



Integrative taxonomy of calcareous sponges (subclass Calcinea) from the Peruvian coast: morphology, molecules, and biogeography

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Received 6 May 2014; revised 2 September 2014; accepted for publication 7 September 2014

Understanding of evolution and systematics of Calcarea (Porifera) have not yet met a corresponding increase in the knowledge of diversity and distribution of these sponges in several parts of the world. Peru is an emblematic example of this lack of taxonomic knowledge, as only three shallow-water species of sponges have hitherto been reported from its 3000 km coast. With the aim of studying sponges of Peru, an integrative taxonomy approach (morphology, molecules, and biogeography) was used in order to achieve sound species identifications. The first findings of Peruvian calcareous sponges are presented here. Eight species are described in the subclass Calcinea, of which five are new to science. The retrieved biogeographical patterns are either locally endemic, widespread, or discontinuous over large areas. *Clathrina antofagastensis* was previously known from Chile, while *C. aurea* and *Ernstia tetractina* had been reported from the Atlantic (Brazil), and thus represent the first genetically confirmed tropical ampho-American distributions of species not yet found on both sides of the Isthmus of Panama. Our results reveal a richer Tropical East Pacific sponge fauna than the Warm Temperate South-Eastern Pacific one.

© 2015 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2015, 173, 787–817.
doi: 10.1111/zoj.12213

ADDITIONAL KEYWORDS: internal transcribed spacer – marine biodiversity – molecular systematics – Pacific Ocean – Peru – Porifera.

INTRODUCTION

Integrative taxonomy involves the use of multiple and complementary disciplines (morphology, and molecular or chemical taxonomy) to evaluate the statuses of taxa (Dayrat *et al.*, 2008; Schlick-Steiner *et al.*, 2010). Today, it appears to be the best approach for producing reliable systematics and phylogenetic studies, and achieve a more natural system of classification (Padial

et al., 2010). This is especially so when organisms have a limited number of easily assessable characters or possess markedly changing morphologies, as is the case for sponges (Dohrmann *et al.*, 2008, 2011, 2012; Rossi *et al.*, 2011; Cárdenas, Pérez & Boury-Esnault, 2012; Gazave *et al.*, 2012; Voigt, Wülfing & Wörheide, 2012; Klautau *et al.*, 2013).

In the molecular era, the first studies on calcareous sponges performed under an integrative perspective aimed to verify the taxonomic status of allegedly cosmopolitan species (Solé-Cava *et al.*, 1991; Klautau, Solé-Cava & Borojevic, 1994; Valderrama *et al.*, 2009).

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However, phylogenetic relationships soon started to become investigated (Manuel *et al.*, 2003, 2004; Rossi *et al.*, 2011; Voigt *et al.*, 2012). In a recent study, species and genera belonging to the order Clathrinida (subclass Calcinea) were re-evaluated based on morphological and molecular data, which resulted in the genera *Ascandra* and *Clathrina* being re-defined, and the erection of four new genera (i.e. *Arthuria*, *Borojevia*, *Brattegardia*, and *Ernstia*; Klautau *et al.*, 2013). In addition, some species of *Guancha*, with triactines and no tetractines, were transferred to *Clathrina*, in spite of their possession of a peduncle (Klautau *et al.*, 2013). An integrative taxonomy approach was also used to strengthen the description of four *Clathrina* species from the Adriatic Sea (Imešek *et al.*, 2013). Somewhat contradictorily, recent advances in understanding the evolution and systematics of Calcarea by applying molecular tools have not yet resulted in a corresponding increase in the knowledge of diversity and distribution of calcarean species in several parts of the world. Peru represents a clear example of this lack of taxonomic information, not just for Calcarea, but also for the entire phylum Porifera, with only three shallow-water species described from its coast.

In response to future needs and challenges in studying marine biodiversity, several international efforts (e.g. Global Taxonomy Initiative) have started with the purpose of strategically inventorying particular underestimated taxa, filling the gaps in regions considered to be poorly known, and developing new taxonomic expertise in countries where it is weak or non-existent (Costello *et al.*, 2010; van Soest *et al.*, 2012; Hooper *et al.*, 2013). The present study is the result of one of these collaborations.

The Peruvian coast (south-east Pacific) holds one of the four major upwelling systems in the world, the Humboldt Current System, which is characterized by its moderate to extremely high primary productivity, and its particularly productive fisheries (Bakun & Weeks, 2008; Chavez & Messié, 2009). According to the Marine Ecoregions of the World system (Spalding *et al.*, 2007), the Peruvian marine ecosystem stretches over two biogeographical provinces, the southern part of the Tropical East Pacific (TEP), which extends from 3 to 6°S, and the northern sector of the Warm Temperate South-eastern Pacific (WTSP), which extends from 6 to 42°S. However, there are no sharp boundaries between biogeographical provinces and at a finer resolution; adjacent biotas may share a transition zone with considerable overlap (Spalding *et al.*, 2007). In addition, the Peruvian ecosystem is subject to high interannual environmental variability caused by the El Niño Southern Oscillation (ENSO), which causes temporary changes in the composition and abundance of biological communities (Paredes *et al.*, 1998;

Tarazona & Valle, 1998; Tarazona *et al.*, 2003; Paredes, Cardoso & Tarazona, 2004; Chavez *et al.*, 2008).

Traditionally, the most investigated marine fauna comprises groups of animals with economic value, and this is also the case in Peru (Tarazona *et al.*, 2003). One exception relates to the Polychaeta (Tarazona, 1974), which is one of the taxonomically best known benthic animals in Peru (Costello *et al.*, 2010; Miloslavich *et al.*, 2011). By contrast, sponges are one of the least studied groups of animals in Peru, despite their numerous functional and structural roles in marine ecosystems (e.g. provision of microhabitat, release of chemicals, enhancement of primary production; Wulff, 2001; Cerrano *et al.*, 2006; Bell, 2008; de Goeij *et al.*, 2013). With more than 3000 km of shoreline, Peru still represents a large hiatus in the South American sponge biodiversity inventory, with only 14 species reported; five Demospongiae from shallow waters (Hyatt, 1877; van Soest, Hooper & Hiemstra, 1991; Desqueyroux-Faúndez & van Soest, 1996; Aguirre *et al.*, 2011), and nine Hexactinellida, all from the deep sea (von Lendenfeld, 1915). No Calcarea have ever been reported from the Peruvian coast.

The aim of this paper was to reduce this lack of knowledge, by determining the species composition and geographical range of calcinean calcareous sponges (subclass Calcinea) from Peru's shallow waters using an integrative taxonomic approach. This goal was pursued by an international cooperation set up in 2007 amongst Belgium (RBINS), Brazil (MNRJ), Peru (UPCH), and Switzerland (MHNG), in the form of two research projects: ESponjas del PERÚ and Capacitação para Pesquisa em Taxonomia, Filogenia e Biogeografia de Poríferos da América do Sul. The first findings of Peruvian calcareous sponges are presented here.

MATERIAL AND METHODS

SAMPLING PROCEDURE

A total of 43 specimens of calcareous sponges (subclass Calcinea) was collected in 28 of the 90 localities investigated along the Peruvian coast, from Tumbes (3°S) to Tacna (18°S) (Fig. 1, Table 1). Several investigated localities are within protected areas (i.e. Paracas National Reserve; Guano Islands and Capes National Reserve System; Tumbes Mangroves National Sanctuary) or identified as priorities for nature conservation (Foca Island; El Nuro; Punta Sal). Consequently, this study received the support of the Protected Natural Areas National Service (SERNANP) to access several sites and in obtaining permits for scientific studies. Samples were collected between 2007 and 2009 at depths varying from the intertidal zone to 30 m by scuba diving. Sponges were photographed *in situ*, along with a 5-cm scale bar and an inventory number. Depth and water temperature were recorded by photographing depth gauges at the time of each sampling.

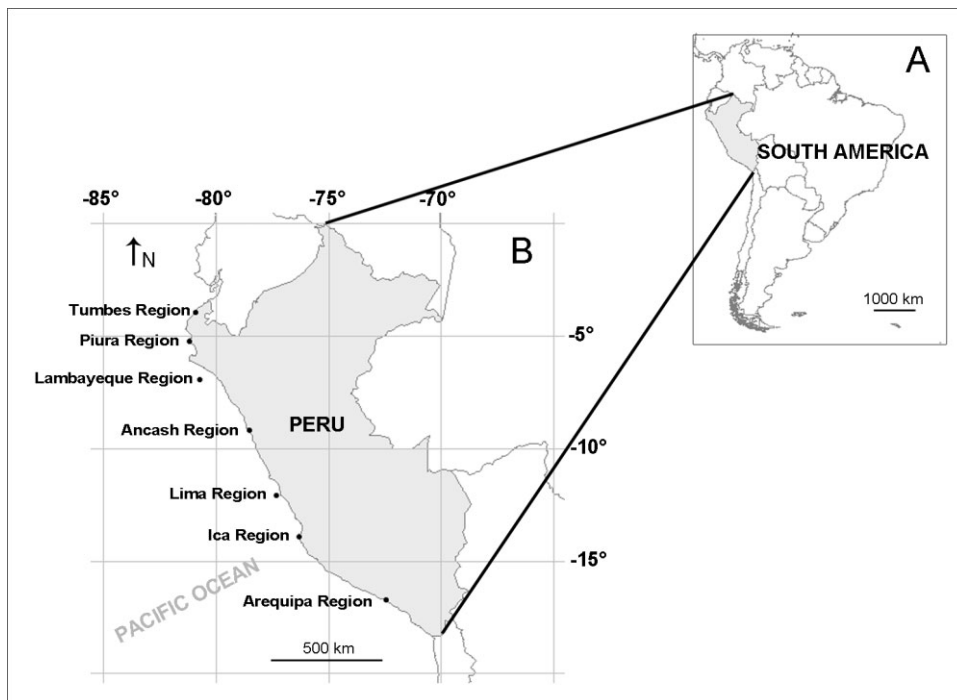


Figure 1. Location of Peru and its seven main regions along the coast.

SPECIMEN PREPARATION

After collection, samples were stored in 96% ethanol. All measurements of the cormus were taken on preserved specimens. Preparations of spicules and sections followed standard procedures (Wörheide & Hooper, 1999; Klautau & Valentine, 2003). Additionally, for scanning electron microscopy (SEM), spicules were spread on cover-slips, mounted on aluminium stubs with silver glue, and sputtered with gold. Sections or *in toto* preparations were photographed with a Canon G11 digital camera mounted on a Zeiss Axioscop optical microscope. Images of preserved whole specimens were taken either with a Zeiss AxioCam ERc 5s camera mounted on a Zeiss Stemi 2000-C dissecting microscope or with a Canon G11 camera. SEM digital micrographs were recorded at the Royal Belgian Institute of Natural Sciences on a FEI/Philips XL30 ESEM scanning electron microscope at 20 to 30 kv. Spicule length and width at the base of each actine were measured (μm) with an ocular micrometer. Data are presented in tabular form, featuring length [minimum, mean, SD, and maximum], width [mean and (SD)] and number of spicules (*N*). Identifications followed the Systema Porifera classification (Borojevic *et al.*, 2002), the revision of the genus *Clathrina* (Klautau & Valentine, 2003), and the new taxonomic proposal for the order Clathrinida (Klautau *et al.*, 2013).

When sufficiently abundant, specimens were fragmented and deposited in the sponge collections of the Universidad Peruana Cayetano Heredia (CZA), Peru;

the Museu Nacional/UFRJ (MNRJ), Brazil; and the Royal Belgian Institute of Natural Sciences (RBINS), Belgium. Additionally, dissociated spicules, preserved in ethanol 93% and dried on SEM stubs, were deposited at RBINS.

DNA EXTRACTION, PCR, SEQUENCING, AND ALIGNMENT

Total DNA was extracted with the guanidine/phenol-chloroform protocol (Lôbo-Hajdu *et al.*, 2004) or with a QIAampR DNA MiniKit (Qiagen). The region comprising partial 18S and 28S, the two spacers [internal transcribed spacer 1 (ITS1) and ITS2], and the 5.8S ribosomal DNA was amplified by PCR with the following primers: 18S (5'-TCATTTAGAGGAAGTAAAGTCG-3') and 28S (5'-GTTAGTTTCTTTTCCTCGCTT-3') (Lôbo-Hajdu *et al.*, 2004). PCR mixes contained: 1 \times buffer (5 \times GoTaq Green Reaction Buffer Flexi, Promega), 0.2 mM deoxyribonucleotide triphosphate, 2.5 mM MgCl_2 , 0.5 $\mu\text{g } \mu\text{L}^{-1}$ bovine serum albumin, 0.33 μM of each primer, one unit of Taq DNA polymerase (Fermentas or Bionline), and 1 μL DNA, made up to 15 μL with Milli-Q water. PCR steps included one first cycle of 4 min at 94 $^\circ\text{C}$, 1 min at 50 or 52 $^\circ\text{C}$, and 1 min at 72 $^\circ\text{C}$, 35 cycles of 1 min at 92 $^\circ\text{C}$, 1 min at 50 or 52 $^\circ\text{C}$, and 1 min at 72 $^\circ\text{C}$, and a final cycle of 6 min at 72 $^\circ\text{C}$. The quality and quantity of the PCR products were visualized by electrophoresis in 1.5% agarose gel. Purification was carried out using a GFX

Table 1. List of the 90 investigated localities with their geographical coordinates Localities where no *Calcinea* were found are marked with *

Locality	Decimal latitude	Decimal longitude
Rocas La Chavelera	-3.9206	-80.9083
Cancas	-3.9421	-80.9438
*Baja Guaraguau	-3.9422	-80.9462
*Cancas Pier	-3.9439	-80.9404
In front of Punta Sal	-3.9333	-80.9333
*In front of Punta Sal, site 2	-3.9452	-80.9463
*Baja de la Antena	-3.9501	-80.9619
*Club Punta Sal Anchorage	-3.9539	-80.9580
*La Antena	-3.9543	-80.9661
*Baja de Diego	-3.9605	-80.9729
*Resort Anchorage	-3.9678	-80.9693
Baja El Burro	-3.9761	-80.985
Punta Sal	-3.9814	-80.9891
*Punta Sal Resort	-3.9841	-80.9865
*Mancora Beach, 'El Point'	-4.1058	-81.0558
*Mancora Pier	-4.1102	-81.0673
El Ñuro site 1, El Ñuro Pier	-4.2167	-81.2139
*El Ñuro site 2, 'Puerto Rico'	-4.2203	-81.1986
*El Ñuro site 3	-4.2223	-81.2013
*El Ñuro site 4, north of Quebrada Verde	-4.2229	-81.2067
*El Ñuro site 5, Baja of Quebrada Verde	-4.2243	-81.2059
El Ñuro site 6, south of Quebrada Verde	-4.2251	-81.2088
El Ñuro site 7, south of Quebrada Verde	-4.2336	-81.2128
*Foca Island site 1	-5.1955	-81.2161
*Foca Island site 2, 'Bajo Norte'	-5.2008	-81.2087
*Foca Island site 3	-5.2019	-81.2083
*Foca Island site 4, 'La Cabrillera'	-5.2026	-81.2111
*Foca Island site 5, 'Islilla'	-5.2048	-81.2068
*Sechura Bay site 1, 'Mantacaballo-Chullachi'	-5.5575	-80.954
*Sechura Bay site 2, 'Mantacaballo-Chullachi'	-5.5728	-80.9358
*Sechura Bay site 3, 'Mantacaballo-Peña Negra'	-5.6147	-80.8412
Sechura Bay site 4	-5.74	-80.9516
*Sechura Bay site 5, 'Puerto Rico'	-5.7804	-81.0688
Sechura Bay site 6, 'Puerto Rico'	-5.7805	-81.068
*Sechura Bay site 7	-5.7931	-80.9524
*Sechura Bay site 8	-5.8409	-80.9524
San Cristobal	-6.9146	-80.7155
Santo Domingo Islet	-6.9194	-80.7359
*El Moño	-6.9198	-80.7131
*Bajo El Chile	-6.9217	-80.7204
*Cristo Salva	-6.9227	-80.7085
San José Inlet	-6.9301	-80.7212
*Independence Bay	-6.9314	-80.7240
Ladrón Bay	-6.9335	-80.7163
El Lagarto Islet	-6.9337	-80.7055
*El Callejón	-6.9340	-80.7189
*Callejón Lagartos	-6.9369	-80.7051
*Macabí Island	-7.8088	-79.4974
*Guañape Island North	-8.5295	-78.9644
*Guañape Island South	-8.5596	-78.9640
*Blanca Island	-9.1020	-78.6130
*Ferrol Islands	-9.1543	-78.6169

Table 1. *Continued*

Locality	Decimal latitude	Decimal longitude
*Colorada Inlet	-9.1863	-78.3892
North of Colorada Inlet	-9.1947	-78.5392
Punta Zamora	-9.2161	-78.5528
*Tortuga Bay	-9.3674	-78.4253
Tortuga Island	-9.3771	-78.439
*Don Martin Island	-11.0196	-77.6704
*San Lorenzo Island site 1	-12.0645	-77.2389
San Lorenzo Island site 2	-12.0671	-77.2292
*San Lorenzo Island site 3	-12.0680	-77.2529
Chilca Island, Pucusana	-12.472	-76.7984
* Grano de Oro site 1, Pucusana	-12.4887	-76.7982
* Grano de Oro site 2, Pucusana	-12.4889	-76.7995
*San Gallan Island site 1	-13.8387	-76.4682
*San Gallan Island site 2	-13.8186	-76.4555
*Candelabro	-13.8274	-76.3021
Lagunillas Beach	-13.8957	-76.3153
Roquedal, Laguna Grande	-14.1533	-76.2504
Bocana, Laguna Grande	-14.1586	-76.2489
Vieja Island, Independence Bay	-14.2898	-76.1746
Santa Rosa Island site 1	-14.3195	-76.1646
Santa Rosa Island site 2	-14.3198	-76.1584
*El Marmol Inlet	-15.3549	-75.1834
*La Baja	-15.3699	-75.2053
*Punta San Juan	-15.3676	-75.1884
*El Avion Islet	-15.3906	-75.1792
Las Tres Hermanas	-15.4423	-75.0708
*Inlet north of Quilca	-16.7017	-72.4483
*Farallon Islet	-16.7395	-72.4199
*Ancupita Inlet	-16.8370	-72.2912
*Punta Hornillos	-16.8805	-72.2884
Las Tres Hermanas	-15.4423	-75.0708
*Blanca Island	-17.0088	-72.1222
*Catarindo Beach	-17.0191	-72.0342
*ENAPU Pier	-17.6454	-71.4846
*Mocho Tres Hermanos	-17.6537	-71.3592
*Puerto Ingles	-17.6639	-71.3583
*Punta Coles, site 1	-17.7000	-71.3808
*Punta Coles, site 2	-17.7052	-71.3780

ENAPU, Empresa Nacional de Puertos S.A.

PCR DNA kit (code: 28–9034-71, GE Healthcare) and the sequencing reaction was performed using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (part no.: 4336917, Applied Biosystems). Forward and reverse strands were automatically sequenced in ABI 3500 (Applied Biosystems). The sequences obtained were edited using the program CHROMAS LITE 2.01 (available from <http://www.technelysium.com.au>) and BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/>) were conducted to confirm their biological source. Sequences were aligned using the Q-INS-i option of the MAFFT v. 7.024b

program (Katoh & Standley, 2013), with a scoring matrix of 200 point accepted mutation/ $k = 2$, gap penalty = 1.53, and offset value = 0. All sequences generated in this work have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov>). Comparative sequences were obtained from the GenBank database (Table 2).

PHYLOGENETIC ANALYSES

Phylogenetic reconstructions were performed using two different methods: maximum likelihood (ML) and

Table 2. Some of the species used in this study with collection sites, voucher numbers, and GenBank accession numbers of DNA sequences

Species	Collection site	Voucher number	GenBank accession number (ITS)
<i>Arthuria hirsuta</i>	Cabo Verde – CV	ZMAPOR 07061	KC843431
<i>Arthuria hirsuta</i>	Cabo Verde – CV	ZMAPOR 07103	KC985143
<i>Arthuria spirallata</i> sp. nov.	Peru – PE	MNRJ 11414	KC985142
<i>Arthuria spirallata</i> sp. nov.	Peru – PE	MNRJ 13652	KC985140
<i>Arthuria spirallata</i> sp. nov.	Peru – PE	MNRJ 12864	KC985141
<i>Arthuria spirallata</i> sp. nov.	Peru – PE	MNRJ 12860	KC985139
<i>Borojevia aspina</i>	Brazil – BR	UFRJPOR 5245	HQ588998
<i>Borojevia brasiliensis</i>	Brazil – BR	UFRJPOR 5214	HQ588978
<i>Borojevia cerebrum</i>	Mediterranean Sea – MS	UFRJPOR 6324	HQ588975
<i>Clathrina antofagastensis</i>	Chile – CH	MNRJ 9289	HQ588985
<i>Clathrina antofagastensis</i>	Peru – PE	MNRJ 11294	KF002722
<i>Clathrina antofagastensis</i>	Peru – PE	MNRJ 13674	KF002723
<i>Clathrina antofagastensis</i>	Peru – PE	MNRJ 13131	KF002721
<i>Clathrina aurea</i>	Brazil – BR	UFRJPOR 5170	HQ588960
<i>Clathrina aurea</i>	Brazil – BR	MNRJ 8990	HQ588958
<i>Clathrina aurea</i>	Peru – PE	MNRJ 13138	KC985132
<i>Clathrina aurea</i>	Peru – PE	MNRJ 13124	KC985131
<i>Clathrina conifera</i>	Brazil – BR	MNRJ 8991	HQ588959
<i>Clathrina coriacea</i>	Norway – NO	UFRJPOR 6330	HQ588986
<i>Clathrina cylindractina</i>	Brazil – BR	UFRJPOR 5413	HQ588993
<i>Clathrina fjordica</i>	Chile – CH	MNRJ 8143	HQ588984
<i>Clathrina aphrodita</i> sp. nov.	Peru – PE	MNRJ 12994	KC985138
<i>Clathrina aphrodita</i> sp. nov.	Peru – PE	MNRJ 14180	KC985137
<i>Clathrina nuroensis</i> sp. nov.	Peru – PE	MNRJ 13032	KC985136
<i>Clathrina peruana</i> sp. nov.	Peru – PE	MNRJ 13144	KC985134
<i>Clathrina peruana</i> sp. nov.	Peru – PE	MNRJ 12839	KC985135
<i>Clathrina peruana</i> sp. nov.	Peru – PE	MNRJ 13127	KC985133

ITS, internal transcribed spacer.

Bayesian inference (BI). ML analysis was performed using PhyML 3.0 (Guindon *et al.*, 2010; available online at <http://www.atgc-montpellier.fr/phyml>), with a general time-reversible (GTR) substitution model and 1000 bootstrap pseudoreplicates. The search for the best evolutionary substitution model was carried out by MODELTEST (Posada & Crandall, 1998), available in the program HyPhy (Pond, Frost & Muse, 2005), including the hierarchical test and the Akaike information criterion. BI was conducted using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), also considering the GTR model, 1 000 000 generations and a burn-in of 100 000. In both phylogenetic reconstructions, a set of three species of *Borojevia* was included as the outgroup.

INSTITUTIONAL ABBREVIATIONS

MHNG, Muséum d'Histoire Naturelle, Geneva (Switzerland); MNHN, Muséum national d'Histoire naturelle, Paris (France); MNRJ, Museu Nacional, Universidade

Federal do Rio de Janeiro, Rio de Janeiro (Brazil); RBINSc, Royal Belgian Institute of Natural Sciences, Brussels (Belgium); UFRJPOR, Department of Zoology, Institute of Biology, Universidade Federal do Rio de Janeiro, Rio de Janeiro (Brazil); CZA, Colección de Zoología Acuática, Universidad Peruana Cayetano Heredia, Lima, (Peru).

SYSTEMATICS

CLASS CALCAREA BOWERBANK, 1864

SUBCLASS CALCINEA BIDDER, 1898

ORDER CLATHRINIDA HARTMAN, 1958

FAMILY CLATHRINIDAE MINCHIN, 1900

GENUS *ARTHURIA* KLAUTAU *ET AL.*, 2013

Diagnosis: Calcinea in which the cormus comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) triactines and tetractines. However, tetractines are more rare. Diactines may be added. Asconoid aquiferous system.

ARTHURIA SPIRALLATA SP. NOV. (FIGS 2, 3; TABLE 3)

Type material: Holotype (ethanol). MNRJ 11414 – fragments from the holotype: RBINSc-IG 32239-POR 11414, CZA 11414, and MHNG 85411. Paratypes (ethanol). MNRJ 11397 – fragments from the paratype: RBINSc-IG 32239-POR 11397, CZA 11397 and MHNG 85395. MNRJ 12860 – fragment from the paratype: RBINSc-IG 32240-POR 12860 and CZA 12860. MNRJ 16745

– fragment from the paratype: RBINSc-IG 32239-POR 16745.

Material examined: Six specimens. MNRJ 11397; San Cristobal, Lobos de Afuera Islands, Guano Islands and Capes National Reserve System (06°54'52.50"S, 80°42'55.90"W); collected by Ph. Willenz and Y. Hooker; 13 m depth; 07.x.2007. MNRJ 11414; San José Inlet,

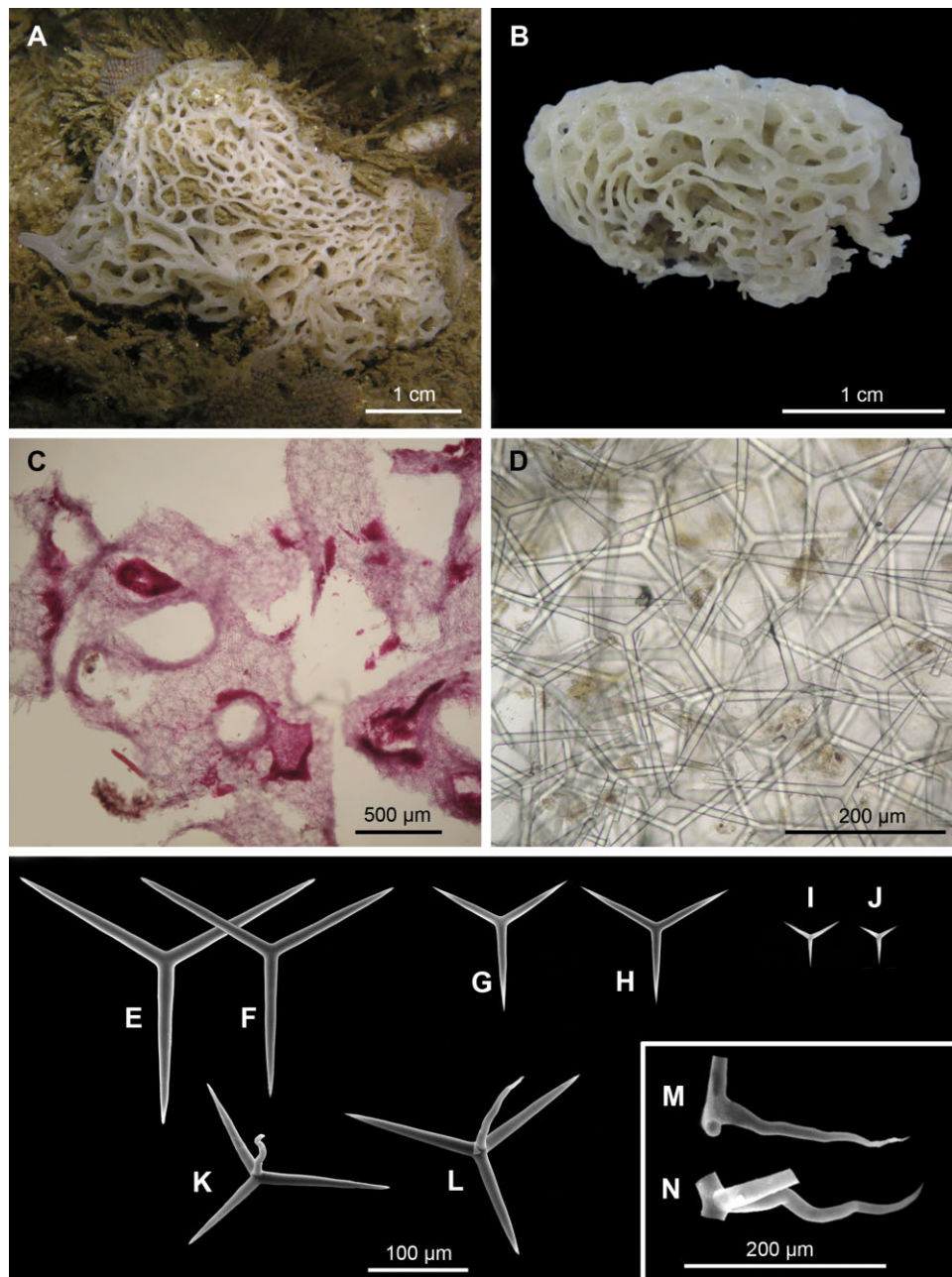


Figure 2. *Arthuria spirallata* sp. nov. (Holotype – MNRJ 11414). A, live specimen (contraction state); B, specimen in ethanol; C, cormus anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E-F, triactine I; G-H, triactine II; I, J, triactine III; K-L, tetractines; M-N, spiraled apical actine of tetractines.

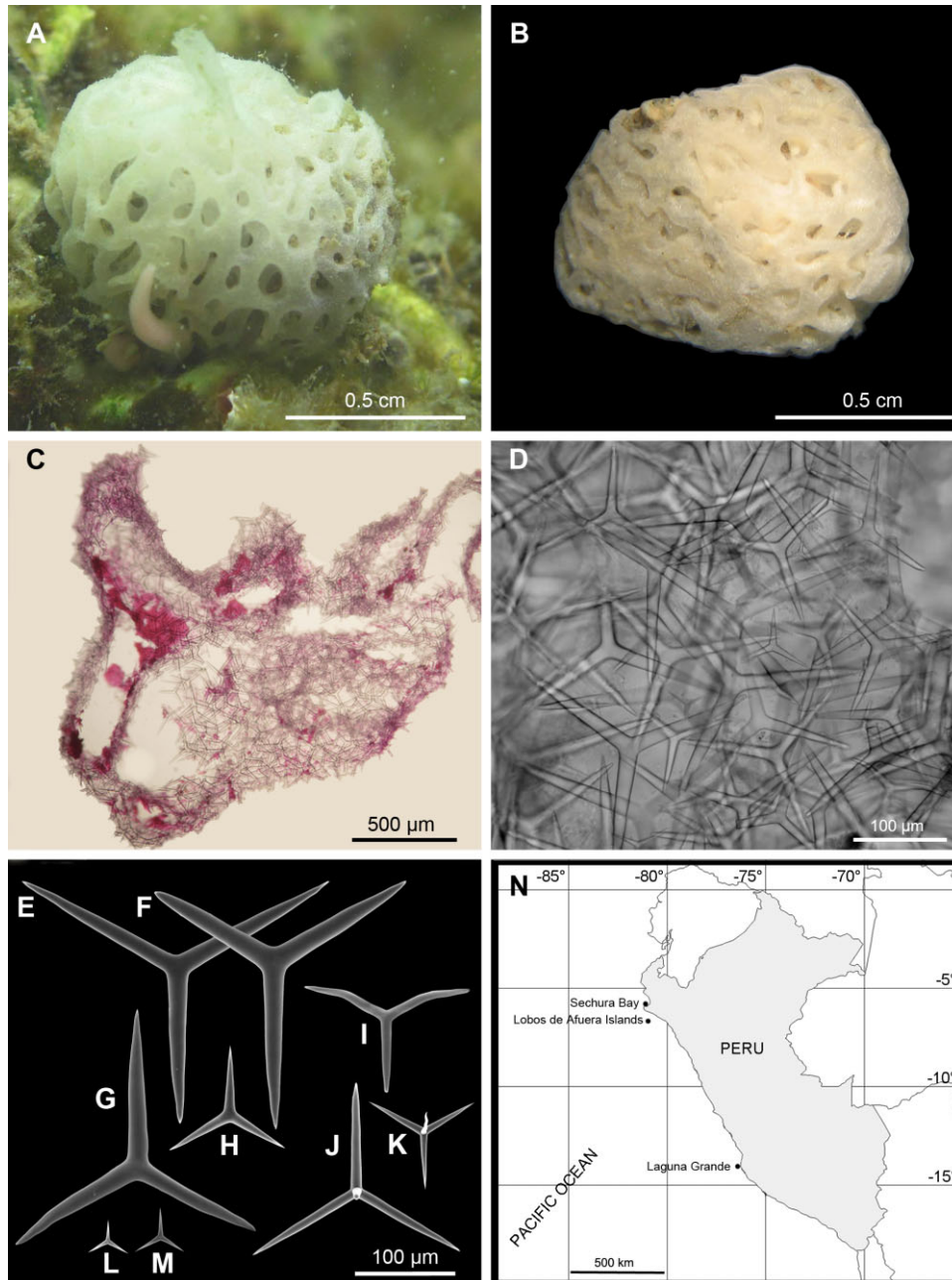


Figure 3. *Arthuria spirallata* sp. nov. (Paratype – MNRJ 12860). A, live specimen; B, specimen in ethanol; C, cornus anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E–G, triactine I; H, triactine II; I, sagittal triactine; J–K, tetractines; L–M, triactine III; N, distribution map along the Peruvian coast.

Lobos de Afuera Islands, Guano Islands, and Capes National Reserve System (06°55'48.50"S, 80°43'16.20"W); collected by Y. Hooker; 9 m depth; 09.x.2007. MNRJ 12860, MNRJ 12864; Roqedal, Laguna Grande, Paracas National Reserve, Ica Region (14°09'11.80"S, 76°15'01.30"W); collected by Y. Hooker and Ph. Willenz; 1–2 m depth; 12.xii.2008. MNRJ 13652; Sechura Bay

site 4, Parachique, Piura Region (05°44'24.10"S, 80°57'05.60"W); collected by Y. Hooker and Ph. Willenz; 19 m depth; 07.xii.2009. MNRJ 16745; El Lagarto Islet, Lobos de Afuera Islands, Guano Islands, and Capes National Reserve System (06°56'01.20"S, 80°42'19.90"W); collected by E. Hajdu; 12 m depth; 04.x.2007.

Table 3. Spicule measurements of *Arthuria spirallata* sp nov.

Specimen	Spicule	Actine	Length (µm)				Width (µm)		N
			Min.	Mean	SD	Max.	Mean	SD	
MNRJ 11414	Triactines I	Basal	132.5	166.4	15.9	190.0	17.8	2.8	30
	Triactines II	Basal	95.0	141.9	16.5	170.0	11.9	1.3	30
	Triactines III	Basal	32.5	57.7	12.8	75.0	6.9	1.2	30
	Tetractines	Basal	110.0	140.0	14.0	157.5	12.1	1.7	15
MNRJ 11397		Apical	50.0	57.5	7.5	65.0	8.3	0.7	3
	Triactines I	Basal	125.0	141.9	10.8	160.0	15.7	0.9	27
	Triactines II	Basal	97.5	116.4	10.9	137.5	10.9	1.1	30
	Triactines III	Basal	45.0	55.5	11.7	80.0	6.6	1.3	30
	Tetractines	Basal	70.0	110.4	30.6	142.5	11.0	1.7	6
MNRJ 16745		Apical	52.0	62.4	10.4	72.8	5.2	1.3	3
	Triactines I	Basal	125.0	156.3	16.1	175.0	15.4	0.9	16
	Triactines II	Basal	120.0	130.9	9.2	150.0	10.7	1.0	30
	Triactines III	Basal	47.5	62.3	8.3	75.00	7.7	0.9	30
	Tetractines	Basal	62.5	115.0	24.9	145.00	10.5	1.9	30
MNRJ 12860		Apical	55.0	66.3	15.9	77.5	8.1	0.9	2
	Triactines I	Basal	132.6	153.7	9.4	176.8	18.3	1.2	30
	Triactines II	Basal	88.4	121.1	15.0	148.2	13.3	1.6	30
	Triactines III	Basal	28.6	64.7	15.7	93.6	9.4	2.0	30
	Tetractines	Basal	88.4	123.1	20.5	158.6	15.6	2.2	14
MNRJ 12864		Apical	–	–	–	–	–	–	–
	Sagittal triactines	Unpaired	85.8	117.6	26.9	161.2	11.6	2.5	9
		Paired	78.0	105.7	14.4	124.8	11.6	2.6	9
	Sagittal tetractines	Unpaired	31.2	88.0	38.9	150.8	9.5	4.0	6
		Paired	39.0	82.8	31.9	132.6	10.0	3.8	6
	Triactines I	Basal	104.0	127.6	9.4	143.0	15.2	1.2	30
	Triactines II	Basal	93.6	119.0	10.8	137.8	12.7	1.0	30
	Triactines III	Basal	36.4	57.5	12.2	80.6	8.4	1.4	30
	Tetractines	Basal	109.2	111.8	3.7	114.4	13.7	0.9	2
		Apical	–	–	–	–	–	–	–
MNRJ 13652	Sagittal triactines	Unpaired	91.0	123.8	17.8	145.6	12.4	1.9	10
		Paired	85.8	108.9	12.5	122.2	11.6	1.6	10
	Sagittal tetractines	Unpaired	62.4	90.7	11.6	111.8	9.2	1.0	18
		Paired	59.8	84.2	11.0	111.8	9.0	1	18
	Triactines I	Basal	98.8	131.2	18.9	184.6	15.1	1.2	30
	Triactines II	Basal	96.2	119.8	10.8	143.0	10.6	1.3	30
	Triactines III	Basal	36.4	58.8	12.0	83.2	7.8	1.4	30
	Tetractines	Basal	70.2	103.4	19.6	135.2	11.6	2.0	9
All specimens		Apical	–	–	–	–	–	–	–
	Sagittal triactines	Unpaired	70.2	116.3	24.5	161.2	12.2	1.8	30
		Paired	65.0	106.0	22.4	156.0	11.9	1.8	30
	Sagittal tetractines	Unpaired	52.0	87.4	18.9	124.8	9.6	1.9	30
		Paired	49.4	80.1	18.2	122.2	9.2	1.7	30
	Triactines I	Basal	98.8	146.2	15.2	190.0	16.3	1.4	–
	Triactines II	Basal	88.4	124.9	9.7	170.0	11.7	1.1	–
	Triactines III	Basal	28.6	59.4	3.4	93.6	7.8	1.0	–
	Tetractines	Basal	62.5	117.3	12.8	158.6	12.4	1.9	–
		Apical	50.0	62.1	4.4	77.5	7.2	1.7	–
Sagittal triactines	Unpaired	70.2	119.2	4.0	161.2	12.1	0.4	–	
	Paired	65.0	106.9	1.8	156.0	11.7	0.2	–	
Sagittal tetractines	Unpaired	31.2	88.7	1.8	150.8	9.4	0.2	–	
	Paired	39.0	82.4	2.1	132.6	9.4	0.5	–	

Type locality: San José Inlet, Lobos de Afuera Islands, Guano Islands, and Capes National Reserve System, Peru.

Colour: Opaque white or translucent light beige in life and beige in ethanol.

Etymology: Named from the Latin *spira*, meaning spiral. Refers to the shape of the apical actine of most tetractines.

Description: Sponge thickly encrusting or massive (3.0 × 1.5 × 0.8 cm) (Figs 2A–C, 3A–C). The massive forms are frequently spherical. Consistency is compressible. Cormus is formed by irregular and frequently tightly anastomosed tubes (0.5–1.0 mm). Water-collecting tubes are present, but in some specimens there is only a single osculum. Granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Without any special organization. It is composed of three size categories of triactines and one size category of tetractines; however, tetractines are very rare (Figs 2D, 3D). Size categories of tetractines are in the same range as triactines.

Spicules (Table 3):

1. Triactines I (large): regular (equiangular and equiradiate). Actines are conical or slightly conical, depending on the specimen, and have sharp tips (Figs 2E, F, 3E–G). They are found mainly outside the tubes.
2. Triactines II (intermediate): regular (equiangular and equiradiate) or sagittal. Actines are slightly conical with sharp tips (Figs 2G, H, 3H, I). This is the most frequent type of spicule. Size highly variable.
3. Triactines III (small): regular (equiangular and equiradiate). Actines are conical with sharp tips. Size highly variable (Figs 2I, J, 3L, M).
4. Tetractines: very rare. Size highly variable (Figs 2K, L, 3J, K). Apical actines are frequently spiralled, but straight forms also occur (Fig. 2M, N).

Reproduction: Unknown

Ecology: This species lives in habitats with moderate to high amounts of sediment. A reasonable quantity of fine sediment was found amongst tubes of the cormus of the holotype specimen (MNRJ 11414). Some individuals were growing on gastropod shells (*Crepidula* sp.) and others underneath boulders, near polychaete reefs. Specimens were found sharing the substrate with bryozoans, lophophorates, and serpulid polychaetes as well as some other calcareous sponges (*Soleneiscus pedicellatus* sp. nov. and *Leucosolenia* sp.). Some polychaetes occurred amongst the tubes of the cormus

of specimen MNRJ 12860. Known bathymetrical distribution extends from 1 to 13 m depth.

Geographical distribution: North (5°S) and south coasts of Peru (14°S) (Fig. 3N).

Remarks: The genus *Arthuria* was recently proposed (Klautau *et al.*, 2013). It comprises eight species: *Arthuria africana* (Klautau & Valentine, 2003) (South Africa), *Arthuria alcatraziensis* (Lanna *et al.*, 2007) (Brazil), *Arthuria canariensis* (Miklucho-Maclay, 1868) (Gulf of Mexico, Azores, Canary and Madeira Islands, Adriatic Sea and European waters), *Arthuria dubia* (Dendy, 1891) (Australia), *Arthuria hirsuta* (Klautau & Valentine, 2003) (South Africa), *Arthuria passionensis* (van Soest, Kaiser & van Syoc, 2011) (Clipperton Island), *Arthuria sueziana* (Klautau & Valentine, 2003) (Red Sea), and *Arthuria tenuipilosa* (Dendy, 1905) (Red Sea and Sri Lanka). *Arthuria spirallata* sp. nov. differs from all of these by the presence of three categories of triactines and one of tetractines (tetractines being very rare). The frequent spiral shape of the apical actines is also very characteristic. Four specimens of *A. spirallata* sp. nov. were sequenced and included in our molecular tree.

GENUS *CLATHRINA* GRAY, 1867

Diagnosis: Calcinea in which the cormus comprises anastomosed tubes. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) and/or parasagittal triactines, to which diactines and tripods may be added. Asconoid aquiferous system.

CLATHRINA ANTOFAGASTENSIS AZEVEDO, HAJDU, WILLENZ & KLAUTAU, 2009 (FIG. 4; TABLE 4)

Citations: Azevedo *et al.*, 2009: 4.

Material examined: Ten specimens. MNRJ 11282 (fragments: RBINSc-IG 32239-POR 11282, CZA 11282 and MHNG 85284); Punta Zamora, Samanco Bay, Chimbote, Ancash Region (09°12'58.10"S, 78°33'09.90"W); collected by Ph. Willenz and Y. Hooker; 4 m depth, 24.ix.2007. MNRJ 11294 (fragments: RBINSc-IG 32239-POR 11294, CZA 11294, and MHNG 85295); north of Colorada Inlet, El Dorado, Chimbote, Ancash Region (09°11'40.80"S, 78°32'21.00"W); collected by E. Hajdu; 2 m depth, 24.ix.2007. MNRJ 11512 (fragments: RBINSc-IG 32239-POR 11512, CZA 11512, and MHNG 85507); San Lorenzo Island site 2, Callao, Callao Region (12°04'01.44"S, 77°13'44.95"W); collected by Y. Hooker and Ph. Willenz; 4 m depth; 27.x.2007. MNRJ 12825; Vieja Island, Independence Bay, Paracas National Reserve, Ica Region (14°17'23.10"S, 76°10'28.40"W); collected by Y. Hooker, Ph. Willenz, and F. Azevedo; 7 m

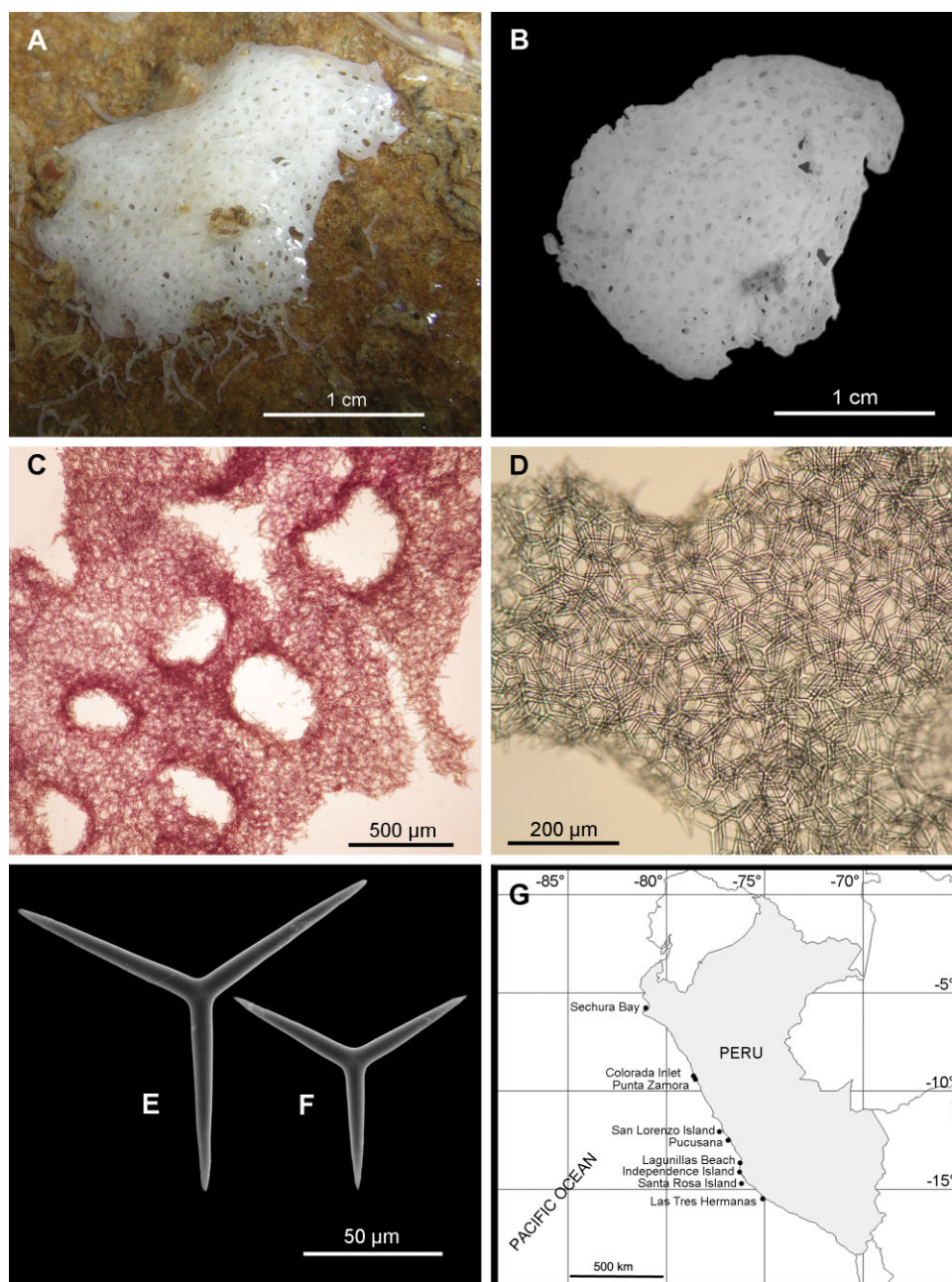


Figure 4. *Clathrina antofagastensis* (MNRJ 13148). A, live specimen; B, specimen in ethanol; C, cormus anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E, triactine I; F, triactine II; G, distribution map along Peruvian coast.

depth, 09.xii.2008. MNRJ 12835 (fragments: RBINSc-IG 32240-POR 12835, CZA 12835, and MHNG 85668); Santa Rosa Island site 2, Independence Bay, Paracas National Reserve, Ica Region ($14^{\circ}19'11.30''S$, $76^{\circ}09'30.10''W$); collected by Y. Hooker, F. Azevedo, and B. C ndor-Luj n; 10 m depth; 10.xii.2008. MNRJ 13125 (fragment: RBINSc-IG 32240-POR 13125); Chilca Island, Pucusana, Lima Region ($12^{\circ}28'19.10''S$,

$0.76^{\circ}47'54.10''W$); collected by E. Hajdu, G. L bo-Hajdu, and F. Azevedo; 7 m depth; 28.xi.2008. MNRJ 13131 (fragment: RBINSc-IG 32240-POR 13131); Las Tres Hermanas, San Juan de Marcona, Ica Region ($15^{\circ}26'32.40''S$, $75^{\circ}04'14.70''W$); collected by F. Azevedo, G. L bo-Hajdu, and E. Hajdu; intertidal; 01.xii.2008. MNRJ 13148 (fragment: RBINSc-IG 32240-POR 13148), MNRJ 16783; Lagunillas Beach, Paracas National

Table 4. Spicule measurements of *Clathrina antofagastensis*

Specimen	Spicule	Length (µm)				Width (µm)		N
		Min.	Mean	SD	Max.	Mean	SD	
MNRJ 11282	Triactines I	65.0	80.9	8.1	95.0	8.1	0.8	30
	Triactines II	35.0	41.3	4.6	50.0	5.6	0.9	30
MNRJ 13125	Triactines I	67.5	84.0	9.0	100.0	8.0	0.6	30
	Triactines II	32.5	41.5	4.7	50.0	5.1	0.3	30
MNRJ 13148	Triactines I	60.0	67.6	5.0	77.5	7.8	1.0	30
	Triactines II	35.0	38.1	2.8	45.0	5.9	0.9	30
All specimens	Triactines I	60.0	77.5	8.7	100.0	8.0	0.2	–
	Triactines II	32.5	40.3	1.9	50.0	5.5	0.4	–

Reserve, Ica Region (13°53'44.68"S, 76°18'55.23"W); collected by E. Hajdu, G. Lôbo-Hajdu, and F. Azevedo; intertidal; 05.xii.2008. MNRJ 13674 (fragments: RBINSc-IG 32241-POR 13674, CZA 13674, and MHNG 85912); Sechura Bay site 6, 'Puerto Rico', Piura Region (05°46'49.70"S, 81°04'04.70"W); collected by Y. Hooker, M. Rios, and Ph. Willenz; 8 m depth; 09.xii.2009.

Type locality: Peninsula Mejillones, Antofagasta, Chile.

Colour: White in life and light beige in ethanol.

Description: Sponge varying from thin to thick encrusting or massive (1.5 × 1.0 × 0.2 cm) (Fig. 4A–C). Consistency is compressible. Cormus is formed by irregular and tightly anastomosed tubes (0.3–0.5 mm). Water-collecting tubes are present. Granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Without any special organization and composed of two size categories of triactines (Fig. 4D).

Spicules (Table 4):

1. Triactines I (large): Regular (equiangular and equiradiate). Actines are conical, straight, or slightly undulated with blunt tips (Fig. 4E).
2. Triactines II (small): Regular (equiangular and equiradiate). Actines are conical, straight, with blunt or sharp tips (Fig. 4F).

Reproduction: Buds were observed at the surface of the specimen MNRJ 13148 in December 2008 and several oocytes were observed inside tubes of the specimen MNRJ 11282 in September 2007.

Ecology: Lives in habitats with moderate to high amounts of sediment, predominantly underneath boulders, protected from sunlight. Some individuals were found growing on gastropod shells and others were near polychaete reefs. Ascidiarians, brachiopods (*Discinisca lamellosa*), bryozoans, hydroids, and polychaetes (e.g.

Phragmatopoma sp. and serpulids) were observed near *C. antofagastensis*. Demosponges (aff. Halichondriidae) and other calcareans (*Leucosolenia* sp. and *Grantia* sp.) were also present. Individuals collected in the intertidal zone were found near barnacles and chitons. Microcrustaceans with eggs and small ophiuroids were found associated with specimen MNRJ 13125. Known bathymetric distribution extends from the intertidal to 10 m depth.

Geographical distribution: North coast of Chile (23°S) and along the Peruvian coast (from 5°, 9°, 12°, 13°, and 15°S (Fig. 4G).

Remarks: *Clathrina antofagastensis* was originally described from and considered provisionally endemic to the north coast of Chile. Its known distribution has now been extended to the north, along the Peruvian littoral. The presence of water-collecting tubes was not observed in the type material; therefore, it was not mentioned in the original description (Azevedo *et al.*, 2009). The *in vivo* photos taken of the Peruvian samples allowed verification of the presence of this structure. In order to confirm the identification, a DNA sequence of the holotype was included in our tree, together with three Peruvian specimens morphologically similar to *C. antofagastensis*. These specimens grouped with the holotype of *C. antofagastensis* with 100% bootstrap support and only 0.5% divergence. In the present work, *C. antofagastensis* was the most abundant species, with the largest geographical extension, found in nine localities along the Peruvian coast.

CLATHRINA AUREA SOLÉ-CAVA, KLAUTAU,
BOURY-ESNAULT, BOROJEVIC & THORPE, 1991
(FIG. 5; TABLE 5)

Citations: Solé-Cava *et al.*, 1991: 385; Muricy *et al.*, 1993: 429; Klautau *et al.*, 1994: 401; Muricy & Moraes, 1998: 215; Muricy & Silva, 1999: 160; Santos, Cantarelli & Tenório, 2002: 398; Klautau & Borojevic, 2001: 401;

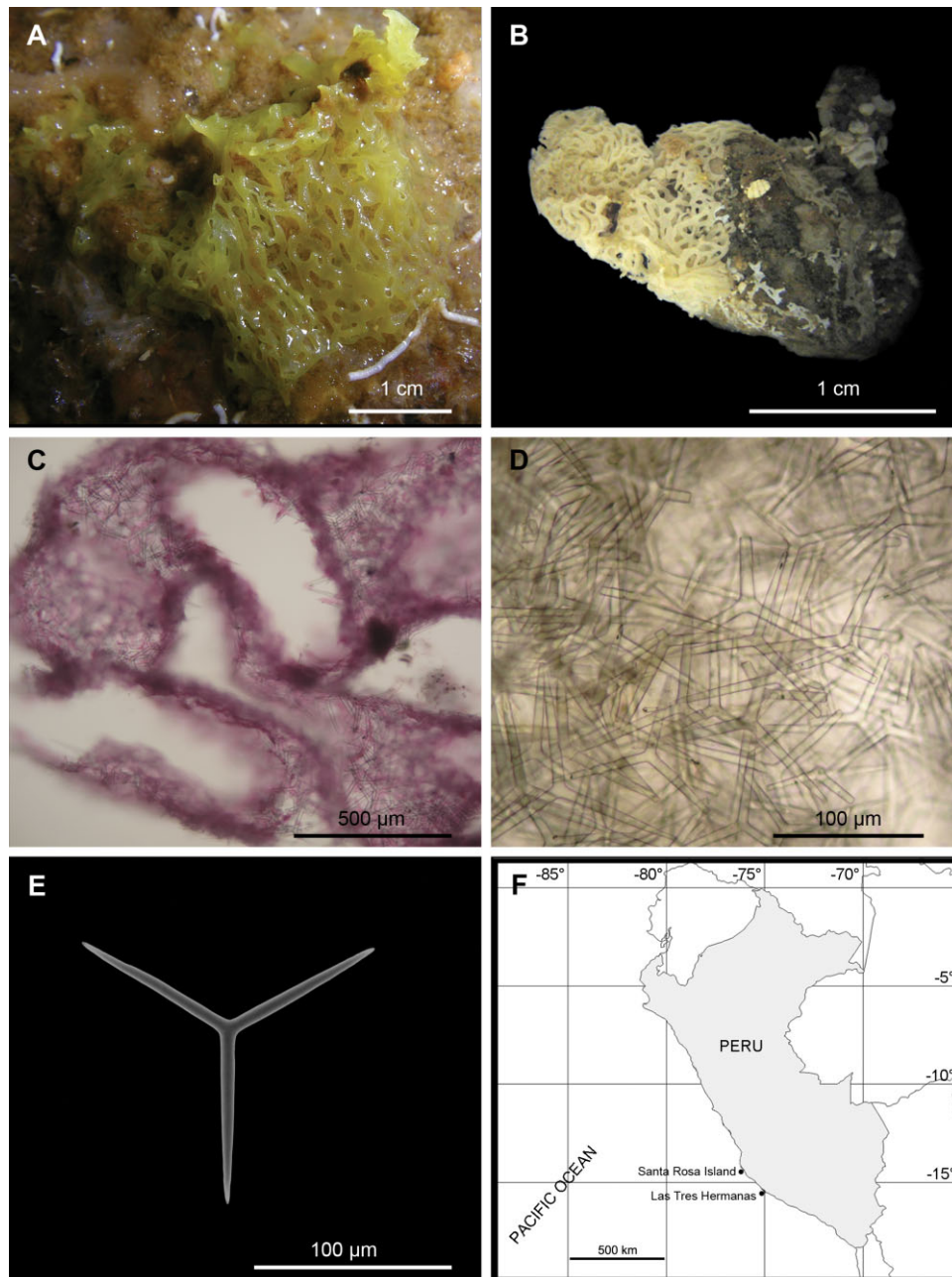


Figure 5. *Clathrina aurea* (MNRJ 12840). A, live specimen; B, specimen in ethanol; C, cormus anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E, triactine I; F, distribution map along the Peruvian coast.

Klautau & Valentine, 2003: 9; Monteiro & Muricy, 2004: 683; Vilanova *et al.*, 2004: 651; Moraes *et al.*, 2006: 166; Muricy & Hajdu, 2006: 85; Lanna *et al.*, 2007: 1554; Muricy *et al.*, 2008: 128; Lanna *et al.*, 2009: 4; Santos *et al.*, 2010: 605; Muricy *et al.*, 2011: 33.

Material examined: Seven specimens. MNRJ 13124 (fragment: RBINSc-IG 32240-POR 13124), MNRJ 13129 (fragment: RBINSc-IG 32240-POR 13129), MNRJ

13130 (fragment: RBINSc-IG 32240-POR 13130), MNRJ 13138 (fragment: RBINSc-IG 32240-POR 13138), MNRJ 13139 (fragment: RBINSc-IG 32240-POR 13139), MNRJ 13143 (fragment: RBINSc-IG 32240-POR 13143); Las Tres Hermanas, San Juan de Marcona, Ica Region (15°26'32.40"S, 75°0.4'14.70"W); collected by F. Azevedo, G. Lôbo-Hajdu, and E. Hajdu; intertidal; 01–02.xii.2008. MNRJ 12840 (fragments: RBINSc-IG 32240-POR 12840, CZA 12840, and MHNG 85673); Santa Rosa Island site

Table 5. Spicule measurements of *Clathrina aurea*

Specimen	Spicule	Length (µm)				Width (µm)		N
		Min.	Mean	SD	Max.	Mean	SD	
MNRJ 12840	Triactines	55.9	77.6	10.4	94.8	6.9	0.9	30
MNRJ 13124	Triactines	62.5	77.5	7.6	92.5	5.7	0.6	30
MNRJ 13138	Triactines	63.2	70.6	4.7	82.6	5.6	0.8	30
All specimens	Triactines	53.1	73.4	7.5	94.8	5.9	0.8	–

1, Paracas National Reserve, Ica Region (14°19'10.20"S, 76°09'52.40"W); collected by Y. Hooker and F. Azevedo; 8 m depth; 10.xii.2008.

Type locality: Arraial do Cabo, Rio de Janeiro, Brazil.

Colour: Bright yellow in life and white in ethanol.

Description: Analysed specimens are very small (< 1.3 × 1.0 × 0.2 cm) and the growth form is thin encrusting. Consistency is soft and fragile. Cormus is formed by irregular, very thin, and loosely anastomosed tubes (0.1–0.4 mm) (Fig. 5A–C). Water-collecting tubes are absent and oscula are abundantly spread throughout the cormus. Granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Without any special organization and exclusively composed of triactines (Fig. 5D).

Spicules (Table 5):

1. Triactines: regular (equiangular and equiradiate). Actines are cylindrical to slightly conical, and undulated with rounded to blunt tips (Fig. 5E).

Reproduction: Unknown

Ecology: Lives in cryptic habitats, such as underneath boulders, with low to moderate amounts of sediment. Low diversity of fauna was found near this species, restricted to a red ascidian, some encrusting demosponges (Chalinidae), and the calcareous sponges *Clathrina peruana* sp. nov. and *Grantia* sp. Known bathymetric distribution ranges from the intertidal zone to 8 m depth.

Geographical distribution: North-east, south-east, and south coasts of Brazil (4°–27°S) and south coast of Peru (14° and 15°S) (Fig. 5F).

Remarks: *Clathrina aurea* was originally described from and considered endemic to the coast of Brazil, where it is the most widespread calcarean species (Solé-Cava *et al.*, 1991; Muricy *et al.*, 2011). This is the first report for this species outside Brazil. Noticeable differences

were observed between the holotype and the Peruvian specimens in relation to the shape of actines. In the holotype, actines are cylindrical with rounded tips, whereas in the Peruvian specimens they can be slightly conical with tips varying from round to blunt. Re-examination of additional Brazilian specimens of *C. aurea* showed that the spicules are also highly variable amongst them, sometimes being slightly conical and blunt. Therefore, to confirm the identification of the Peruvian specimens as *C. aurea*, Brazilian specimens were included in our molecular tree analysis with Peruvian specimens; they grouped with 100% bootstrap support and 1% divergence, confirming the presence of *C. aurea* in Peru. The occurrence of *C. aurea* in Peru was highly unexpected. Despite several recent surveys (2004–2009) by Hajdu, Willenz, and collaborators, this species is apparently not present in any region between the Brazilian and the Peruvian coasts. In order to further verify this unlikely biogeographical scenario, a genetic analysis at the population level is being conducted to evaluate the genetic connectivity between these distant populations of *C. aurea*.

CLATHRINA APHRODITA SP. NOV. (FIG. 6; TABLE 6)

Type material: Holotype (ethanol). MNRJ 13021 – fragments from the holotype: RBINSc-IG 32241-POR 13021, CZA 13021, and MHNG 85809. Paratypes (ethanol). MNRJ 11440 – fragments from the paratype: RBINSc-IG 32239-POR 11440, CZA 11440, and MHNG 85440, MNRJ 12977 – fragments from the paratype: RBINSc-IG 32241-POR 12977, CZA 12977, and MHNG 85766, MNRJ 13022 – fragments from the paratype: RBINSc-IG 32241-POR 13022, CZA 13022, and MHNG 85810.

Material examined: Nine specimens. MNRJ 11440; El Ñuro site 6, south of Quebrada Verde, Piura Region (04°13'30.40"S, 81°12'31.60"W); collected by Y. Hooker and M. Rios; 1 m depth; 14.x.2007. MNRJ 11476 (fragments: RBINSc-IG 32239-POR 11476, CZA 11476, and MHNG 85472); Rocas La Chavelera, Cancas, Tumbes Region (03°55'14.10"S, 80°54'29.90"W); collected by Y. Hooker and M. Rios; 9 m depth; 17.x.2007. MNRJ 11486

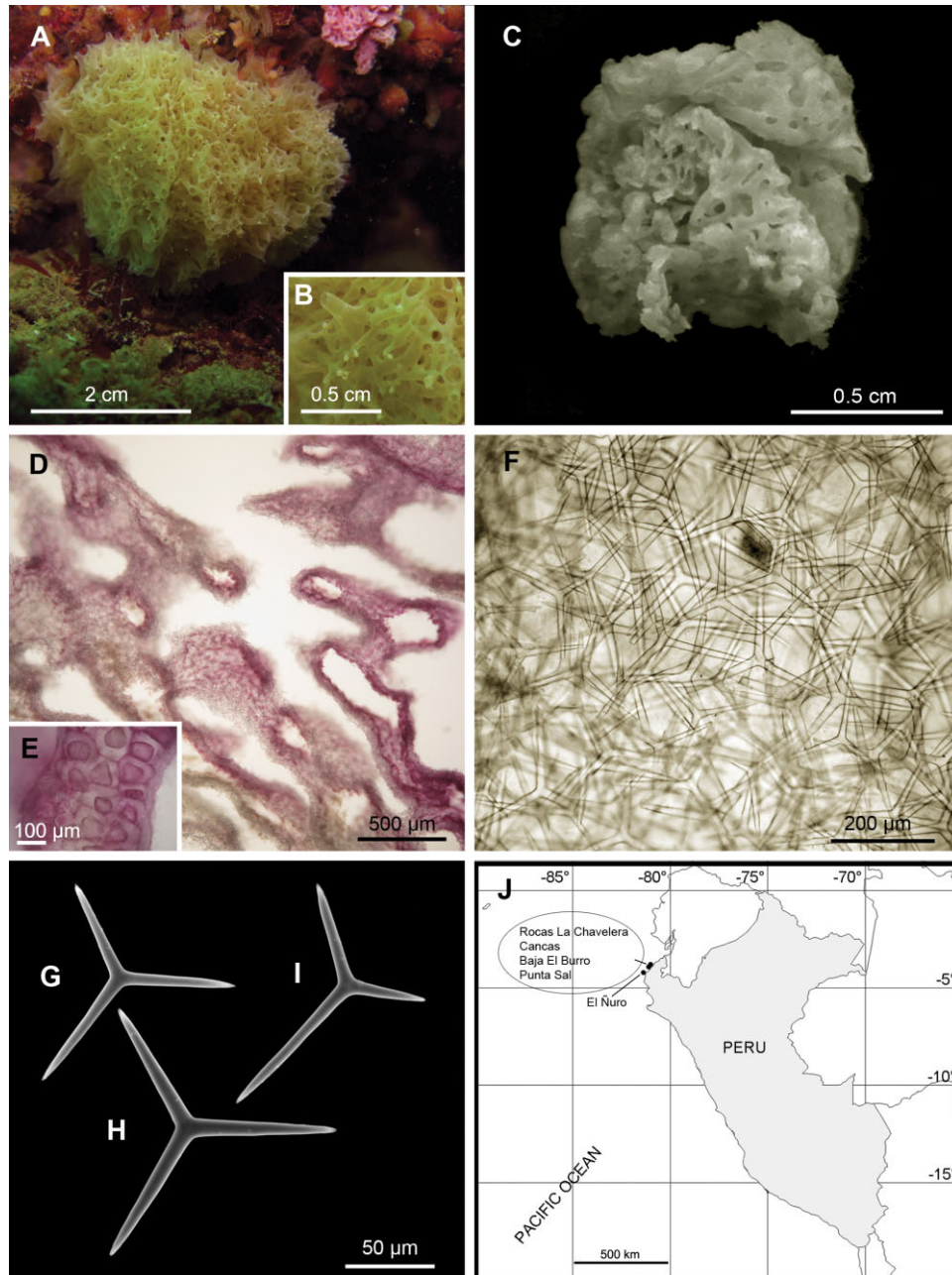


Figure 6. *Clathrina aphrodita* sp. nov. (Holotype – MNRJ 13021). A, live specimen; B, detail of spherical buds at the sponge surface; C, specimen in ethanol; D, corium anastomosis (cross section); E, detail of oocytes inside hexagonal follicles in the corium; F, spicules in the wall of a tube (tangential section); G–H subregular, slightly conical, triactines; I, regular, conical, triactine; J, distribution map along the Peruvian coast.

(fragments: RBINSc-IG 32239-POR 11486, CZA 11486, and MHNG 85482); Cancas, Tumbes Region (03°56'31.70"S, 80°56'37.60"W); collected by Y. Hooker, 11 m depth, 17.x.2007. MNRJ 12974 (fragments: RBINSc-IG 32239-POR 12974, CZA 12974, and MHNG 85763); Baja El Burro, Punta Sal, Tumbes Region (03°58'34.10"S, 80°59'06.00"W); collected by Y. Hooker, B. Ibañez, and Ph. Willenz; 13–19 m depth; 21.xi.2009.

MNRJ 12994 (fragments: RBINSc-IG 32241-POR 12994, CZA 12994, and MHNG 85783), MNRJ 12977; El Nuro site 7, south of Quebrada Verde, Piura Region (04°14'01.00"S, 81°12'46.00"W); collected by Y. Hooker, F. Menendez, and Ph. Willenz; 5 m depth; 19.xi.2009. MNRJ 13021, MNRJ 13022; Punta Sal, Tumbes Region. (03°58'52.90"S, 80°59'20.60"W); collected by Y. Hooker and Ph. Willenz; 15 m depth; 23.xi.2009. MNRJ 14180

Table 6. Spicule measurements of *Clathrina aphrodita* sp. nov.

Specimen	Spicule	Length (µm)				Width (µm)		N
		Min.	Mean	SD	Max.	Mean	SD	
MNRJ 11440	Triactines	70.0	90.7	10.0	110.0	9.2	1.2	30
MNRJ 12977	Triactines	80.0	92.7	6.9	107.5	9.7	1.2	30
MNRJ 13021	Triactines	60.0	89.7	10.0	105.0	10.1	1.4	30
All specimens	Triactines	60.0	91.0	1.5	100.0	9.7	0.5	–

(fragment: RBINSc-IG 32241-POR 14180); in front of Punta Sal, Tumbes Region (03°56'42.54"S, 80°56'44.70"W); collected by W. Viera; 12 m depth; 08.xii.2009.

Type locality: Cancas, Tumbes Region, Peru.

Colour: Translucent beige or light pink (reproducing specimen) in life, and dirty beige or grey in ethanol.

Etymology: Refers to the beauty of this sponge.

Description: Massive sponge with subspherical shape (2.5 × 2.0 × 1.0 cm) (Fig. 6A, C, D). Consistency is friable and compressive. Cormus is formed by irregular and frequently tightly anastomosed tubes (0.2–0.5 mm), although loosely anastomosed tubes were also observed in a few specimens. Several conspicuous oscula were present at the surface. Water-collecting tubes and granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Without any special organization and composed of regular and subregular triactines (Fig. 6F).

Spicules (Table 6):

1. Triactines: Regular (equiangular and equiradiate) or subregular (equiangular with two or three actines of different sizes). Actines are conical or slightly conical, and straight with blunt tips (Fig. 6G–I).

Reproduction: One specimen, with a pink colour, was filled with oocytes (MNRJ 13022, in November 2009) individually packed inside hexagonal follicles (Fig. 6E). Two other specimens (MNRJ 12977 and 13021, both collected in November 2009) were white in colour, with abundant spherical buds at the surface of the external tubes (Fig. 6B). Asexual reproduction by budding in *Calcinea* has only been reported by Johnson (1978) for specimens identified as *Clathrina blanca* (Miklucho-Maclay, 1868). The budding process begins with the constriction of tubes localized at the surface of the sponge (reviewed in Ereskovsky, 2010).

Ecology: Lives in habitats with moderate amounts of sediment, although there was no evidence of sediment amongst the tubes of the cormus. Some individuals were found under boulders protected from sunlight, whereas others, encrusting the substrate, were exposed to light. Specimens were found near colonial organisms such as ascidians, bryozoans, and zoanthids, as well as some algae (red dichotomic and calcareous) and demosponges (Chalinidae). A polychaete and some bivalve eggs were found associated with the tubes of the specimens MNRJ 11476 and MNRJ 12994, respectively. Known bathymetric distribution extends from 1 to 19 m depth.

Geographical distribution: North coast of Peru, in Tumbes (3°S) and Piura (4°S) regions (Fig. 6J).

Remarks: The genus *Clathrina* was recently redefined (Klautau *et al.*, 2013) and now comprises 31 species. Amongst these species, *Clathrina cribrata* Rapp, Klautau & Valentine, 2001 (Norway) is the one that most resembles *Clathrina aphrodita* sp. nov., in the presence of numerous and conspicuous oscula at its surface. However, the oscula of *C. cribrata* has a sieve, which is not found in *C. aphrodita* sp. nov. In addition, the skeleton of *C. cribrata* is exclusively composed of regular triactines, whereas *C. aphrodita* sp. nov. has conspicuous and abundant subregular triactines, which have two or three actines of different lengths.

***CLATHRINA NUROENSIS* SP. NOV. (FIG. 7; TABLE 7)**

Type material: Holotype (ethanol): MNRJ 13032 – fragments from the holotype: RBINSc-IG 32241-POR 13032, CZA 13032, and MHNG 85820.

Material examined: MNRJ 13032; El Ñuro site 1, El Ñuro Pier, Piura Region (04°13'00.00"S, 81°10'50.00"W); collected by Y. Hooker, C. Segami, B. Ibañez, and Ph. Willenz; 5 m depth; 24.xi.2009.

Type locality: El Ñuro Pier, Piura Region, Peru.

Colour: White in life and light beige in ethanol.

Etymology: Named after the type locality (El Ñuro).

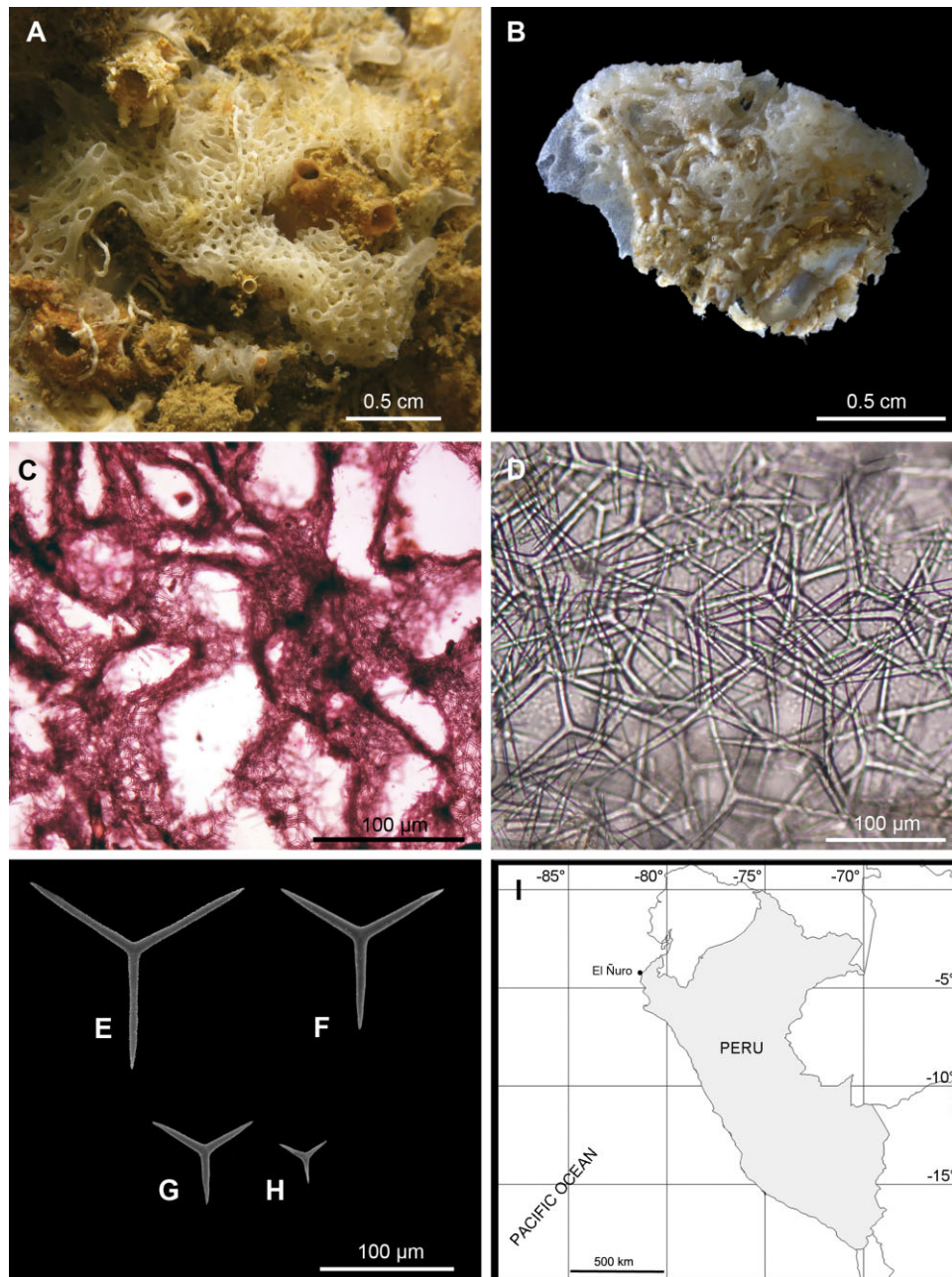


Figure 7. *Clathrina nuroensis* sp. nov. (Holotype – MNRJ 13032). A, live specimen; B, specimen in ethanol; C, corium anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E, triactine I; F, triactine II; G–H, triactine III; I, distribution map along the Peruvian coast.

Description: A single specimen was collected, which was thickly encrusting ($1.0 \times 0.7 \times 0.3$ cm) (Fig. 7A), but became completely flattened after ethanol preservation (Fig. 7B). Consistency is friable. Corium is formed by irregular and apparently tightly anastomosed tubes (0.2–0.5 mm) (Fig. 7A–C). Water-collecting tubes are present. Granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Without any special organization and composed of three categories of triactines (Fig. 7D).

Spicules (Table 7):

1. Triactines I: Regular (equiangular and equiradiate). Variable in size. Actines are slightly conical, straight, with sharp tips (Fig. 7E).

Table 7. Spicule measurements of *Clathrina nuroensis* sp. nov.

Specimen	Spicule	Length (µm)				Width (µm)		N
		Min.	Mean	SD	Max.	Mean	SD	
MNRJ 13032	Triactines I	75.0	88.7	7.5	100.0	7.6	0.5	30
	Triactines II	62.5	77.3	6.5	95.0	8.1	1.1	30
	Triactines III	27.5	40.1	5.3	50.0	6.7	1.2	30

2. Triactines II: Regular (equiangular and equiradiate) or sagittal. Actines are conical, straight, with sharp tips (Fig. 7F).

3. Triactines III: Regular (equiangular and equiradiate). Very similar to triactines II, but smaller. Actines are conical, straight, with sharp tips (Fig. 7G, H).

Ecology: Lives in habitats with high amounts of sediment (predominantly composed of broken shells and polychaete tubes). Lophophorate, ramified colonial bryozoans, encrusting tunicates, and serpulid polychaetes were found sharing the same substrate. The calcareous sponges *Soleneiscus pedicellatus* sp. nov., *Leucosolenia* sp., *Sycon* sp., and *Grantia* sp. also occurred near this species. Bathymetrical distribution is restricted to 5 m depth.

Geographical distribution: North coast of Peru (4°S) (Fig. 7I).

Remarks: Considering all species within *Clathrina*, only two species have their skeleton composed of three size categories of triactines: *Clathrina laminoclathrata* Carter, 1886 (Australia) and *Clathrina nuroensis* sp. nov. However, the average sizes of spicules of *C. laminoclathrata* given by Klautau & Valentine (2003) (i.e. triactines: 188/18 µm, 132/13 µm, 72/8 µm) markedly exceed those of *C. nuroensis* sp. nov. (i.e. triactines: 89/8 µm, 77/8 µm, 40/7 µm), which justifies the status as a new species. Furthermore, the geographical distance between Australia and Peru is too large to postulate conspecificity.

CLATHRINA PERUANA SP. NOV. (FIG. 8; TABLE 8)

Type material: Holotype (ethanol). MNRJ 12839 – fragments from the holotype: RBINSc-IG 32240-POR 12839, CZA 12839, and MHNG 85672. Paratypes (ethanol). MNRJ 13127 – fragment from the paratype: RBINSc-IG 32240-POR 13127. MNRJ 13141 – fragment from the paratype: RBINSc-IG 32240-POR 13141.

Material examined: Six specimens. MNRJ 11277 (fragments: RBINSc-IG 32239-POR 11277, CZA 11277, and MHNG 85279); Tortuga Island, Casma, Ancash Region

(09°22'37.62"S, 78°26'20.22"W); collected by Ph. Willenz and Y. Hooker; 9 m depth; 23.ix.2007. MNRJ 12839; Santa Rosa Island site 1, Independence Bay, Paracas National Reserve, Ica Region (14°19'10.20"S, 76°09'52.40"W); collected by Y. Hooker and F. Azevedo; 8 m depth; 10.xii.2008. MNRJ 12849; Bocana, Laguna Grande, Paracas National Reserve, Ica Region (14°09'31.10"S, 76°14'55.90"W); collected by Y. Hooker; 6 m depth; 12.xii.2008. MNRJ 13127, MNRJ 13141, MNRJ 13144 (fragment: RBINSc-IG 32240-POR 13144); Las Tres Hermanas, San Juan de Marcona, Ica Region (15°26'32.40"S, 75°04'14.70"W); collected by E. Hajdu, G. Lôbo-Hajdu, and F. Azevedo; intertidal; 01–02.xii.2008.

Type locality: Santa Rosa Island, Paracas National Reserve, Ica Region, Peru.

Etymology: Named after its wide distribution along the Peruvian coast.

Colour: Opaque white in life and brown in ethanol.

Description: Encrusting to slightly massive sponge (1.0 × 0.8 × 0.2 cm) (Fig. 8A–C). Consistency is soft and fragile. Cormus is formed by irregular and loosely anastomosed tubes (0.3–0.7 mm). Water-collecting tubes are present. Granular cells are abundant (Fig. 8E insert). Aquiferous system is asconoid.

Skeleton: Without any special organization, with only triactines (Fig. 8D).

Spicules (Table 8):

1. Triactines: regular (equiangular and equiradiate) (Fig. 8F). Actines are cylindrical, slightly undulated toward the distal part, with sharp or blunt tips (Fig. 8G, H).

Reproduction: Unknown

Ecology: Lives in habitats with low to moderate amounts of sediment. Evidence of sediment was found amongst the tubes of the cormus of specimen MNRJ 12849. Individuals occurred underneath boulders protected from sunlight. A low diversity of fauna was found near this

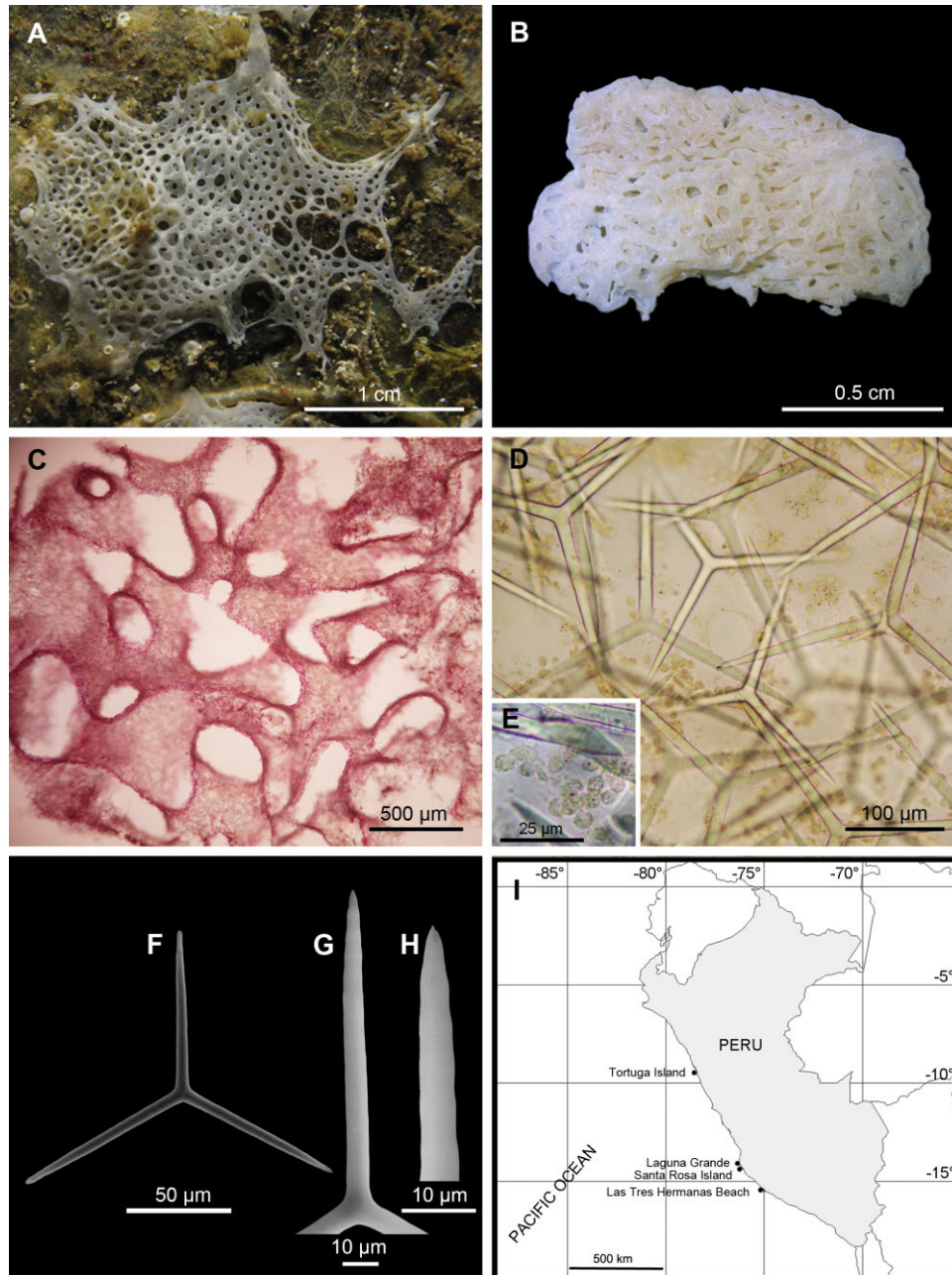


Figure 8. *Clathrina peruana* sp. nov. (Holotype – MNRJ 12839). A, live specimen; B, specimen in ethanol; C, cormus anastomosis (cross section); D, spicules in the wall of a tube (tangential section); E, detail of granular cells; F, triactine; G-H, detail of an actine; I, distribution map along the Peruvian coast.

species: only serpulid polychaetes and demosponges. Some intertidal specimens (MNRJ 13127, MNRJ 13141, and MNRJ 13142) were always found side by side with *C. aurea*. An ophiroid was found amongst the tubes of the cormus of specimen MNRJ 11277. Bathymetrical distribution extends from the intertidal to 8 m depth.

Geographical distribution: Central (9°S) and south (15°S) coasts of Peru (Fig. 8I).

Remarks: Amongst all 31 described species of *Clathrina*, six share white colour, irregular and loosely anastomosed tubes, and only one category of triactine with cylindrical actines: *C. cribrata* (Norway); *Clathrina cylindractina* Klautau, Solé-Cava & Borojevic, 1994 (Brazil); *Clathrina heronensis* Wörheide & Hooper, 1999 (Australia); *Clathrina hispanica* Klautau & Valentine, 2003 (Spain); *Clathrina parva* Wörheide & Hooper, 1999 (Australia), and *Clathrina wistariensis* Wörheide &

Table 8. Spicule measurements of *Clathrina peruana* sp. nov.

Specimen	Spicule	Length (µm)				Width (µm)		N
		Min.	Mean	SD	Max.	Mean	SD	
MNRJ 12839	Triactines	80.6	101.1	9.7	122.2	7.7	0.6	30
MNRJ 13127	Triactines	80.6	102.6	11.0	122.2	7.2	0.7	30
MNRJ 13144	Triactines	85.8	110.6	12.5	145.6	7.7	1.4	30
All specimens	Triactines	80.6	104.8	5.1	145.6	7.5	0.3	–

Hooper, 1999 (Australia). *Clathrina peruana* sp. nov. is easily distinguished from *C. cylindractina*, *C. cribrata*, and *C. hispanica* by the presence of water-collecting tubes and granular cells, which are absent in the three latter species. The three Australian species, *C. heronensis*, *C. parva*, and *C. wistariensis*, similarly to *C. peruana* sp. nov., also have granular cells. However, *C. heronensis* and *C. wistariensis* do not have water-collecting tubes. Although *C. parva* shares water-collecting tubes and granular cells with *C. peruana* sp. nov., it has consistent differences in the shape and size of the spicules. The actines in *C. peruana* sp. nov. (105/8 µm) are shorter and thinner than in the holotype of *C. parva* (143/14 µm: Wörheide & Hooper, 1999; 129/10 µm: Klautau & Valentine, 2003). Moreover, *C. peruana* sp. nov. has strongly undulated actines near the tips, whereas the undulation is very subtle in *C. parva*.

GENUS *ERNSTIA* KLAUTAU ET AL., 2013

Diagnosis: Calcinea in which the cormus comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) and/or sagittal triactines and tetractines. Tetractines are the most abundant spicules or occur at least in the same proportion as the triactines. Tetractines frequently have very thin (needle-like) apical actines. Diactines may be present. Asconoid aquiferous system.

ERNSTIA TETRACTINA (KLAUTAU & BOROJEVIC, 2001)
(FIG. 9; TABLE 9)

Material examined: Two specimens. MNRJ 11344 (fragments: RBINSc-IG 32239-POR 11344, CZA 11344, and MHNG 85343); Ladrón Bay, Lobos de Afuera Islands, Guano Islands and Capes National Reserve System (06°56'00.59"S, 80°42'58.70"W); collected by Ph. Willenz and Y. Hooker; 11 m depth; 04.x.2007. MNRJ 11347 (fragments: RBINSc-IG 32239-POR 11347, CZA 11347, and MHNG 85346); Santo Domingo Islet, Lobos de Afuera Islands, Guano Islands and Capes National Reserve System (06°55'09.80"S, 80°44'09.40"W); collected by Ph. Willenz and Y. Hooker; 20 m depth; 05.x.2007.

Colour: White in life and light beige in ethanol.

Description: Thinly encrusting sponge (2.5 × 1.0 × 0.2 cm). Consistency is soft and fragile. Cormus is formed by irregular and loosely anastomosed tubes (0.2–0.5 mm) (Fig. 9A–C). Water-collecting tubes are present (1.5 mm, diameter). Granular cells are absent. Aquiferous system is asconoid.

Skeleton: Without any special organization, composed of abundant tetractines and few triactines (Fig. 9E).

Spicules (Table 9):

1. Triactines: regular (equiangular and equiradiate). Actines are conical, straight, with sharp tips.
2. Tetractines: regular (equiangular and equiradiate). Actines are conical, straight, with sharp tips (Fig. 9F). Apical actine is straight, smooth, very thin, and frequently longer than the basal ones (Fig. 9F).

Reproduction: Unknown

Ecology: Lives in habitats with low amounts of sediment. Individuals were found underneath boulders, protected from sunlight. A low diversity of fauna was found near this species. Chitons and serpulid polychaetes as well as a finely incrusting yellow demosponge were found sharing the same substrate; however, these were fairly distant from *E. tetractina*. Some sea urchin spines were also found nearby. Bathymetrical distribution varies from 11 to 20 m depth.

Geographical distribution: South-eastern coast of Brazil (23°S) and Lobos de Afuera Islands in Peru (6°S) (Fig. 9H).

Remarks: The genus *Ernstia* was recently proposed (Klautau et al., 2013). It comprises five species: *Ernstia adusta* (Wörheide & Hooper, 1999) (Australia), *Ernstia quadriradiata* (Klautau & Borojevic, 2001) (Brazil), *Ernstia sagamiana* (Hōzawa, 1929) (Japan), *Ernstia septentrionalis* (Rapp et al., 2001) (Norway), and *Ernstia tetractina* (Klautau & Borojevic, 2001) (Brazil). *Ernstia tetractina* was originally described from and considered endemic to the coast of Brazil, where its

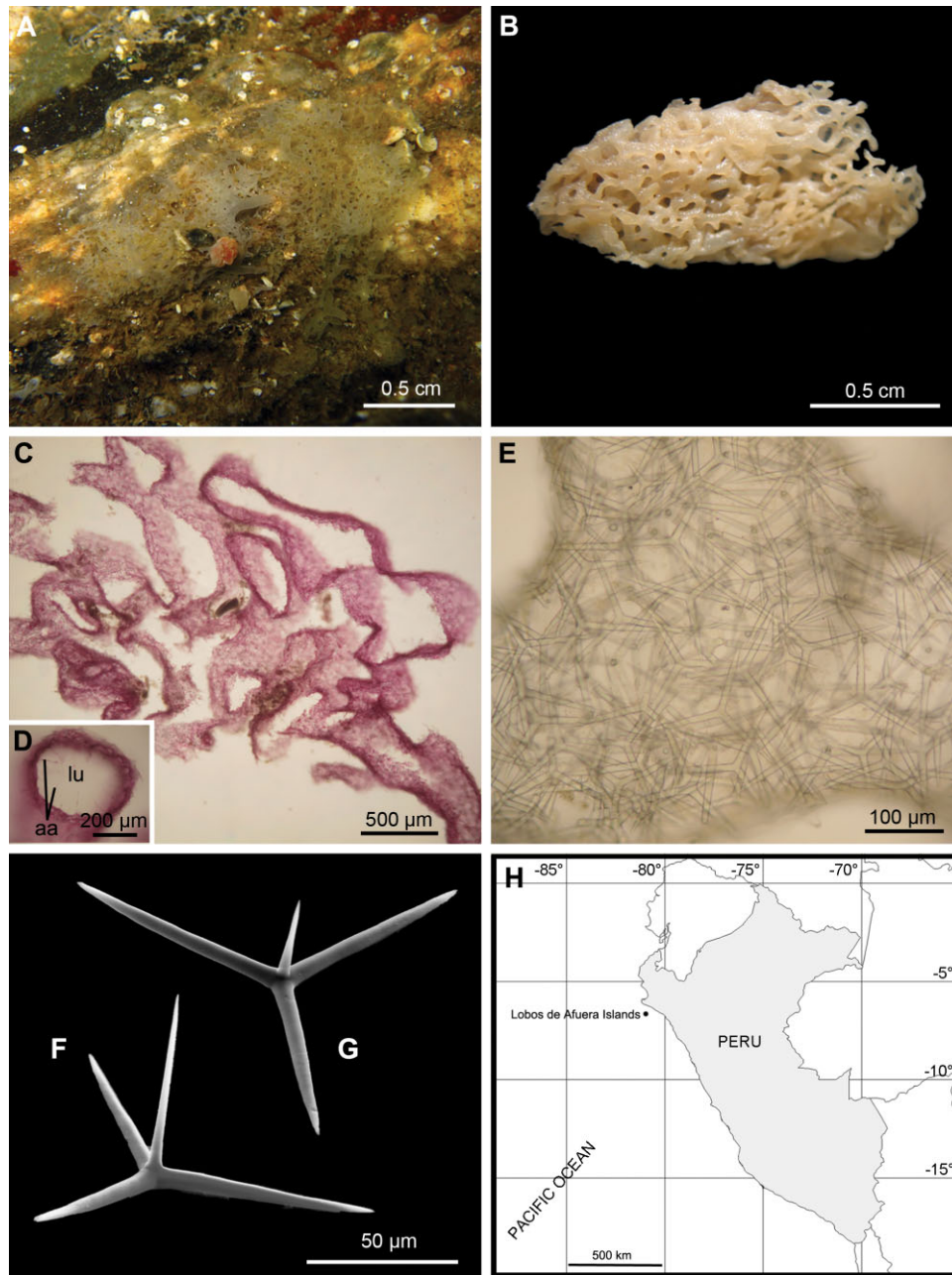


Figure 9. *Ernstia tetractina* (MNRJ 11344). A, live specimen; B, specimen in ethanol; C, cormus anastomosis (cross section); D, detail of a tube (lu = lumen; aa = apical actines); E, spicules in the wall of a tube (tangential section); F-G, tetractine; H, distribution map along the Peruvian coast.

distribution is restricted to the south-east coast (Klautau & Borojevic, 2001). Its first report outside Brazil raises the need for a population genetics study.

FAMILY DENDYIDAE DE LAUBENFELS, 1936

A very important point that must be discussed here is the case of the family Dendyidae. This family was proposed by de Laubenfels in 1936 to include species

of *Dendya*. In 1990, Borojevic and collaborators proposed a new family, Soleneiscidae, later corrected to Soleneiscidae, to include species of *Soleneiscus* and *Dendya* (Borojevic, Boury-Esnault & Vacelet, 1990; Borojevic *et al.*, 2002). It seems that those authors overlooked the family Dendyidae, which has priority over Soleneiscidae. Therefore, we synonymize here Soleneiscidae with Dendyidae. However, as the original diagnosis of Dendyidae is very incomplete and does

Table 9. Spicule measurements of *Ernstia tetractina*

Specimen	Spicule	Actine	Length (µm)				Width (µm)		N
			Min.	Mean	SD	Max.	Mean	SD	
MNRJ 11344	Triactines	Basal	70.0	91.8	8.6	107.5	8.2	0.9	30
	Tetractines	Basal	77.5	92.2	8.6	107.5	8.7	1.0	30
MNRJ 11347		Apical	28.6	66.1	25.0	104.0	4.5	0.8	16
	Triactines	Basal	80.0	96.9	7.6	115.0	8.6	1.3	30
	Tetractines	Basal	65.0	95.2	9.2	110.0	8.8	1.2	30
Both specimens		Apical	45.0	91.3	24.9	130.0	3.4	0.8	21
	Triactines	Basal	70.0	94.4	3.6	115.0	8.4	0.3	–
	Tetractines	Basal	65.0	93.7	2.1	110.0	8.8	0.1	–
		Apical	28.6	78.7	17.8	130.0	4.0	0.8	–

not include *Soleneiscus*, we propose to keep the diagnosis given by Borojevic *et al.* (2002).

GENUS *SOLENEISCUS* BOROJEVIC, BOURY-ESNAULT & VACELET, 1990

Diagnosis: Dendyidae that grow in the form of an individual ascon tube, with several ascon tubes growing upright from basal stolon-like tubes, or in the form of creeping, distally ramified but only rarely anastomosing tubes.

***SOLENEISCUS PEDICELLATUS* SP. NOV.**
(FIG. 10; TABLE 10)

Type material: Holotype (ethanol). MNRJ 16746 – fragments from the holotype: RBINSc-IG-POR 16746. Paratypes (ethanol). MNRJ 16781 – fragment from the paratype: RBINSc-IG-POR 16781. MNRJ 16782 – fragment from the paratype: RBINSc-IG-POR 16782.

Material examined: Three specimens. MNRJ 16746 and MNRJ 16781; San José Inlet, Lobos de Afuera Islands, Lambayeque region (06°55'48.50"S, 80°43'16.20"W); collected by Y. Hooker and Ph. Willenz; 7–9 m depth; 09.x.2007. MNRJ 16782; El Lagarto Islet, Lobos de Afuera Islands, Guano Islands, and Capes National Reserve System (06°56'01.20"S, 80°42'19.90"W); collected by E. Hajdu; 12 m depth; 04.x.2007.

Type locality: San José Islet, Lobos de Afuera Islands, Guano Islands, and Capes National Reserve System, Peru.

Etymology: Named from the Latin *pedicello*, meaning small foot. Refers to the stalk of the sponge.

Colour: White in life as well as in ethanol.

Description: Small solitary individuals (0.7 cm high) gathered in abundance and densely covering the substrate in patches reaching 25 cm² (Fig. 10A–C). Consistency is fragile. Tubular body is very similar to olynthi (juvenile *Calcarea*), with simple apical osculum (without ornamentation) and stalk (1.0 mm) attached to substrate. Some individuals bud from the stalk of others, but there is no anastomosis between adults. Granular cells were not observed. Aquiferous system is asconoid.

Skeleton: Composed of only parasagittal triactines (Fig. 10D). The unpaired actine is always basipetally directed. The three actines sometimes have different lengths.

Spicules (Table 10):

1. Triactines: parasagittal. Actines are slightly conical, straight, with tips varying from blunt to sharp. The unpaired actine is basipetally orientated and is always longer than the paired ones. Frequently, it is also thicker than the paired actines (Fig. 10E–G).

Reproduction: Unknown

Ecology: This species lives in habitats with moderate to high amounts of sediment. Individuals were found underneath boulders, covering a gastropod shell (*Crepidula*) and a calcareous algae (red algae). Bryozoans, serpulid polychaetes, encrusting demosponges (*Chalinidae*) and calcareous sponges (*A. spirallata* sp. nov. and *Leucosolenia* sp.) were found close to this species. *Leucosolenia* sp. grows attached to *S. pedicellatus* sp. nov. Bathymetrical distribution extends from 7 to 12 m depth.

Geographical distribution: Lobos de Afuera Islands (6°S) (Fig. 10H).

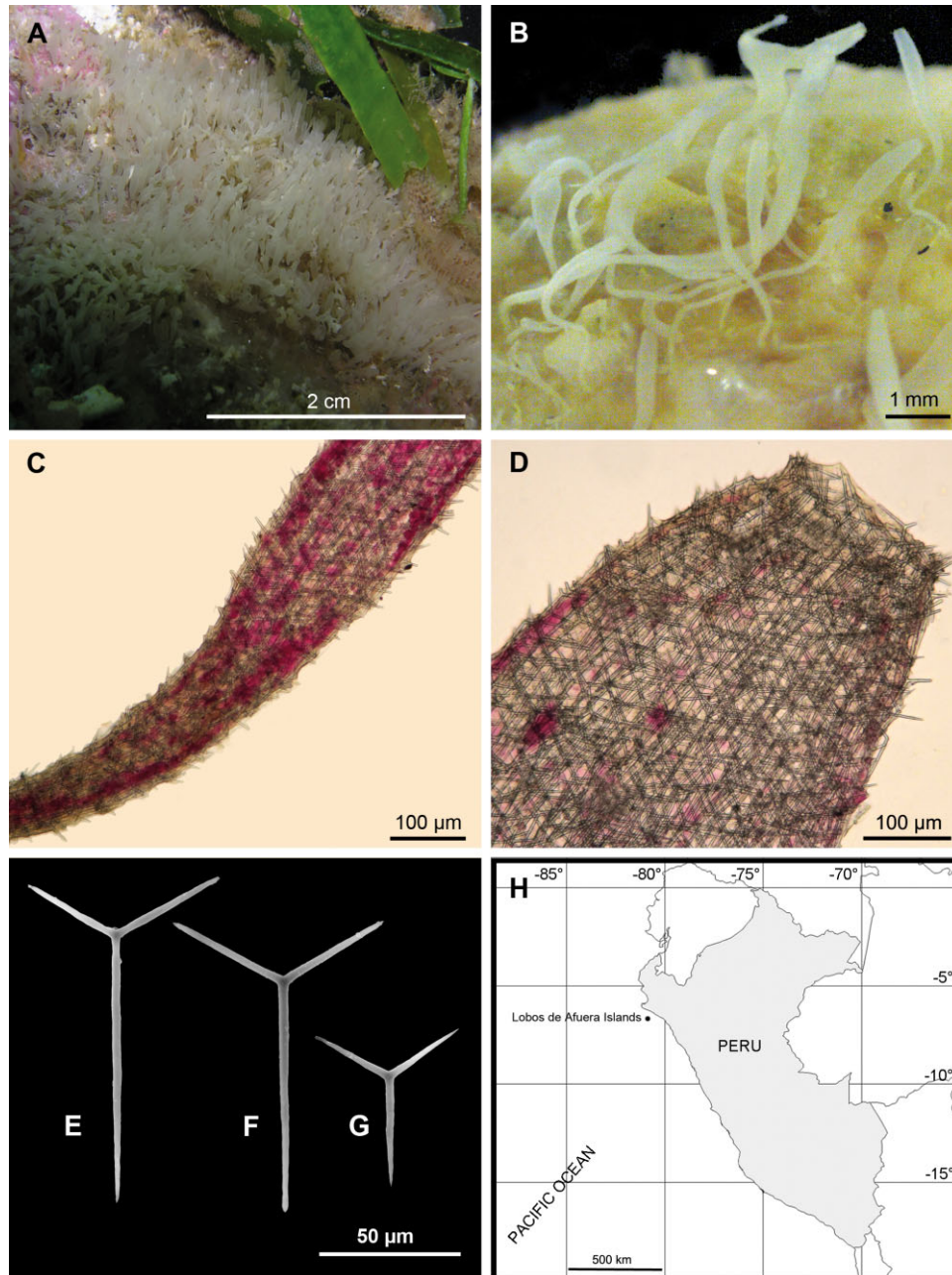


Figure 10. *Soleneiscus pedicellatus* sp. nov. (Holotype – MNRJ 16746). A, live specimen; B, specimen in ethanol; C, basal and median regions of tubular body (cross section); D, detail of apical region of tubular body (cross section); E–F, parasagittal triactines; G, subregular triactine; H, distribution map along the Peruvian coast.

Remarks: *Soleneiscus pedicellatus* sp. nov. is the eighth species of the genus *Soleneiscus* and the first one with only triactines. As in other species of *Soleneiscus*, parasagittal spicules are common. It will not be a surprise if in the future *S. pedicellatus* sp. nov. proves to be part of a new genus. New findings on the molecular phylogeny of Clathrinida have shown that

former *Clathrina* species grouped according to their spicule composition (Rossi *et al.*, 2011; Klautau *et al.*, 2013). For example, species with only triactines formed a monophyletic clade, separated from species with tetractines. Unfortunately, it was not possible to obtain DNA sequences of this species to test our hypothesis.

Table 10. Spicule measurements of *Soleneiscus pedicellatus* sp. nov.

Specimen	Spicule	Actine	Length (µm)				Width (µm)		N
			Min.	Mean	SD	Max.	Mean	SD	
MNRJ 16746	Triactines	Unpaired	62.4	83.5	10.4	109.2	3.1	0.6	30
		Paired	33.8	42.3	6.1	70.2	2.7	0.4	30
MNRJ 16781	Triactines	Unpaired	62.4	78.1	6.5	88.4	2.7	0.4	30
		Paired	36.4	44.2	5.9	57.2	2.6	0.0	30
Both specimens	Triactines	Unpaired	62.4	80.8	3.8	109.2	2.9	0.3	–
		Paired	33.8	43.3	1.3	70.2	2.7	0.1	–

KEY FOR CALCINEA FROM PERU

1. A Body formed by anastomosed tubes (cormus).....2
 B Body without anastomosis (solitary).....*Soleneiscus pedicellatus* sp. nov.
2. A Skeleton composed only of triactines.....3
 B Skeleton composed of triactines and tetractines.....7
3. A Sponge yellow in life.....*Clathrina aurea*
 B Sponge white in life.....4
4. A Only one category of triactine.....5
 B More than one category of triactine.....6
5. A Only regular triactines and presence of granular cells.....*Clathrina peruana* sp. nov.
 B Subregular triactines and no granular cells.....*Clathrina aphrodita* sp. nov.
6. A Two categories of triactines with only conical actines.....*Clathrina antofagastensis*
 B Three categories of triactines with slightly conical actines.....*Clathrina nuroensis* sp. nov.
7. A Tetractines are rare. Apical actine is spiralled.....*Arthuria spirallata* sp. nov.
 B Tetractines are the most abundant spicules. Apical actine is straight.....*Ernstia tetractina*

MOLECULAR RESULTS

The total length of our sequences, after the final alignment, was 839 bp (including gaps). From these sequences, 404 were conserved sites, 429 variable sites, and 44 singletons. The same topology (BI tree shown in Fig. 11) was obtained by both methods of reconstruction analyses (ML and BI). Three major clades (A, B, and C) with high support values (ML bootstrap: 100; BI posterior probability: 1) were recovered.

Clade A corresponded to six known species of the genus *Clathrina* and three new ones (*C. aphrodita* sp. nov., *C. nuroensis* sp. nov., and *C. peruana* sp. nov.). *Clathrina antofagastensis* and *C. aurea* were found in Peru for the first time. Other morphologically similar clathrinas were also included in our molecular analysis (*Clathrina conifera*, *Clathrina coriacea*, *Clathrina cylindractina*, and *Clathrina fjordica*). The three new species possess a skeleton exclusively composed of triactines and clustered together with other clathrinas with the same skeleton composition, confirming the phylogenetic signal of the skeleton (Rossi *et al.*, 2011; Klautau *et al.*, 2013). Clade B corresponded to two species of *Arthuria*, including the type species, *A. hirsuta*, and the new species *A. spirallata* sp. nov. Both species bear skeletons composed of triactines as the main spic-

ules, and rare tetractines (*A. hirsuta* also has diactines). Clade C corresponded to three known species of *Borojevia* (*Borojevia aspina*, *Borojevia brasiliensis*, and *Borojevia cerebrum*) that were used as outgroups. These three species have skeletons composed of triactines, tetractines bearing spined apical actines, and tripods. Unfortunately, the two other species, *S. pedicellatus* sp. nov. and *E. tetractina* were not successfully sequenced and could not be tested in our phylogenetic reconstruction.

DISCUSSION

In the present study eight species of Calcinea were found in Peruvian waters, five of which are new to science (Fig. 12). As in other parts of South America, *Clathrina* was the most species-rich genus, with five species, notwithstanding the recent erection of the new genera *Arthuria*, *Borojevia*, *Brattegardia*, and *Ernstia* for sponges formerly assigned to a more loosely defined *Clathrina* (Klautau *et al.*, 2013).

RESTRICTED DISTRIBUTIONS

With six species of Calcinea found so far (*A. spirallata* sp. nov., *C. antofagastensis*, *C. aphrodita* sp. nov.,

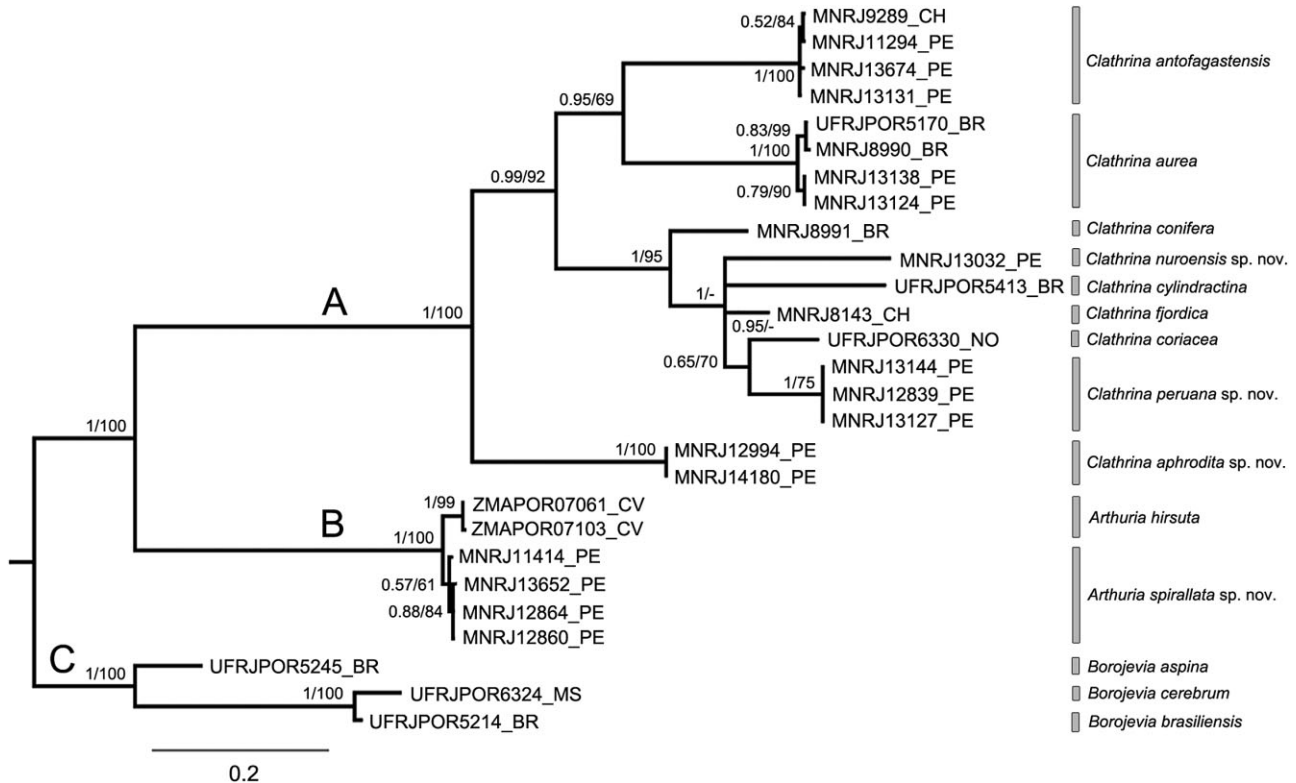


Figure 11. Bayesian 50% majority rule consensus tree (1 000 000 trees sampled; burn-in =10 000 trees) inferred from the ITS rDNA alignment under the GTR model. Bayesian posterior probabilities (BI) and bootstrap (ML) are given near the branches.

C. nuroensis sp. nov., *E. tetractina*, *S. pedicellatus* sp. nov.), the northern part of Peru (southern limit of the Tropical East Pacific province) appears to be richer than the central and southern parts, where only two (*C. antofagastensis*, *C. peruana* sp. nov.), and four species were found (*A. spirallata* sp. nov., *C. peruana* sp. nov., *C. antofagastensis*, *C. aurea*), respectively. This richness pattern appears to be true for Demospongiae as well (Ph. Willenz, Y. Hooker & E. Hajdu, unpubl. data).

Three out of eight species described here are 'single locality' records (or nearly so), provisionally endemic to the northern Peruvian coastline, collected in the transition zone between the TEP and the WTSP biogeographical provinces (Spalding *et al.*, 2007; Hooker, Prieto-Rios & Solís-Marín, 2013). The region between Tumbes and Piura (including Lobos de Afuera and Lobos de Tierra Islands) has long been considered as a transitional zone (Ekman, 1953; Hooker, 2009). Such areas are generally biologically rich as a consequence of faunal mixture from at least two neighbouring biogeographical regions. *Clathrina aphrodita* sp. nov. and *C. nuroensis* sp. nov. were collected on the southern limit of the TEP province, whereas *S. pedicellatus* sp. nov. was obtained from the Lobos de Afuera Islands, which are situated at the northern limit of the WTSP province.

These localities are only 400 km away from each other, and it is unclear whether both northern, as well as the southern, species reflect biogeographically distinct faunas or an endemic element. Some clues might be found in the study of the Calcaronea and Demospongiae collected at the same time and still under examination.

WIDE DISTRIBUTIONS

The remaining five species reported here have considerably larger distributions, from a 750-km range for *C. peruana* sp. nov., to 1000 km for *A. spirallata* sp. nov., 2300 km for *C. antofagastensis*, and culminating with the markedly discontinuous patterns recorded for *C. aurea* and *E. tetractina*, formerly known from the Brazilian coast only (Solé-Cava *et al.*, 1991; Klautau & Borojevic, 2001; Muricy & Hajdu, 2006; Lanna *et al.*, 2007; Muricy *et al.*, 2011). The presence of the first of these species in Peru is now also confirmed by molecular tools (present results).

A relatively wide distribution of sponges within the WTSP province alone is a new finding by itself. Most of the Peruvian and central–northern Chilean coast are characterized by extensive sandy beaches,



Figure 12. Distribution of the eight Peruvian *Calcinea*. 1, *Arthuria spirallata* sp. nov.; 2, *Clathrina antofagastensis*; 3, *Clathrina aurea*; 4, *Clathrina aphrodita* sp. nov.; 5, *Clathrina nuroensis* sp. nov.; 6, *Clathrina peruana* sp. nov.; 7, *Ernstia tetractina*; 8, *Soleneiscus pedicellatus* sp. nov. Type localities of species previously described in Chile and Brazil are also indicated (2, 3 and 7).

interrupted only by a few inlets, peninsulas ('puntas'), and small islands. In this scenario, hard-bottom shallow-water species with low dispersal capabilities may have their connectivity hampered, which possibly accounts for our inability to find further widely distributed *Calcinea* species. In any case, the three species reported above somehow managed to cope with these obstacles.

The distribution patterns found for *C. antofagastensis*, *C. peruana* sp. nov., and *A. spirallata* sp. nov. are within the proposed limits of the WTSP province, in spite of not extending all the way down to its southern boundary. It is common to find austral elements occurring as far north as the middle of the WTSP province, as documented in Desqueyroux-Faúndez & Moyano (1987); de Paula *et al.* (2012), and Hajdu *et al.* (2013). This is the expected outcome of the north-bound flow of the Humboldt Current along with more localized upwelling, which assures a temperate climate and an easy route to the north for species distributed much farther south in the Magellanic province (Thiel *et al.*, 2007). Nonetheless, the three species listed above have not been found in the Magellanic province yet, in spite of the marked increase in latter years of the effort invested in inventorying the sponge fauna in these latitudes (e.g.

Hajdu, Desqueyroux-Faúndez & Willenz, 2006; Esteves, Lôbo-Hajdu & Hajdu, 2007; Azevedo *et al.*, 2009; Willenz *et al.*, 2009a, b; Carvalho, Desqueyroux-Faúndez & Hajdu, 2011; Hajdu *et al.*, 2013). Accordingly, they are more probably warm temperate biotic elements, but the likelihood of a (sub)tropical affinity is hard to dismiss completely, as the knowledge of calcareous sponges in the Tropical Eastern Pacific is, at best, meagre (e.g. van Soest *et al.*, 2012).

Examples of species occurring in the Magellanic province and widely distributed in the WTSP include *Cliona chilensis* and *Clionaopsis platei* (Thiele, 1905). De Paula *et al.* (2012) found specimens of *Cliona chilensis* from Antofagasta (c. 23°S) to be genetically indistinguishable from individuals obtained from the Chilean fjords (42–43°S). It remains to be verified whether the large *Cliona* aff. *chilensis* collected from southern Peru (17°S; Ph. Willenz, Y. Hooker & E. Hajdu, unpubl. data) belongs to the same species. In addition, a rather smaller, yellow *Cliona* sp. was also obtained as far north as 4°S.

There are some possible scenarios to explain these wide WTSP distributions. In the case of *C. antofagastensis*, we find it rather likely that the lack of systematic sampling conducted along the northern Chilean coast and at the southern tip of Peru's coast explains its apparent distribution gap between 15°24' and 23°S. However, although this hypothesis may account for the observed distributions of *C. peruana* sp. nov. and *A. spirallata* sp. nov. if they are truly temperate species, this may not be the case if they are instead ENSO vagrants. If this latter case scenario is correct, it is very surprising that both species appear to be absent from Peru's extreme north (TEP province). Moreover, the lack of faunistic inventories for calcareous sponges to the north of 3°S precludes a more definitive argument. A last hypothesis rests on both species' possible true endemism. Similarly distributed species might have evolved as a consequence of the complex oceanographic scenario observed off the WTSP province. The association of the Humboldt Current, the Peru-Chile Countercurrent, and the Equatorial Undercurrent along with upwelling phenomena, the Oxygen Minimum Zone, and ENSO events, is still not entirely understood (Graco *et al.*, 2007). This scenario could permit a temporary colonization of intermediary populations, followed by long periods of ceased connectivity to donor areas.

DISCONTINUOUS DISTRIBUTIONS

Amphi-American distribution patterns are a common phenomenon amongst species of Porifera, mainly in southern South America or northern North America, where Atlantic and Pacific marine biotas have a chance to meet. The former case is reflected in the recognition of a Magellanic province spanning western and

south-eastern South America (according to the Marine Ecoregions of the World biogeographical classification system), with a few tens of species shared by both oceans. These include taxa spread over several orders of Demospongiae, such as *Axinella crinita* Thiele, 1905 (Axinellidae; Thiele, 1905; Burton, 1932), *Callyspongia fortis* (Ridley, 1881) (Callyspongiidae; Ridley, 1881; Cuartas, 1991), and *Geodia magellani* (Sollas, 1886) (Geodiidae; Sollas, 1886; Burton, 1932); as well as a few Calcarea, for instance *Clathrina fjordica* Azevedo, Hajdu, Willenz & Klautau, 2009 (Clathrinidae; Azevedo *et al.*, 2009; F. Azevedo, E. Hajdu & M. Klautau, unpubl. data) and *Leucosolenia australis* Brøndsted, 1931 (Leucosoleniidae; Tanita, 1942; Azevedo *et al.*, 2009). Nevertheless, verification of hypotheses resting upon morphological characters and molecular data has been conducted solely for *Cliona chilensis* (Clionidae) and *Borojevia* aff. *brasiliensis* (Clathrinidae). De Paula *et al.* (2012) employed COI mtDNA and ITS rDNA as molecular markers, and found Chilean and Argentinean populations of *Cliona chilensis* inhabiting localities 2500 km apart from each other to belong to the same clade (their Clade A). On the basis of ITS rDNA, F. Azevedo *et al.* (unpubl. data) found Chilean and Argentinean populations of *Borojevia* aff. *brasiliensis* stretching over a similar geographical range to be conspecific.

In boreal latitudes, Atlantic–Pacific connectivity is achieved via the Arctic Province, which bridges the geographical gap between the Cold Temperate North-west Atlantic and Cold Temperate North-east Pacific provinces. Faunistic interchange appears less pronounced here however, the harsh climate acts as an impediment for proper faunistic inventory in the area. Despite this, a few species appear to be true ampho-American examples in boreal latitudes, for instance *Halichondria panicea* (Pallas, 1766), *Haliclona rufescens* (Lambe, 1893), and *Suberites montalbidus* Carter, 1880.

The ampho-American distributions reported here for Calcinea deviate from the boreal examples given above in that a major discontinuity appears to be a real attribute in the distribution ranges of *C. aurea* and *E. tetractina*. In the case of the latter, verification of this is pending confirmation via molecular tools. Similar discontinuous ampho-American distributions were reported by Wulff (1996) on the basis of morphological taxonomy applied to sponges from both sides of the Isthmus of Panama. However, the identity of the *Halichondria* spp. studied by that author is not 100% clear. Similarly, the proposal for a trans-isthmian distribution of *Spirastrella* cf. *mollis* has been subsequently contradicted by integrative taxonomic studies conducted by Boury-Esnault *et al.* (1999), who found these putative conspecific sponge populations to belong to two highly divergent species, namely *Spirastrella hartmani* Boury-Esnault *et al.*, 1999 and *Spirastrella*

sabogae Boury-Esnault *et al.*, 1999. By contrast, Wulff's (1996) record of *Haliclona caerulea* (Hechtel, 1965) on both sides of the isthmus was corroborated by De Weerd's (2000) observations. More recently, Nichols & Barnes (2005) found a series of clades in *Placospongia*, three of which were sampled in Panama. One clade was found to be restricted to the TEP province, another to the Tropical North-western Atlantic province, and the third and commonest one, occurred on both provinces. The molecular evidence (ITS) did not match the morphological data according to Nichols & Barnes (2005) and so this ampho-American clade remains unnamed. Another suspected ampho-American sponge was recently reported by Carballo & Cruz-Barraza (2010), who found the former Tropical West Atlantic endemic *Mycale* aff. *magnirhaphidifera* van Soest, 1984 in the Mexican Pacific.

As it stands, genetically backed-up tropical ampho-American sponge species remain of very rare occurrence, and the presently reported findings for *C. aurea* are therefore highly unusual. It is possible however that increased effort dedicated to the integrative taxonomic study of TEP province sponges may yield important surprises in the near future.

ACKNOWLEDGEMENTS

The ESPER Project granted to RBINS was funded by the Global Taxonomy Initiative from the Belgian Development Cooperation; the Proyecto Esponjas was funded by the National Counsel of Technological and Scientific Development (CNPq/PROSUL). The Servicio Nacional de Areas Naturales Protegidas (SERNANP), Agrorural (ex Proabonos), and the Dirección de Hidrografía y Navegación del Perú are acknowledged for permitting access to Lobos de Afuera Islands (Guano Islands and Capes National Reserve System) and Paracas National Reserve and for providing logistical support. We thank Gisele Lôbo-Hajdu (UERJ) and the field staff (M. Rios, B. Ibañez, F. Menendez, W. Vieira, and C. Segami) for assistance in collecting samples on different expeditions. We thank J. C. Fernandes for providing identification of associated Demospongiae. M. K. and E. H. are thankful to CNPq and the Carlos Chagas Filho Research Support Foundation of Rio de Janeiro State (FAPERJ). B. C. L. and F. A. received scholarships from CNPq and FAPERJ, respectively. Ph. W. obtained travel funds from the Fonds National de la Recherche Scientifique. This paper is part of the DSc requirements of Fernanda Correia Azevedo at the Zoology Graduate Program of the National Museum of the Federal University of Rio de Janeiro.

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