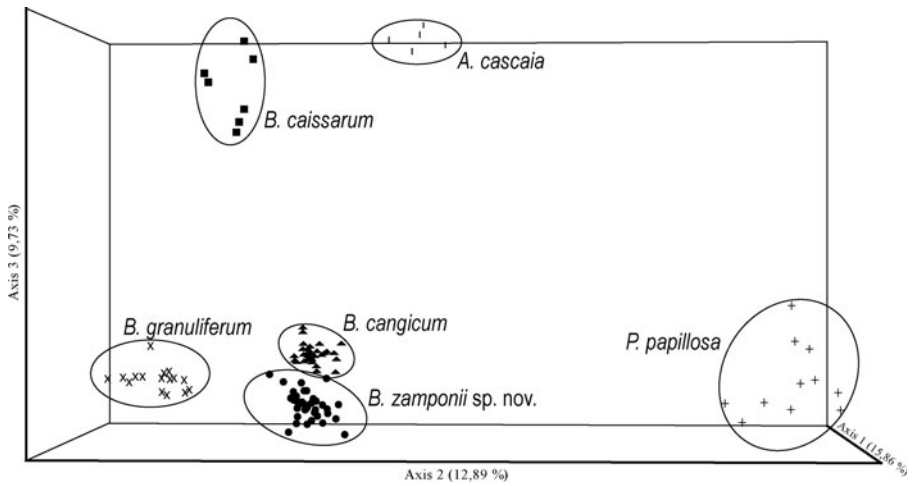

Fig. 3. Three-dimensional representation of a factorial correspondence analysis based on 19 allozyme loci.

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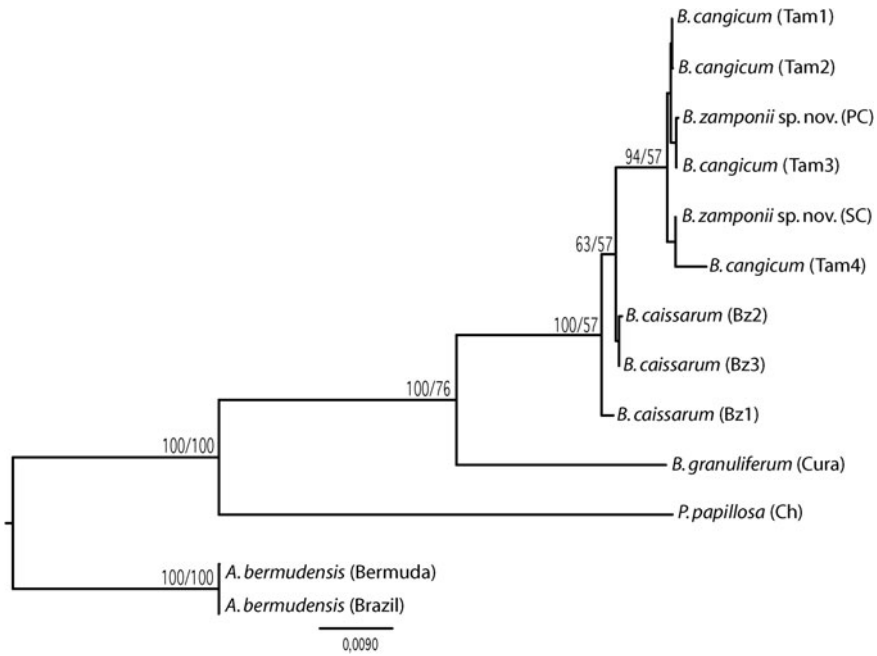

Fig. 4. Phylogenetic tree constructed by the neighbour-joining (NJ) method with Jukes–Cantor distances and pairwise deletion. Numbers on branch are: 1000 bootstrap replicates NJ/1000 bootstrap replicates maximum likelihood.

ADDITIONAL MATERIAL

UFPE (GPA 092), two specimens, MNRJ (6275), one specimen, Atlantic Ocean, Mar del Plata (38°05′S 57°32′W), Acantilados, intertidal, Coll. P.B. Gomes, 24 November 1999, preserved in formalin; UFPE (GPA 093), one specimen, Atlantic Ocean, Mar del Plata (38°05′S 57°32′W), Escollera Norte, intertidal, Coll. A.C. Excoffon, 14 November 1999, preserved in formalin.

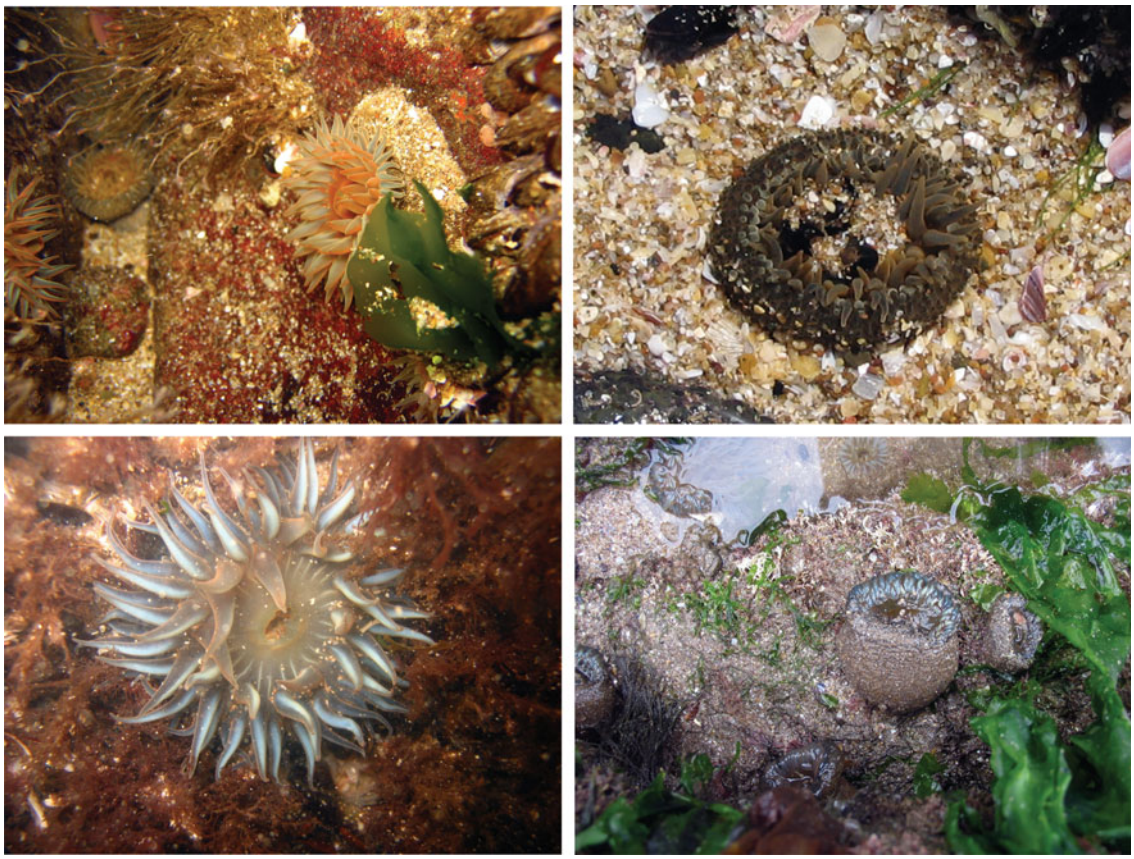

Fig. 5. External morphology of *Bunodosoma zamponii* sp. nov. on the intertidal zone of Mar del Plata. Photograph by Gabriel Genzano.

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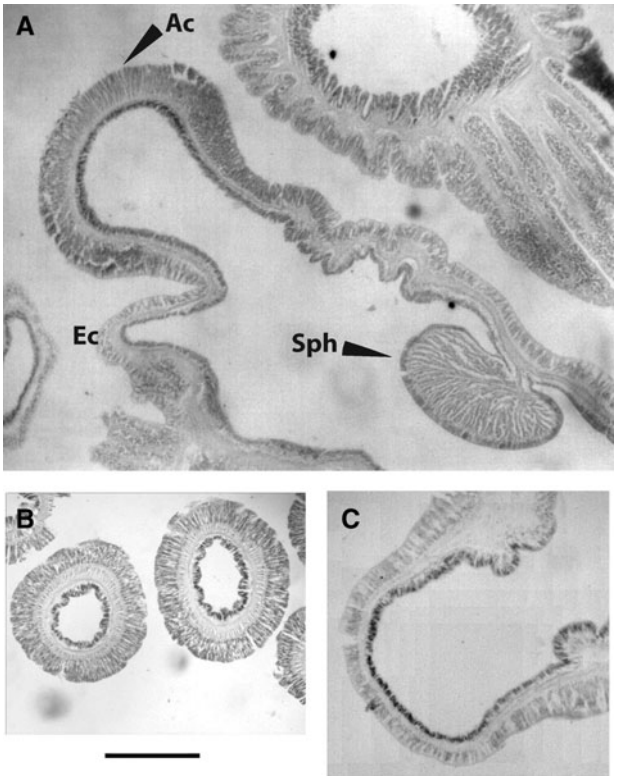

Fig. 6. Internal anatomy of *Bunodosoma zamponii* sp. nov.: (A) marginal sphincter muscle circumscribed, palmated (Sph), acrorhagus (Ac) and ectoderm (Ec); (B) cross-section through tentacles; (C) cross-section through a vesicle. Scale bars: A, 700 μm; B, 1.1 mm; C, 0.8 mm.

DIAGNOSIS

Actiniidae with rows of non-adhesive vesicles on the column from margin to limbus. Margin with marginal projections each one with a single holotrichous acrorhagus on the oral surface and simple or compound vesicles on the adoral surface. Projections arranged in two alternate crowns of slightly different size. Fosse present. Column orange to cream with dark grey vesicles, or dark orange with olive green vesicles. Tentacles arranged in five cycles, approximately 96 total, brownish or crimson red. Size and coloration distinguish live specimens of *B. zamponii* from other *Bunodosoma* species, except *B. cangicum* from which it only differs on the arrangement of the mesenteries.

DESCRIPTION

Column

Cylindrical with a great capacity of elongation. Live specimens vary from 3 to 6 cm long, width of the base between 1.4 and 3.8 cm. Contracted specimens often dome-shaped. Fosse deep. Margin denticulate, with vesicle-covered marginal projections arranged in two alternate crowns of slightly different size; each projection bears a single holotrichous acrorhagus on the oral surface. Acrorhagi simple, rounded, of cream or opaque white colour, arranged in two alternate cycles with 48 acrorhagi each (Figure 5). External cycle endocoelic and large (diameter 1.1 cm), internal cycle exocoelic and small (0.75 cm of diameter). In some specimens, the second cycle may be absent or incomplete. Column covered from margin to just above the limbus with endocoelic and exocoelic non-adhesive vesicles, mostly with no clear arrangement due to the state of contraction of the specimens, but in some specimens, especially near the margin, a clear arrangement can be observed forming approximately 96 longitudinal rows. Vesicles rounded, sometimes compound only near the margin and on marginal projections, without nematocyst batteries. Column orange to cream with dark grey vesicles or dark orange with olive green vesicles. Adherent base roughly circular, bigger in diameter than the column, cream with orange or pale brown radial lines.

Oral disc and tentacles

Number of tentacles from 96 to about 102, in 5 cycles. The outermost cycle, exocoelic, alternating with the largest row of acrorhagi. Tentacles of the outermost cycle are the same length as innermost tentacles (from 1.5 to 2.5 cm long). Tentacles pale or dark brownish or crimson red typically without marks. Oral disc brownish or pale cream, mesentery insertions visible as orange or cream lines. Oral disc diameter from 2 to 5 cm, central mouth, rounded, atop an oral cone. Actinopharynx creamy white.

Internal anatomy

Actinopharynx extends half to three-quarters of the length of the column, with folds. Two siphonoglyphs, extending below the end of the actinopharynx, each one attached to a pair of directive mesenteries. Equal number of mesenteries distally and proximally (46 to 52 pairs, usually 48). Mesenteries hexamerously arranged in four cycles, all

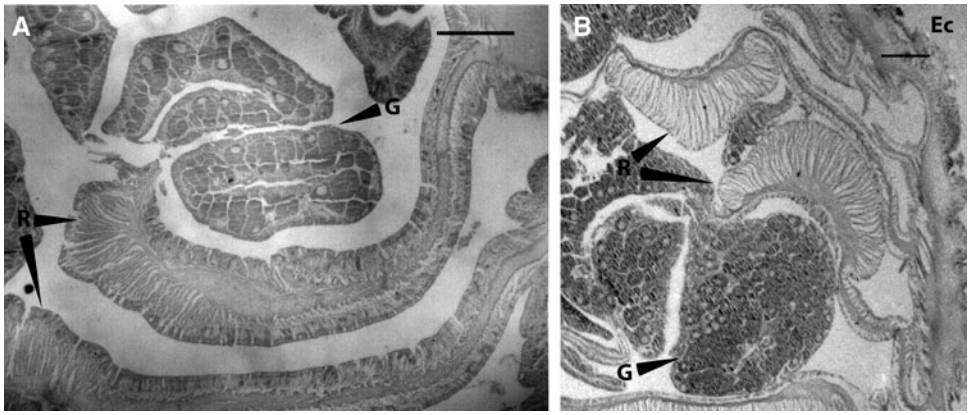

Fig. 7. Internal anatomy, cross-section showing mesenteries with retractor muscles (R), gonads (G) and ectoderm (Ec). (A) *Bunodosoma zamponii* sp. nov.; (B) *B. cangicum*. Scale bar: 200 μm.

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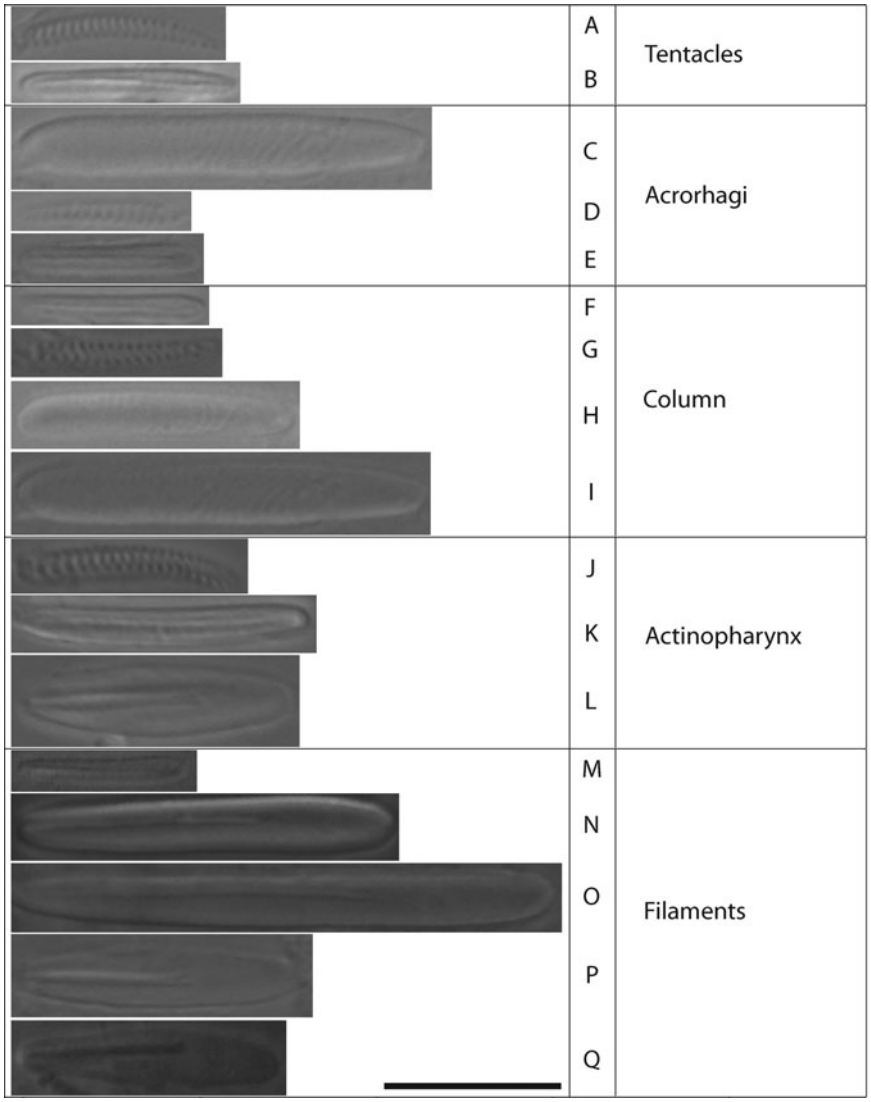

Fig. 8. Cnidae of *Bunodosoma zamponii* sp. nov.: (A) spirocyst; (B) b-rhabdoid; (C) holotrich; (D) spirocyst; (E) b-rhabdoid; (F) b-rhabdoid; (G) spirocyst; (H) holotrich; (I) holotrich; (J) spirocyst; (K) b-rhabdoid; (L) p-rhabdoid; (M) b-rhabdoid 1; (N) b-rhabdoid 2; (O) b-rhabdoid 3; (P) p-rhabdoid A; (Q) p-rhabdoid B1a. Scale bar: 10 μm.

perfect. The last cycle is only attached to the actinopharynx just below the oral disc. Near the aboral end, all mesenteries join together, forming a basal node. Gonochoric. All mesenteries, except the directives, are fertile. Gametogenic tissue may be poorly developed or absent in mesenteries of the last cycle. In younger mesenteries, the gametogenic tissue is positioned more distally. The male gametogenic tissue appears rounded and pale white or yellow in fixed specimens. The female tissue, when poorly developed, is similar to that of the male. Well-developed, female gametogenic tissue is grey and not as round.

Marginal sphincter strong, circumscribed, palmate (Figure 6A), rounded or oval. Endodermal circular musculature well developed at mid-column. Vesicles in the column show no histological differentiation (Figure 6C). Parietobasilar muscles diffuse, with a short pennon (Figure 7A). Retractor muscles circumscribed to diffuse strong (Figure 7A). Tentacles and oral disc with ectodermal longitudinal musculature (Figure 6B). As in other species of the genus, tissues without zooxanthellae.

Cnidom

Spirocysts, b-rhabdoids, p-rhabdoids A, p-rhabdoids B1a, holotrichs (Figure 8). See Table 3 for sizes and distribution.

Distribution and natural history

Atlantic Ocean, Argentina, from Santa Clara del Mar (37°50′S 57°32′W) to Mar del Plata (38°05′S 57°32′W). The species inhabits crevices, clefts and tide pools in the intertidal and subtidal zones. *Bunodosoma zamponii* sp. nov. is the most abundant species where it occurs (Zamponi *et al.*, 1998b). Much work has been done on the species (all still considering the species as *Phymactis clematis*) about its autoecology (Acuña & Zamponi, 1995; Acuña, 1997) and morphological variation (Zamponi & Perez, 1996; Acuña & Zamponi, 1997). The species is polyphagous opportunistic (Acuña & Zamponi, 1996). It is gonochoric, with an oviparous–pelagic–planktotrophic pattern (Excoffon & Zamponi, 1991). No fission scars or anatomical irregularities have been reported for the species. Many studies have been conducted about the morphological, functional and biochemical

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Table 3. Size and distribution of *Bunodosoma zamponii* sp. nov. cnidae. All measurements are in μm; range: length × width; mean ± standard deviation; N, total number of capsules measured; F, frequency; VC, very common; C, common; S, scarce; R, rare; ratio, ratio of number of specimens in which each cnidae was found to number of specimens examined.

Tissue/cnidae type	Range	Length	Width	N	F	Ratio
TENTACLES						
Hpirocysts	(10.0–25.4) × (2.0–2.4)	17.12 ± 3.82	2.31 ± 1.57	40	VC	10/10
b-rhabdoids	(13.0–31.8) × (2.0–3.0)	21.75 ± 4.77	2.42 ± 0.48	40	C	10/10
ACRORHAGI						
Holotrichs	(29.9–55.1) × (3.0–6.4)	41.32 ± 8.26	4.74 ± 0.90	40	C	10/10
Spirocysts	(19.1–39.6) × (2.0–4.2)	26.26 ± 4.36	3.47± 4.36	40	C	10/10
b-rhabdoids	(14.0–23.3) × (2.0–4.2)	16.83 ± 2.98	2.66 ± 0.69	25	S	10/10
COLUMN						
b-rhabdoids	(10.6–24.0) × (2.0–3.0)	16.59 ± 2.96	2.30 ± 0.44	67	C	10/10
Spirocysts	(14.8–30.0) × (2.0–3.0)	23.08 ± 4.04	2.31 ± 0.39	25	R	5/10
Holotrichs[a]	(17.0–25.0) × (2.0–5.0)	21.89 ± 1.86	4.11 ± 0.39	35	C	10/10
Holotrichs	(30.0–46.0) × (3.0–6.0)	36.50 ± 4.95	4.50 ± 0.85	20	R	4/10
ACTINOPHARYNX						
Spirocysts	(17.0–22.8) × (2.1–2.4)	20.69 ± 2.62	2.29 ± 0.14	20	R	10/10
b-rhabdoids	(18.0–32.4) × (2.4–4.24)	24.43 ± 4.22	3.23 ± 0.90	35	C	10/10
p-rhabdoids	(23.3–27.6) × (4.0–5.0)	25.2 ± 1.84	4.8 ± 0.14	25	C	10/10
MESENTERIAL FILAMENTS						
b-rhabdoids 1	(37.0–53.0) × (4.0–6.0)	43.31 ± 4.54	5.19 ± 0.54	25	C	9/10
b-rhabdoids 2	(21.0–27.0) × (2.0–3.0)	22.4 ± 2.13	2.87 ± 0.35	25	S	8/10
b-rhabdoids 3	(8.4–19.0) × 2.0	14.47 ± 2.47	2.0 ± 0.0	30	C	9/10
p-rhabdoids A	(19.0–27.0) × (4.0–6.0)	22.2 ± 2.18	4.72 ± 0.59	25	C	8/10
p-rhabdoids B1a	(15.0–20.0) × (5.0–6.0)	17.67 ± 1.42	5.2 ± 0.14	20	R	3/10

a, very common in the middle column, absent or sporadic near the oral and pedal disc.

characterization of the sphincter of *B. zamponii* sp. nov. (Patronelli *et al.*, 2005, 2008; Olivera *et al.*, 2009).

ETYMOLOGY

Bunodosoma zamponii is named after Dr Mauricio O. Zamponi, in recognition of his numerous contributions to the study of cnidarians, especially in Argentina.

TAXONOMIC REMARKS

This species has a circumscribed sphincter, typical non-adhesive vesicles and old, strong fertile mesenteries typical of the genus *Bunodosoma*. The specimens of *P. papillosa* collected in Chile and used in the present study presented a diffuse sphincter and no reproductive tissue in the first and second mesentery cycles, which is similar to previous descriptions of the species (McMurrich, 1904; Carlgren, 1899, 1920, 1945, 1959; Stotz, 1979; Haussermänn, 2004). The fact that the new species is the most common on the intertidal zone of Mar del Plata and that there are no other species that resemble the genus *Phymactis* in the area nor in the Cnidarian Collection of UNMdP lead us to believe that the records of *P. papillosa* (= *P. clematis*) from Argentina were misidentifications. *Phymactis papillosa* probably does not occur on the Atlantic coast of South America, as already suggested by Carlgren (1939).

Bunodosoma zamponii sp. nov. differs from other species of the genus by a combination of characters such as retractor muscle type, number of mesenteries and tentacles, reproductive tissue distribution and cnidom. From the species of *Bunodosoma* that occur in the South Atlantic Ocean, *B. zamponii* sp. nov. resembles *B. granuliferum* and *B. biscayense* (Fischer, 1874) by having 96 mesenteries disposed in 4 cycles. However, these two species have the column marked with alternating dark and white longitudinal bands (Watzl, 1922; Pax, 1924; den Hartog, 1987), which are not present in *B. zamponii* sp. nov. (Figure 5). Furthermore, *B. zamponii* sp. nov. can be distinguished from those two species by cnidae differences and gonad distribution.

The cnidae distribution of the new species is very similar to that of other *Bunodosoma* species. Nevertheless, the presence of spirocysts in the filaments and column observed in *B. zamponii* sp. nov. differentiate this species from *B. caissarum* and *B. cangicum*.

Bunodosoma zamponii sp. nov. is very similar to *B. cangicum*, a species present along the coasts of Brazil and Uruguay. Their major morphological differences are the mesentery cycle number (four in *Bunodosoma zamponii* sp. nov., against three in *B. cangicum*) and retractor muscle type (strong and circumscribed in *B. cangicum*; Figure 7). Table 4 summarizes the major differences among the different species.

Den Hartog (1987) proposed modifications to the diagnosis of the genus *Bunodosoma* establishing that the number of mesentery cycles should be four or five and that the retractor muscle of the mesenteries should be diffuse (den Hartog, 1987: 555–556). This diagnosis is not compatible with the species of *Bunodosoma* from South America. *Bunodosoma zamponii* sp. nov., *B. caissarum* and *B. cangicum* present a circumscribed or circumscribed–diffuse retractor muscle and the last species has only three mesentery cycles.

In Mar del Plata and Santa Clara del Mar *B. zamponii* sp. nov. shares the hard substrate with *Aulactinia marplatensis* Zamponi, 1977 and *Oulactis muscosa* (Drayton in Dana, 1846). The new species differs from all others in the field by the coloration and the presence of non-adhesive vesicles on the column. The presence of acrorhagi also distinguishes *B. zamponii* sp. nov. from *Aulactinia marplatensis*.

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Table 4. *Bunodosoma* spp. and *Phymactis papillosa*. Main morphological characters.

Species	b-rhabdoids on acrorhagi	Spirocyst in column and actinopharynx	Sphincter	Number of tentacles	Number of mesenteries	Number of mesentery cycles	Mesentery arrangement	Column colour	Retractor muscles	Gonad disposition
P. papillosa[a]	Very common, long	Absent	Diffuse	96	Up to 400	Up to 7	Not informed	Red, green, blue or brown	Diffuse, band-like	Two oldest cycles and directives sterile
B. zampanii	Scarce or rare, short	Present	Circumscribed	96–102	96	4	6 + 6 + 12 + 24	Orange to cream	Circumscribed–diffuse	All mesenteries fertile except directives
B. cangicum	Scarce or rare, short	Absent	Circumscribed	96	96	3	12 + 12 + 24	Orange to cream	Strong and circumscribed	All mesenteries fertile except directives
B. caissarum[b]	Scarce or rare, short	Absent	Circumscribed	192	192	4	12 + 12 + 24 + 48	Red	Strong and circumscribed	1st, 2nd and 3rd cycles fertile except directives
B. granuliferum[c]	Scarce or rare, short	Present in the column; absent in actinopharynx	Circumscribed	96	96	4	6 + 6 + 12 + 24	Green with dark and white bands	Diffuse or restricted–diffuse	All mesenteries fertile except directives
B. biscayenses[d]	Scarce or rare, short	Absent	Circumscribed	96	96	4	6 + 6 + 12 + 24	Greyish with dark and white bands	Diffuse or restricted–diffuse	Almost all strong mesenteries fertile including the 1st cycle and directives

a, Haussermänn, 2004 and personal observation; b, Belém, 1988 and personal observation; c, Pax, 1924; d, den Hartog, 1987.

DISCUSSION

Both genetic and morphological data clearly show that *Phymactis papillosa* (formerly cited as *Phymactis clematis*) specimens from Argentina are not only very distinct from *P. papillosa* from Chile, the species' type locality, but, more importantly, belong to a different genus (*Bunodosoma*).

Only Carlgren (1949) reported *P. papillosa* along the whole Chilean coast as far south as Tierra del Fuego. No other study to date has found this species south of the Golfo de Penas (48°S) (Haussermänn, 2004), indicating that a phylogeographical break might occur in that region. Surface ocean currents are recognized as one of the most important factors for the dispersal of benthic marine animals, since many larvae are planktonic and expected to have great dispersal potential. Nevertheless, how much dispersal really occurs is not always well known and studies describing species with long-lived larvae that have low dispersal and species with no planktonic development with high dispersal capability have been reported (Miller & Ayre, 2008). Phylogeographical breaks, where there is a discontinuity in the distribution of a species that coincides with a geographical feature, seem to play an important role for benthic marine animals (Hellberg, 2009). Golfo de Penas has already been reported as a dispersal barrier for some marine animals (Lancellotti & Vásquez, 1999). It is possible that the cold and low salinity waters in that area (Försterra & Haussermänn, 2003) could be responsible for the southward restriction in *P. papillosa* distribution.

The external features of the genera *Phymactis* and *Bunodosoma* are very similar, increasing the difficulty in making a distinction between them, nevertheless other, more subtle, morphological features differentiate the two genera (den Hartog, 1987; Haussermänn, 2004). Species of *Phymactis* have the first and second mesentery cycles sterile, a diffuse sphincter and few holotrichs in the acrorhagi, with abundant long b-rhabdoids instead (Carlgren, 1934, 1949, 1959; Stotz, 1979; Haussermänn, 2004). *Bunodosoma* members have a variety of degrees of circumscribed sphincter (den Hartog, 1987; Haussermänn, 2004; Fautin *et al.*, 2007). Morphological analyses carried out in this study confirm all these differences, since all *Bunodosoma* species studied differ from *P. papillosa* on these characters. The specimens from Argentina presented the same type of sphincter and cnidae distribution of other *Bunodosoma* species and also all strong mesenteries, except the directives, were fertile (see description above).

The low genetic differentiation found between the Santa Clara del Mar and Punta Cantera *B. zamponii* sp. nov. populations (D = 0.02; 50 km distance) and between the Tamandaré and Búzios *B. cangicum* populations (D = 0.01; 3500 km distance) are similar to those found between other species of the genus. Russo & Solé-Cava (1991) found a low divergence (D = 0.05) between *B. caissarum* populations that were 180 km distant. The same was observed for *B. cavernata* (Bosc, 1802) (D = 0.11; 2000 km) and *B. granuliferum* (D = 0.16; 1600 km) populations from the Gulf of Mexico and the Caribbean, respectively (McCommas & Lester, 1980; McCommas, 1982). These values indicate that *Bunodosoma* species have a high potential for long distance dispersal. Studies on the reproductive biology of *B. caissarum* showed that they bear long-lived planktotrophic larvae (Belém, 1987). Although there are no studies on *B. cangicum* reproduction patterns or larval biology, the Argentinean

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B. zamponii sp. nov. and *B. caissarum* have the same kind of larvae which can live for five days in the plankton (Belém, 1987; Excoffon & Zamponi, 1991), enabling them for long distance dispersal.

Bunodosoma cangicum and *Bunodosoma zamponii* sp. nov. are genetically, morphologically and ecologically more similar to each other than either of them is to *B. caissarum* or *B. granuliferum*. This indicates that *B. cangicum* and *Bunodosoma zamponii* sp. nov. have probably diverged very recently. It is possible that, due to its hydrographical characteristics and the high sedimentation rate, the La Plata River might have played an important role in the isolation of these two species. In some cases habitat specificity can be more important than larval dispersion when it comes to gene flow among populations (Miller & Ayre, 2008; Ayre *et al.*, 2009). In fact, large estuaries such as the Amazon River delta are an important source of phylogeographical breaks for costal marine species (Rocha *et al.*, 2002; Lima *et al.*, 2005; Currie & Small, 2006). Although the Amazon River estuary did not seem to be an important barrier to gene flow (Vianna *et al.*, 2003) for the sea anemone species *Actinia bermudensis*, that species is known to withstand large salinity variations, inhabiting estuaries (Stephenson, 1935; Corrêa, 1964; Douek *et al.*, 2002) where *Bunodosoma* species are never found (Gomes, 2002). So far few papers have analysed the role of the La Plata River as a gene flow barrier for marine animals but Zamponi *et al.* (1998a) have already called attention to this estuary as a potential barrier to cnidarian dispersal, since the Brazilian and Argentinean coasts only share three species of sea anemones out of more than 50 presently recognized species in the two areas.

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REFERENCES

Acuña F.H. (1997) Ecología trófica de actiniarios (Cnidaria, Anthozoa) intermareales: selección de la talla de las presas. *Physis—A* 53, 1–5.

Acuña F.H. and Zamponi M.O. (1995) Ecology of intertidal sea anemones. Density, dispersion and autoecology of *Phymactis clematis* Dana,1849 (Anthozoa: Actiniaria). *Ciencias Marinas* 21, 1–12.

Acuña F.H. and Zamponi M.O. (1996) Ecología trófica de las anémonas intermareales *Phymactis clematis* Dana, 1849, *Aulactinia marplatensis* (Zamponi, 1977) y *A. reynaudi* (Milne-Edwards, 1857) (Actiniaria: Actiniidae): relaciones entre las anémonas y sus presas. *Ciencia Marina* 22, 397–413.

Acuña F.H. and Zamponi M.O. (1997) The use of cnidocysts for ecological races identification from sea anemones populations (Anthozoa, Actiniidae). *Iheringia—Série Zoologia* 82, 9–18.

Acuña F.H., Zamponi M.O. and Perez C.D. (1996) Metodología para la cuantificación de algunas estructuras taxonómicas en Actiniaria (Cnidaria, Anthozoa). *Iheringia—Série Zoologia* 80, 27–31.

Acuña F.H., Excoffon A.C., McKinstry S.R. and Martinez D.E. (2007) Characterization of *Aulactinia* (Actiniaria: Actiniidae) species from Mar del Plata (Argentina) using morphological and molecular data. *Hydrobiologia* 592, 249–256.

Ayre D.J., Minchinton T.E. and Perrin C. (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Molecular Ecology* 18, 1887–1903.

Belém M.J.C. (1987) *Aspectos da biologia de* Bunodosoma caissarum *Corrêa, 1964 (Cnidaria, Anthozoa, Actiniidae) do litoral do Rio de Janeiro, com ênfase na estimativa de seu comportamento reprodutivo*. PhD thesis. Universidade de São Paulo, São Paulo, Brazil.

Belém M.J.C. (1988) Anatomy and biology of *Bunodosoma caissarum* Corrêa, 1964 (Cnidaria, Anthozoa, Actiniidae). 1. Systematic position and revision of morphology and microanatomy. *Anais da Academia Brasileira de Ciencias* 60, 365–375.

Belém M.J.C. and Monteiro D.C. (1981) Fauna de cnidários do Rio de Janeiro. III. *Anthopleura varioarmata* Watzl, 1922 (Actiniaria, Endomyaria), uma nova ocorrência de Actiniidae. *Seminários de Biologia Marinha–Academia Brasileira de Ciências*, pp. 193–203.

Belkhir K., Borsa P., Chikhi L., Raufaste N. and Bonhomme F. (2002) *GENETIX 4.04, logiciel sous Windows TM pour la génétique des populations*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Billingham M. and Ayre D.J. (1996) Genetic subdivision in the subtidal, clonal sea anemone *Anthothoe albocincta*. *Marine Biology* 125, 153–163.

Brattstrom H. and Johanssen A. (1983) Ecological and regional zoogeography of marine benthic fauna of Chile. Report No. 49 of the Lund University Chile Expedition, 1948–59. *Sarsia* 68, 289–339.

Bucklin A. and Hedgecock D. (1982) Biochemical genetic evidence for a third species of *Metridium* (Coelenterata: Actiniaria). *Marine Biology* 66, 1–7.

Carlgren O. (1899) Zoantharien. *Hamburger Magalhaensische Sammelreise* 4, 1–48.

Carlgren O. (1920) Förteckning över de i skånska resan omnämnda djurarterna. In *Linnés Skånska Resa med anmärkningar utgiven av Jöran Sahlgren*. Stockholm: P.A. Norstedt and Söners, pp. 19–25.

Carlgren O. (1922) Actiniaria und Zoantharia von Juan Fernandez und der Osterinsel. In Skottsberg C. (ed.) *The natural history of Juan Fernandez and Easter Island. 3 Part 2*. Uppsala: Almquist & Wiksells Boktryckeri, pp. 145–160.

Carlgren O. (1924) On *Boloceroides*, *Bunodeopsis* and their supposed allied genera. *Arkiv förZoologi* 17A, 1–20.

Carlgren O. (1934) Zur revision der Actiniarien. *Arkiv för Zoologi* 26A, 1–36.

Carlgren O. (1939) Actiniaria and Zoantharia of the Scottish National Antarctic Expedition, 1902–1904. *Transactions of the Royal Society of Edinburgh* 49, 791–800.

Carlgren O. (1945) Further contributions to the knowledge of the cnidom in the Anthozoa especially in the Actiniaria. *Kungliga Fysiografiska Sällskapets Handlingar* 56 NF, 1–24.

Carlgren O. (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar* 4, 1–121.

Carlgren O. (1951) The actinian fauna of the Gulf of California. *Proceedings of the United States National Museum* 101, 415–449.

Carlgren O. (1959) Corallimorpharia and Actiniaria with description of new genus and species from Peru. Reports of the Lund University Chile Expedition 1948–49. *Lund Universitets Arsskrift* 38, 1–39.

Carter M.A. and Thorpe J.P. (1981) Reproductive, genetic and ecological evidence that *Actinia equina* var. *mesembrianthemum* and var. *fragacea* are not conspecific. *Journal of the Marine Biological Association of the United Kingdom* 61, 79–93.

Carter-Verdeilhan C.D. (1965) Actinias de Montemar, Valparaiso. *Revista de Biologia Marina* 12, 129–157.

Corrêa D.D. (1964) *Corallimorpharia e Actiniaria do Atlântico Oeste Tropical*. PhD thesis. Universidade de São Paulo, Brazil.

Currie D.R. and Small K.J. (2006) The influence of dry-season conditions on the bottom dwelling fauna of an east Australian sub-tropical estuary. *Hydrobiologia* 560, 345–361.

Daly M. (2003) On the anatomy, terminology and homology of acrorhagi and pseudoacrorhagi. *Zoologische Mededelingen* 345, 89–102.

Daly M. (2004) Anatomy and taxonomy of three species of sea anemones (Cnidaria: Anthozoa: Actiniidae) from the Gulf of California, including *Isoaulactinia hespervolita* Daly, n. sp. *Pacific Science* 58, 377–390.

Daly M., Chaudhuri A., Gusmão L. and Rodríguez E. (2008) Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogenetics and Evolution* 48, 292–301.

Damato M.E. and Corach D. (1996) Genetic diversity of populations of the fresh-water shrimp *Macrobrachium borellii* (Caridea, Palaemonidae) evaluated by RAPD analysis. *Journal of Crustacean Biology* 16, 650–655.

Douek J., Barki Y., Gateño D. and Rinkevich B. (2002) Possible cryptic speciation within the sea anemone *Actinia equina* complex detected by AFLP markers. *Zoological Journal of the Linnaean Society* 136, 315–320.

Excoffon A.C. and Zamponi M.O. (1991) La biologia reproductiva de *Phymactis papillosa* Dana, 1849 (Actiniaria: Actiniidae); gametogénesis, períodos reproductivos, desarrollo embrionario y larval. *Spheniscus* 9, 25–39.

Excoffon A.C., Genzano G.N. and Zamponi M.O. (1999) Macrobenthos associated with a population of *Anthothoe chilensis* (Lesson, 1830) (Cnidaria, Actiniaria) in Mar del Plata Harbor, Argentina. *Ciencias Marinas* 25, 177–191.

Farris J.S., Kallersjo M., Kluge A.G. and Bult C. (1995) Constructing a significance test for incongruence. *Systematic Biology* 44, 570–572.

Fautin D.G. (2011) *Hexacorallins of the World*. Available at: http://geoporbal.kgs.ku.edu/hexacoral/anenone2/index.cfm (accessed 28 October 2011).

Fautin D.G., Hickman C.P. Jr, Daly M. and Molodtsova T. (2007) Shallow-water sea anemones (Cnidaria: Anthozoa: Actiniaria) and tube anemones (Cnidaria: Anthozoa: Ceriantharia) of the Galápagos Islands. *Pacific Science* 61, 548–573.

Felsenstein J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17, 368–376.

Forsman Z.H., Barshis D.J., Hunter C.L. and Toonen R.J. (2009) Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC Evolutionary Biology* 9, 45.

Forsman Z.H., Guzman H.M., Chen C.A., Fox G.E. and Wellington G.M. (2005) An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): is *S. glynni* endangered or introduced? *Coral Reefs* 24, 343–347.

Försterra G. and Haussermänn V. (2003) First report on large scleractinian (Cnidaria: Anthozoa) accumulations in cold-temperate shallow water of South Chilean fjords. *Zoologische Verhandelingen, Leiden* 345, 117–128.

Fukami H., Chen C.A., Budd A.F., Collins A., Wallace C., Chuang Y., Chen C., Dai C., Iwao K., Sheppard C. and Knowlton N. (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS One* 3, 1–9.

Garese A., Guzmán H.M. and Acuña F.H. (2009) Sea anemones (Cnidaria: Actiniaria and Corallimorpharia) from Panama. *Revista de Biologia Marina y Oceanografia* 44, 791–802.

Genzano G.N., Acuña F.H., Excoffon A.C. and Perez C.D. (1996) Cnidarios bentónicos de la provincia de Buenos Aires. Listado sistemático, distribución y estrategias de colonización. *Actas Jornadas Pampeanas Ciencias Naturales* 6, 113–121.

Gomes P.B. (2002) Anêmonas-do-mar (Cnidaria, Actiniaria) de Pernambuco (Brasil). In Tabarelli M. and Silva J.M.C. (eds) *Diagnóstico da biodiversidade de Pernambuco*. Volume II. Recife, Brazil: SECTMA e Editora Massangana, pp. 343–364.

Gomes P.B., Belém M.J. and Schlenz E. (1998) Distribution, abundance and adaptations of three species of Actiniidae (Cnidaria, Actiniaria) on an intertidal beach rock in Carneiros beach, Pernambuco, Brasil. *Miscellanea Zoologica* 21, 65–72.

Grohmann P.A. (1998) Anêmonas-do-mar (Cnidaria, Anthozoa, Actiniaria) de Vitória, ES. In *XII Congresso Brasileiro de Zoologia, Universidade Federal de Pernambuco*, Brazil, p. 9.

Gusmão L.C. (2010) *Systematics and evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs*. PhD thesis. Ohio State University, United States of America.

Haldane J.B.S. (1954) An exact test for randomness of mating. *Genetics* 52, 631–635.

Hare M.P., Karl S.A. and Avise J.C. (1996) Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. *Molecular Biology and Evolution* 13, 334–345.

den Hartog J.C. (1987) A redescription of the sea anemone *Bunodosoma biscayensis* (Fischer, 1984) (Actiniaria, Actiniidae). *Zoologische Mededelingen* 61, 533–558.

Haussermänn V. (2004) Redescription of *Phymactis papillosa* (Lesson, 1830) and *Phymantea pluvia* (Brayton in Dana, 1846) (Cnidaria: Anthozoa), two actiniid sea anemones from the Southeast Pacific with a discussion of the genera *Phymacti*, *Bunodosoma* and *Phymantea*. *Zoologische Mededelingen, Leiden* 78, 345–381.

Hellberg M.F. (2009) Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution and Systematics* 40, 291–310.

Hillis D.M., Moritz C., Porter C.A. and Baker R.J. (1991) Evidence for biased gene conversion in concerted gene evolution of ribosomal DNA. *Science* 251, 308–310.

Jukes T.H. and Cantor C.R. (1969) Evolution of protein molecules. In Munro H.N. (ed.) *Mammalian protein metabolism*. New York: Academic Press, New York, pp. 21–123.

Kiernan J.A. (1990) *Histological and histochemical methods: theory and practice*. Oxford: Pergamon.

Knowlton N. (2000) Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420, 73–90.

Kumar S., Tamura K. and Nei M. (2004) Mega 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5, 150–163.

Lancellotti D.A. and Vásquez J.A. (1999) Biogeographical patterns of benthic macroinvertebrates in the Sutheastern Pacific littoral. *Journal of Biogeography* 26, 1001–1006.

Lessios H.A. (1992) Testing electrophoretic data for agreement with Hardy–Weinberg expectations. *Marine Biology* 112, 517–523.

Lima D., Freitas J.E.P., Araujo M.E. and Solé-Cava A.M. (2005) Genetic detection of cryptic species in the frill goby *Bathygobius soporator*. *Journal of Experimental Marine Biology and Ecology* 320, 211–223.

Lôbo-Hajdu G., Guimarães A.C., Mendes A.M., Lamarão F.R.M., Vieiralves T., Mansure J.J. and Albano R.M. (2004) Intragenic, intra- and interspecific variation in the rDNA ITS of Porifera revealed by PCR-single-strand comformation polymorphism (PCR-SSCP). *Bolletino dei Musei e degli Instituti Biologici dell'Università di Genova* 68, 413–423.

Manchenko G.P. (1994) *Handbook of detection of enzymes on electrophoretic gels*. Ann Arbor, MI: CRC Press Inc.

Manchenko G.P., Dautova T.N. and Latypov Y.Y. (2000) High level of genetic divergence between sympatric color morphs of the littoral sea anemone *Anthopleura orientalis* (Anthozoa: Actiniaria). *Biochemical Systematics and Ecology* 28, 737–750.

McCommas S.A. (1982) Biochemical genetics of the sea anemone *Bunodosoma cavernata* and the zoogeography of the Gulf of Mexico. *Marine Biology* 68, 169–173.

McCommas S.A. and Lester L.J. (1980) Electrophoretic evaluation of the taxonomic status of two species of sea anemone. *Biochemical Systematics and Ecology* 8, 289–292.

McFadden C.S., Grosberg R.K., Cameron B.B., Karlton D.P. and Secord D. (1997) Genetic relationships within and between clonal and solitary forms of the sea anemone *Anthopleura elegantissima* revisited: evidence for the existence of two species. *Marine Biology* 128, 127–139.

McManus M.G., Place A.R. and Zamer W.E. (1997) Physiological variation among clonal genotypes in the sea anemone *Haliplanella lineata*: growth and biochemical content. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 192, 426–443.

McMurrich J.P. (1904) The Actiniae of the Plate Collection. *Zoologische Jahrbuecher* 6 Supplement, 215–306.

Miller K.J. and Ayre D.J. (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. *Journal of Animal Ecology* 77, 713–724.

Miller M.P. (1997) *Tools for population genetic analysis. Version 1.3*. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Monteiro F.A., Solé-Cava A.M. and Thorpe J.P. (1997) Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. *Marine Biology* 129, 425–433.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590.

Olivera E.G., Patronelli D.L. and Zamponi M.O. (2009) Morphological and functional study of the marginal sphincter of the sea anemones *Phymactis clematis* and *Aulactinia marplatensis* from intertidal of Mar del Plata, Argentina. *Iheringia* 99, 313–316.

Paranhos J.D.N., Pinto S.L., Silva C.B., Amaral L.F.B. and Farias R.R.S. (1999) Levantamento preliminar dos Cnidaria da praia de Barra Grande—PI. In *XII Encontro de Zoologia do Nordeste, Feira de Santana*, Brazil, p. 184.

Patronelli D., Olivera D. and Zamponi M.O. (2005) Influence of intertidal environment on muscle activity in different species of sea anemones (Actiniaria). *Animal Biology* 55, 101–109.

Patronelli D., Olivera E.G., Zamponi M.O. and Crupkin M. (2008) Caracterización morfológica, funcional y bioquímica del esfínter marginal de la anémona de mar *Phymactis clematis* (Cnidaria). *Investigaciones Marinas* 29, 73–77.

Patronelli D., Zamponi M.O., Bustos A. and Vega F. (1987) Morphological adaptations in the marginal sphincter of anemone *Phymactis clematis* Dana, 1849 from different environment. *Biochemical Physiology* 88a, 337–340.

Pax F. (1924) Actiniarien, Zoantharien und Ceriantharien von Curaçao. *Kungliga Zoologisch Genootschap Natura Artis Magistra* 23, 93–122.

Pollero R.J. (1983) Lipid and fatty acid characterization and metabolism in the sea anemone *Phymactis clematis* (Dana). *Lipids* 18, 12–17.

Posada D. and Crandall K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.

Raymond M. and Rousset F. (1995) An exact test for population differentiation. *Evolution* 49, 1280–1283.

Rivadeneira M.M. and Oliva E. (2001) Patrones asociados a la conducta de desplaziamiento local en *Phymactis papillosa* Drayton (Anthozoa: Actiniidae). *Revista Chilena de Historia Natural* 74, 855–863.

Rocha L.A., Bass A.L, Robertson D.R. and Bowen B.W. (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae) *Molecular Ecology* 11, 243–251.

Russo C.A.M. and Solé-Cava A.M. (1991) Genetic variation and differentiation between populations of a tropical sea-anemone (*Bunodosoma caissarum* Corrêa). *Revista de Biologia Tropical* 39, 41–46.

Russo C.A.M., Solé-Cava A.M. and Thorpe J.P. (1994) Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. *Marine Biology* 119, 267–276.

Saitou N. and Nei M. (1987) The neighbor joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406–425.

Schama R., Solé-Cava A.M. and Thorpe J.P. (2005) Genetic divergence between East and West Atlantic populations of *Actinia* sea anemones (Cnidaria: Actiniidae). *Marine Biology* 146, 435–443.

Schmidt H. (1969) Die nesselkapseln der Aktinien und ihre differentialdiagnostische Bedeutung. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 19, 284–317.

Schmidt H. (1972) Prodromus zu einer Monographie der mediterranen Aktinien. *Zoologica* 42, 1–120.

Schmidt H. (1974) On evolution in the Anthozoa. *Proceedings of the Second International Coral Reef Symposium* 1, 533–560.

Sebens K. and Paine R. (1978) Biogeography of anthozoans along the West coast of South America: habitat, disturbance, and prey availability. *Proceedings of the International Symposium of Marine Biogeography and Evolution of South Hemisphere* 1, 219–237.

Sneath P.H.A. and Sokal R.R. (1973) *Numerical taxonomy*. San Francisco, CA: W.H. Freeman Press.

Solé-Cava A.M. and Thorpe J.P. (1989) Biochemical correlates of genetic variation in marine lower invertebrates. *Biochemical Genetics* 27, 303–312.

Solé-Cava A.M., Thorpe J.P. and Kaye J.G. (1985) Reproductive isolation with little genetic divergence between *Urticina* (= *Tealia*) *felina* and *U. eques* (Anthozoa: Actiniaria). *Marine Biology* 85, 279–284.

Stephenson T.A. (1935) *The British sea anemones*. Volume 2. London: The Ray Society.

https://doi.org/10.1017/S0025315411002049 Published online by Cambridge University Press

Stoletzki N. and Schierwater B. (2005) Genetic and color morphs differentiations in the Caribbean sea anemone *Condylactis gigantea*. *Marine Biology* 147, 747–754.

Stotz W.B. (1979) Functional morphology and zonation of three species of sea anemones from rocky shores in Southern Chile. *Marine Biology* 50, 181–188.

Swofford D.L. (2000) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Sunderland, MA: Sinauer Associates.

Swofford D.L. and Selander R.B. (1981) BIOSYS-1, a FORTRAN programme for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72, 281–283.

Thompson J.D., Higgins D.G. and Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.

Thorpe J.P. and Solé-Cava A.M. (1994) The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta* 23, 3–18.

Valentin J.L. (2000) *Ecologia numérica: uma introdução à análise multivariada de dados ecológicos*. Rio de Janeiro: Interciência LTDA.

Vianna P., Schama R. and Russo C.A.M. (2003) Genetic divergence and isolation by distance in the West Atlantic sea anemone *Actinia bermudensis* (McMurrich, 1889). *Journal of Experimental Marine Biology and Ecology* 297, 19–30.

Ward R.D. and Beardmore J.A. (1977) Protein variation in the plaice (*Pleuronectes platessa*). *Genetic Research* 30, 45–62.

Watzl O. (1922) Die Actiniarien der Bahamainseln. *Arkiv för Zoologi* 14, 1–89.

Zamponi M.O. (1977) La anemofauna de Mar del Plata y localidades vecinas. I. Las anemonas Boloceroidaria y Endomyaria (Coelentherata: Actiniaria). *Acta Neotrópica* 23, 137–153.

Zamponi M.O. (1989) *Los Cnidaria y su interaccion pelagico-bentonica*. PhD thesis. Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

Zamponi M.O. (1993) El ambiente intermareal subtemplado frio como un posible «pool» de tipos reproductivos. *Physis—A* 51, 13–15.

Zamponi M.O. (2000) El estuario del Río de La Plata: una barrera geográfica para los cnidarios bentónicos marinos? *Biociências* 8, 127–136.

Zamponi M.O. (2005) Estudio de la reproducción sexual de las anémonas de mar (Actiniaria) y la estrategia del hombre pobre (the poor man's game). *Revista Real Academia Galega de Ciencias* 24, 5–28.

Zamponi M.O. and Perez C.D. (1996) Comparative morphological study of different species of Actiniaria between the intertidal zone from Mar del Plata and Santa Clara del Mar (Argentine). I. *Phymactis papillosa* Dana, 1849 (Anthozoa, Actiniidae). *Biociências* 4, 91–102.

Zamponi M.O., Belém M.J.C., Schlenz E. and Acuña F.H. (1998a) Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic Coast. *Physis—A* 55, 31–45.

Zamponi M.O., Genzano G.N., Acuña F.H. and Excoffon A.C. (1998b) Studies of benthic cnidarian populations along a transect off Mar del Plata (Argentina). *Russian Journal of Marine Biology* 24, 7–13.

and

Zouros E. and Foltz D.W. (1984) Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia* 25, 583–591.

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