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Adriatic calcarean sponges (Porifera, Calcarea), with the description of six new species and a richness analysis

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Abstract. In this study we analyze the calcarean sponge diversity of the Adriatic Sea, the type locality of some of the first described species of calcarean sponges. Morphological and molecular approaches are combined for the taxonomic identification. Our results reveal six species new to science and provisionally endemic to the Adriatic Sea (*Ascandra spalatensis* sp. nov., *Borojevia croatica* sp. nov., *Leucandra falakra* sp. nov., *L. spinifera* sp. nov., *Paraleucilla dalmatica* sp. nov., and *Sycon ancora* sp. nov.), one species previously known only from the Southwestern Atlantic (*Clathrina conifera*), and three already known from the Adriatic Sea (*Ascaltis reticulum*, *Borojevia cerebrum*, and *Clathrina primordialis*). We confirm the presence of the alien species *Paraleucilla magna* in the Adriatic and again record *Clathrina blanca*, *C. clathrus*, and *C. rubra*. We emend the description of the genus *Ascaltis*, propose a lectotype for *Borojevia cerebrum* and synonymise *B. decipiens* with *B. cerebrum*. A checklist of all calcarean species previously and currently known from the Adriatic Sea (39 species) is given. The Central Adriatic is indicated as the richest calcarean sponge fauna sector; however, the biodiversity of this class is underestimated in the whole Adriatic Sea and new systematic surveys are desirable.

Keywords. Porifera, Calcarea, Adriatic Sea, molecular taxonomy, morphological taxonomy.

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Introduction

Although the biodiversity of sponges of the Mediterranean Sea has been studied for a long time (e.g., Schmidt 1862, 1864; Haeckel 1872), some regions and sponge taxa have remained rather neglected. The Adriatic Sea is one of the seven eco-regions of the Mediterranean Province (Spalding *et al.* 2007) and is considered a biodiversity hotspot (Bianchi *et al.* 2012) of major ecological importance. It forms a very narrow, semi-enclosed basin in the northernmost part of the Mediterranean Sea, subdivided into three sectors: Northern Adriatic, Central Adriatic and Southern Adriatic (Bianchi & Morri 2000). The karst limestone is known for forming unique habitats such as caves, overhangs and pits, which are rather inaccessible and often inhabited by a number of invertebrate groups, including calcarean sponges. They are generally less investigated, mainly because of a smaller number of experts involved in their taxonomy, and consequently a large number of species is still unknown. Several calcarean species are known from the Mediterranean, including the Adriatic (see Pansini & Longo 2008), but literature data on these Adriatic species is very scarce or difficult to access, moreover lacking a comprehensive and detailed morphological and molecular descriptions.

Dohrmann *et al.* (2006), Rossi *et al*. 2011 and Voigt *et al.* (2012) demonstrated that modern methods of DNA taxonomy are necessary to highlight the phylogenetic signals in morphological features of, mostly ambiguous, calcarean sponges. Thus, expanding the number of analysed species helps in defining the species-specific morphological features related to certain phylogenetic traits (Rossi *et al.* 2011). A clear and precise taxonomy, based on molecular analyses, represents a strong foundation for accurate systematics changes on different taxonomic levels (Klautau *et al.* 2013).

Some of the first studies on the class Calcarea were done along the Dalmatian coast (e.g., Schmidt 1862, 1864; Haeckel 1870, 1872). Therefore, the Adriatic Sea is the type locality of many of the first known species of calcarean sponges (Haeckel 1870, 1872). Nevertheless, a large number of the original descriptions are fragmentary and many type specimens were lost over time. Hence, in addition to the importance of species diversity records for this eco-region, it is crucial to establish new collections and descriptions of Adriatic calcarean sponges, preferably with detailed morphological and molecular analyses.

In the present work, we study the calcarean sponge diversity of the Adriatic Sea and re-describe some of Haeckel's species using morphological and DNA taxonomy. Furthermore, we performed an analysis of species richness and compiled a checklist comprising all species previously known from the Adriatic Sea (32 species), together with the new data provided here (7 species). Our results indicate that the calcarean sponge fauna of the Adriatic Sea is still underestimated and new systematic surveys including molecular markers are very welcome.

Materials and methods

The material studied is preserved in the following collections:

BMNH = The Natural History Museum, London, UK
GW = Gert Wörheide
IRB = Institut Ruđer Bošković, Zagreb, Croatia
MNRJ = Museu Nacional do Rio de Janeiro, Brazil
PMJ = Phyletisches Museum Jena, Germany
PMR = Prirodoslovni Muzej Rijeka, Croatia

QM = Queensland Museum, Australia
SAM = South Australian Museum, Australia
UFRJPOR = Sponge collection of the Biology Institute of the Universidade Federal do Rio de Janeiro, Brazil
ZMAPOR = Zoölogisch Museum, Instituut voor Systematiek en Populatiebiologie, Amsterdam, The Netherlands
ZMB = Museum für Naturkunde and der Universität Humboldt zu Berlin, Germany

Whenever possible, specimens were divided among two different collections. In such cases the specimens received two register numbers. Both numbers are indicated in the text.

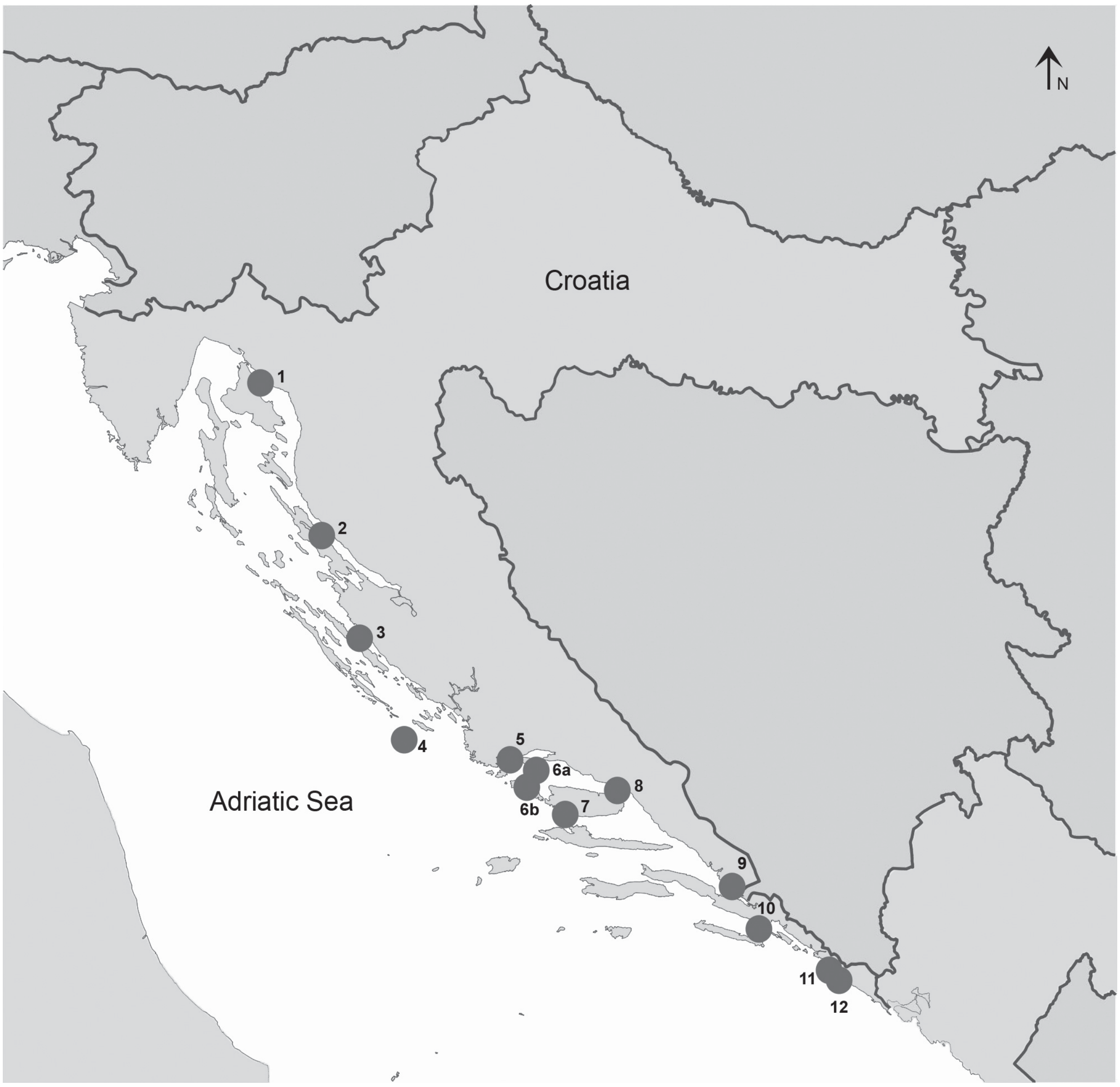

Fig. 1. Map of the Croatian coast. Studied locations along the coast are marked with gray circles and numbers. **1**. Near Selce. **2**. Island of Pag. **3**. Near Zadar. **4**. Island of Blitvenica. **5**. Near Split. **6a–b**. Island of Čiovo. **7**. Island of Brač. **8**. Vrulja Cove. **9**. Port of Ploče. **10**. Prapratno Cove. **11**. Near Dubrovnik. **12**. Island of Lokrum.

Sampling and morphological analyses

Calcarean sponges were collected by SCUBA in 12 localities along the Croatian coastline (Fig. 1). Sponges were fixed and preserved in 96% ethanol. Spicule preparations and sections followed standard procedures (Wörheide & Hooper 1999; Klautau & Valentine 2003).

Length and width at the base of each actine of the spicules were measured. The results are presented in tabular form, featuring length (minimum [min], mean, standard deviation [sd] and maximum [max]), width (minimum [min], mean, standard deviation [sd] and maximum [max]) and sample size (n). Photomicrographs were taken with a digital camera mounted on a Zeiss Axioskop microscope. Micrographs were taken with a JEOL, JSM-6510 scanning electron microscope (SEM) at the Biology Institute (Universidade Federal do Rio de Janeiro).

Specimens are deposited in the sponge collections of the Biology Institute / Universidade Federal do Rio de Janeiro, Brazil, the Natural History Museum in Rijeka, Croatia, and at the Ruđer Bošković Institute, Croatia (Table 1).

DNA isolation, amplification, cloning and sequencing

Total DNA was extracted from 0.05–0.3 g of tissue, using the E.Z.N.A. Forensic DNA Kit (Omega Bio-tek) or G-spin Genomic DNA Extraction Kit (Intron) following the manufacturer's protocol. The ITS1-5.8S-ITS2 rDNA region was amplified by polymerase chain reaction (PCR) using primers 18SF (5′-TCATTTAGAGGAAGTAAAAGTCG-3′) plus 5.8SR (5′-GCGTTCAAAGACTCGATGATTC-3′) (Lôbo-Hajdu *et al.* 2004) and ITS2F (5′-CGGCTCGTGCGTCGATGAAGAAC-3′) plus ITS2R (5′-CGCCGTTACTGGGGGAATCCCTGTTG-3′) (Harcet *et al.* 2010). Partial 28SrDNA gene sequences were amplified with two pairs of primers, NL4F (5′-GACCCGAAAGATGGTGAACTA-3′) plus NL4R (5′-ACCTTGGAGACCTGATGCG-3′) (Nichols 2005), and primers CAL-28SFW (5′-GKCGGATCCGAAYGGACCG-3′) plus CAL28SRV (5′-CCTCTAATCATTCGCTTTACC-3′) designed at the Laboratory of Molecular Genetics of the Ruđer Bošković Institute, based on multiple alignments with the sequences available from GenBank.

PCR reactions were performed under the following conditions: 3 min/95 °C, 30 cycles (30 s/94 °C, 45 s/55 °C, 90 s/70 °C) and final elongation 10 min/72 °C. Reaction mixtures containing 2.5 µl of 10×PCR buffer, 3 µl $MgCl_2$ (25 mM), 0.8 µl of each primer (10 mM), 0.5 µl dNTPs (10 mM each), 1 unit of Taq-DNA polymerase and 100–150 ng template. The quality and quantity of the amplified DNA were estimated by agarose gel electrophoresis. PCR products were purified from gel using QIAquick Gel Extraction Kit (Qiagen) and directly sequenced using the ABI PRISM 3100 automatic sequencer and ABI PRISM BigDye Terminator v. 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). In a few cases, the outcomes of the sequencing reaction were mixed peaks caused by non-target DNA contamination, such as bacteria or algae, which were identified using BLAST network service (http://www.ncbi.nlm.nih.gov/). These regions were ligated with the pGEM-T Vector Kit (Promega), cloned into XL1-Blue competent cells and up to three clones were purfied using the QIAprep Spin Miniprep Kit (Qiagen) and sequenced.

Sequence alignment and phylogenetic reconstruction

Cloned sequences were assembled using Lasergene processing software (DNASTAR Inc., Madison, WI, USA) and checked manually for sequencing errors. The BLAST network service (http://www.ncbi.nlm.nih.gov/) was used for sequence homology searches. Multiple alignments were performed with the Q-INS-i option of the MAFFT program (Katoh & Standley 2013), using score matrix 200 PAM/k=2, gap penalty 1.53 and offset value 0. Alignments were run through a Gblocks v. 0.91b server under less stringent parameters (Castresana 2000) to exclude poorly aligned regions from further analyses. Two

Table 1. Specimens included in the phylogenetic analyses with collection sites, voucher numbers and GenBank accession numbers. *Specimens with newly generated DNA sequences.

Species	Collection site	Voucher number	GenBank accession number	
CALCINEA			**ITS**	28S
Arthuria hirsuta	Cape Verde	ZMAPOR07061	KC843431	-
Arthuria hirsuta	Cape Verde	ZMAPOR 07103	KC985143	-
Arthuria spirallata	Peru	MNRJ 13652	KC985140	-
Arthuria spirallata	Peru	MNRJ 11414	KC985142	-
Ascaltis reticulum	Mediterranean Sea	UFRJPOR6258	HQ588973	-
Ascaltis reticulum	Mediterranean Sea	UFRJPOR6260	HQ588977	-
*Ascaltis reticulum**	Adriatic Sea	PMR-13739 =UFRJPOR6870	KP740022	KP739998
Ascandra contorta	Mediterranean Sea	UFRJPOR6327	HQ588970	-
Ascandra corallicola	Norway	UFRJPOR6329	HQ588994	-
Ascandra falcata	Mediterranean Sea	UFRJPOR5856	HQ588962	-
Ascandra falcata	Mediterranean Sea	UFRJPOR6320	HQ588963	-
Ascandra spalatensis sp. nov.*	Adriatic Sea	PMR-17806 =UFRJPOR7540	KP740024	KP740003
Borojevia cf. *aspina*	Brazil	UFRJPOR5211	HQ588969	-
Borojevia cf. *aspina*	Brazil	UFRJPOR5245	HQ588998	-
Borojevia brasiliensis	Brazil	UFRJPOR5214	HQ588978	-
Borojevia brasiliensis	Brazil	UFRJPOR5230	HQ588999	-
Borojevia cerebrum	Mediterranean Sea	UFRJPOR6322	HQ588964	-
Borojevia cerebrum	Mediterranean Sea	UFRJPOR6323	HQ588971	-
Borojevia cerebrum	Mediterranean Sea	UFRJPOR6324	HQ588975	-
*Borojevia cerebrum**	Adriatic Sea	IRB-CLB26	KP740029	KP740008
*Borojevia cerebrum**	Adriatic Sea	IRB-CLB32	KP740031	KP740010
*Borojevia cerebrum**	Adriatic Sea	PMR-17808	KP740030	KP740009
*Borojevia cerebrum**	Adriatic Sea	IRB-CLB33 = UFRJPOR7539	KP740032	KP740011
Borojevia croatica sp. nov.*	Adriatic Sea	PMR-13740 = UFRJPOR6864	KP740020	KP739995
Borojevia croatica sp. nov.*	Adriatic Sea	PMR-13741 = UFRJPOR6865	KP740021	KP739997
Borojevia croatica sp. nov.*	Adriatic Sea	IRB-CLB6	KP740023	KP740002
Borojevia croatica sp. nov.*	Adriatic Sea	IRB-CLB17	KP740026	KP740005
Borojevia croatica sp. nov.*	Adriatic Sea	IRB-CLB18	KP740027	KP740006
Borojevia croatica sp. nov.*	Adriatic Sea	IRB-CLB19	KP740028	KP740007
Clathrina adusta	GBR, Wistari Reef	QM G313665	-	JQ272288
Clathrina aphrodita	Peru	MNRJ 14180	KC985137	-
Clathrina aphrodita	Peru	MNRJ 12994	KC985138	-
Clathrina aurea	Brazil	MNRJ 8998	HQ588968	-
Clathrina aurea	Brazil	MNRJ 8990	HQ588958	-
Clathrina antofagastensis	Chile	MNRJ 9289	HQ588985	-
Clathrina antofagastensis	Peru	MNRJ 11294	KF002722	-
Clathrina blanca	Adriatic Sea	PMR-14307	KC479087	KC479085
*Clathrina blanca**	Adriatic Sea	PMR-13744	KP740017	KP740000
Clathrina clathrus	Mediterranean Sea	UFRJPOR6315	HQ588974	-
Clathrina clathrus	Mediterranean Sea	UFRJPOR6325	HQ588965	-
Clathrina clathrus	Mediterranean Sea	UFRJPOR6326	HQ588972	-
Clathrina clathrus	Adriatic Sea	PMR-14308	KC479089	KC479083
*Clathrina clathrus**	Adriatic Sea	IRB-CLB12	KP740025	KP740004
*Clathrina clathrus**	Adriatic Sea	PMR-13745	KP740015	KP740001
Clathrina conifera	Brazil	MNRJ 8997	HQ588957	-
Clathrina conifera	Brazil	MNRJ 8991	HQ588959	-
*Clathrina conifera**	Adriatic Sea	PMR-13738 = UFRJPOR6869	KP740019	KP739994
*Clathrina conifera**	Adriatic Sea	PMR-17807	KP740033	KP740012
*Clathrina conifera**	Adriatic Sea	IRB-S2 = UFRJPOR7541	KP740034	KP740013
*Clathrina conifera**	Adriatic Sea	IRB-S3 = UFRJPOR7542	KP740035	KP740014
Clathrina coriacea	Norway	UFRJPOR6330	HQ588986	-
Clathrina fjordica	Chile	MNRJ 8143	HQ588984	-
Clathrina helveola	Australia	QMG313680	HQ588988	AM180987.1
Clathrina hispanica	Mediterranean Sea	UFRJPOR6305	KC843432	-
Clathrina lacunosa	Norway	UFRJPOR6334	HQ588991	-
Clathrina lacunosa	Norway	UFRJPOR6335	HQ588992	-
Clathrina luteoculcitella	Australia	QMG313684	-	AM180988.1
Clathrina peruana	Peru	MNRJ 13144	KC985134	-
Clathrina peruana	Peru	MNRJ 12839	KC985135	-
Clathrina primordialis	Adriatic Sea	PMR-14305	KC479086	KC479084
*Clathrina primordialis**	Adriatic Sea	IRB-CLB3 = UFRJPOR6863	KP740016	KP739996

Clathrina ramosa	Chile	MNRJ 10313	HQ588990	-
Clathrina rubra	Adriatic Sea	PMR-14306	KC479088	KC479082
*Clathrina rubra**	Adriatic Sea	IRB-CLC2	KP740018	KP739999
Clathrina wistariensis	Australia	QMG313663	-	AM180990
Lelapiella incrustans	Vanuatu	QM G313914	-	JQ272306
Leucaltis clathria	GBR, DJ's reef	QM G316022	-	JQ272302
Levinella prolifera	GBR, Hook Reef	QM G313818	-	JQ272292
Murrayona phanolepis	Coral Sea, Osprey Reef	QM G313992	-	JQ272304
Murrayona phanolepis	Coral Sea, Bougainville Reef	QM G316290	-	AM180998
Soleneiscus radovani	GBR, Wistari Reef	QM G313661	-	JQ272289
Soleneiscus stolonifer	GBR, Wistari Reef	QM G313668	-	JQ272290
CALCARONEA			**ITS**	**28S**
Grantia compressa	-	-	-	AY563538
Grantiopsis cylindrica	GBR, Lizard Island	GW 973	-	JQ272263
Grantiopsis heroni	GBR, Wistari Reef	QM G313670	-	JQ272261
Leucandra aspera	-	-	-	AY563535
Leucandra falakra sp. nov.*	Adriatic Sea	PMR-13748/UFRJPOR8349	KT447551	KT447560
Leucandra nicolae			-	JQ272268
Leucandra spinifera sp.nov.*	Adriatic Sea	PMR-13742/UFRJPOR6861	KT447552	KT447562
Leucandra spinifera sp.nov.*	Adriatic Sea	IRB-SG3/UFRJPOR8348	KT447553	KT447561
Leucascandra caveolata	GBR	QM G316057	-	JQ272259
Paraleucilla dalmatica sp. nov.*	Adriatic Sea	PMR-13747	KT447556	KT447565
Paraleucilla dalmatica sp. nov.*	Adriatic Sea	IRB-SD5/UFRJPOR8346	KT447557	KT447566
Paraleucilla magna	Brazil	GW 824	-	JQ272267
Paraleucilla magna	South Atlantic	-	-	AM181005
*Paraleucilla magna**	Adriatic Sea	PMR-13743	KT447554	KT447563
*Paraleucilla magna**	Adriatic Sea	IRB-P14	KT447555	KT447564
Sycettusa aff. *hastifera*	Red Sea	GW 893	-	JQ272282
Sycettusa cf. *simplex*	Western Indian Ocean	ZMA POR11566	-	JQ272279
Sycettusa tenuis	GBR, Heron Reef	QM G313685	-	JQ272281
Sycon ancora sp. nov.*	Adriatic Sea	PMR-17809/UFRJPOR8345	KT447558	KT447567
Sycon ancora sp. nov.*	Adriatic Sea	IRB-SD12/UFRJPOR8347	KT447559	KT447568
Sycon capricorn	-	QM G316025	AJ633889	-
Sycon capricorn	GBR, Ribbon Reef 3	QM G316187	-	JQ272272
Sycon carteri	Australia	SAM PS 0142	-	JQ272260
Sycon ciliatum	-	-	AJ627187	AY563532
Sycon raphanus	-	-	-	AY563537
Syconessa panicula	GBR, Wistari Reef	QM G313671	-	JQ272276
Syconessa panicula	GBR, Wistari Reef	QM G313672	-	AM181007
Teichonopsis labyrinthica	Australia	SAM PS 0228	-	JQ272264
Ute ampullacea	GBR, Wistari Reef	QM G313669	-	JQ272266
Ute aff. *syconoides*	GBR, Yonge Reef	QM G313694	-	JQ272271
Ute aff. *syconoides*	Tasmania	QM G323233	-	JQ272269

different methods were applied for phylogenetic reconstruction: maximum likelihood (ML) and Bayesian inference (BI). The Akaike Information Criterion (AIC) implemented in jModeltest 3.7 (Guindon & Gascuel 2003; Darriba *et al.* 2012) was used to select the best-fit models of sequence evolution. The models were chosen for each dataset as follows: for 28S analysis, GTR+I+G and TrN+I+G models were chosen for Calcinea and Calcaronea, respectively; for ITS analysis, the TrN+G model was chosen for both datasets. Phylogenetic analyses were performed in PhyML 3.0 (Guindon *et al.* 2010), where datasets were analysed by the ML method. Bootstrap tests of phylogeny were performed with 1000 replicates. Bayesian MCMC analyses were performed in MrBayes v. 3.1.2. (Huelsenbeck & Ronquist 2001), considering the same models for given datasets. Two parallel runs each comprising four Markov chains were run for 1,000,000 generations with a sampling frequency of one in every 100 trees; a consensus tree was constructed based on the trees sampled after burn-in of 100,000. Phylogenetic trees were generated separately for each dataset, rooted at midpoint and displayed in FigTree v. 1.4.2 (http://

tree.bio.ed.ac.uk/software/figtree/). Obtained sequences were submitted to GenBank under the accession numbers KP739994–KP740035 and KT447551–KT447568 (Table 1).

Analysis of species richness

A richness analysis of all calcarean species present in the Adriatic Sea was performed using DIVA-GIS version 7.5, a free computer program for mapping and analyzing biodiversity data (http://www.diva-gis.org/) (Hijmans *et al*. 2012). Menu options chosen for this analysis were: species (parameters), 0.5 × 0.5 degrees (cell size) and simple (point to grid procedure).

Results

Species list

Ascaltis reticulum (Schmidt, 1862)
Ascandra spalatensis sp. nov.
Borojevia cerebrum (Haeckel, 1872)
Borojevia croatica sp. nov.
Clathrina conifera Klautau & Borojević, 2001
Clathrina primordialis (Haeckel, 1872)
Leucandra falakra sp. nov.
Leucandra spinifera sp. nov.
Paraleucilla dalmatica sp. nov.
Sycon ancora sp. nov.

Taxonomy

Class Calcarea Bowerbank, 1864
Subclass Calcinea Bidder, 1898
Genus ***Ascaltis*** Haeckel, 1872

Ascaltis reticulum (Schmidt, 1862)
Fig. 2; Table 2

Nardoa reticulum Schmidt, 1862: 18.
Tarrus reticulatus Haeckel, 1870: 244.
Nardopsis reticulum Haeckel, 1870: 247.
Ascandra reticulum Haeckel, 1872: 87.
Olynthus reticulum Haeckel, 1872: 88.
Clistolynthus reticulum Haeckel, 1872: 88.
Soleniscus reticulum Haeckel, 1872: 88.
Nardorus reticulum Haeckel, 1872: 88.
Tarrus reticulum Haeckel, 1872: 88.
Auloplegma reticulum Haeckel, 1872: 88.
Ascometra reticulum Haeckel, 1872: 88.
Ascandra retiformis Haeckel, 1872: 88.
Ascandra reticulata Haeckel, 1872: 88.
Clathrina reticulum Minchin, 1896: 359.
Ascandra hermesi Breitfuss, 1897a: 39.
Leucosolenia hermesi Dendy & Row, 1913: 722.

Nardoa reticulum – Schmidt 1869: 91; 1870: 73.
Ascandra reticulum – Vosmaer 1881: 5. — Lendenfeld 1891: 39. — Breitfuss 1897b: 214; 1898a: 23; 1898b: 92. — Brøndsted 1914: 530.

Leucosolenia reticulata – Dendy & Row 1913: 723. — Breitfuss 1932: 243.
Leucosolenia reticulum – Dendy & Row 1913: 723. — Breitfuss 1930: 275; 1932: 243; 1935: 14. — Topsent 1934: 9; 1936: 22. — Hôzawa 1940: 32. — Arndt 1941: 4. — Tanita 1942: 82; 1943: 386. — Burton 1963: 200.
Ascandra retiformis – Breitfuss 1932: 243.
Leucosolenia hermesi – Tanita 1942: 82.
Clathrina reticulum – Borojević 1967: 189. — Borojević & Peixinho 1976: 993. — Borojević & Boury-Esnault 1987: 12. — Klautau & Valentine 2003: 36. — Longo & Pronzato 2011: 230. — Muricy *et al.* 2011: 34.
Ascaltis reticulum – Klautau *et al.* 2013: 452.

Original type locality

ADRIATIC SEA: Zara (Croatian: Zadar) and Sebenico (Croatian: Šibenik).

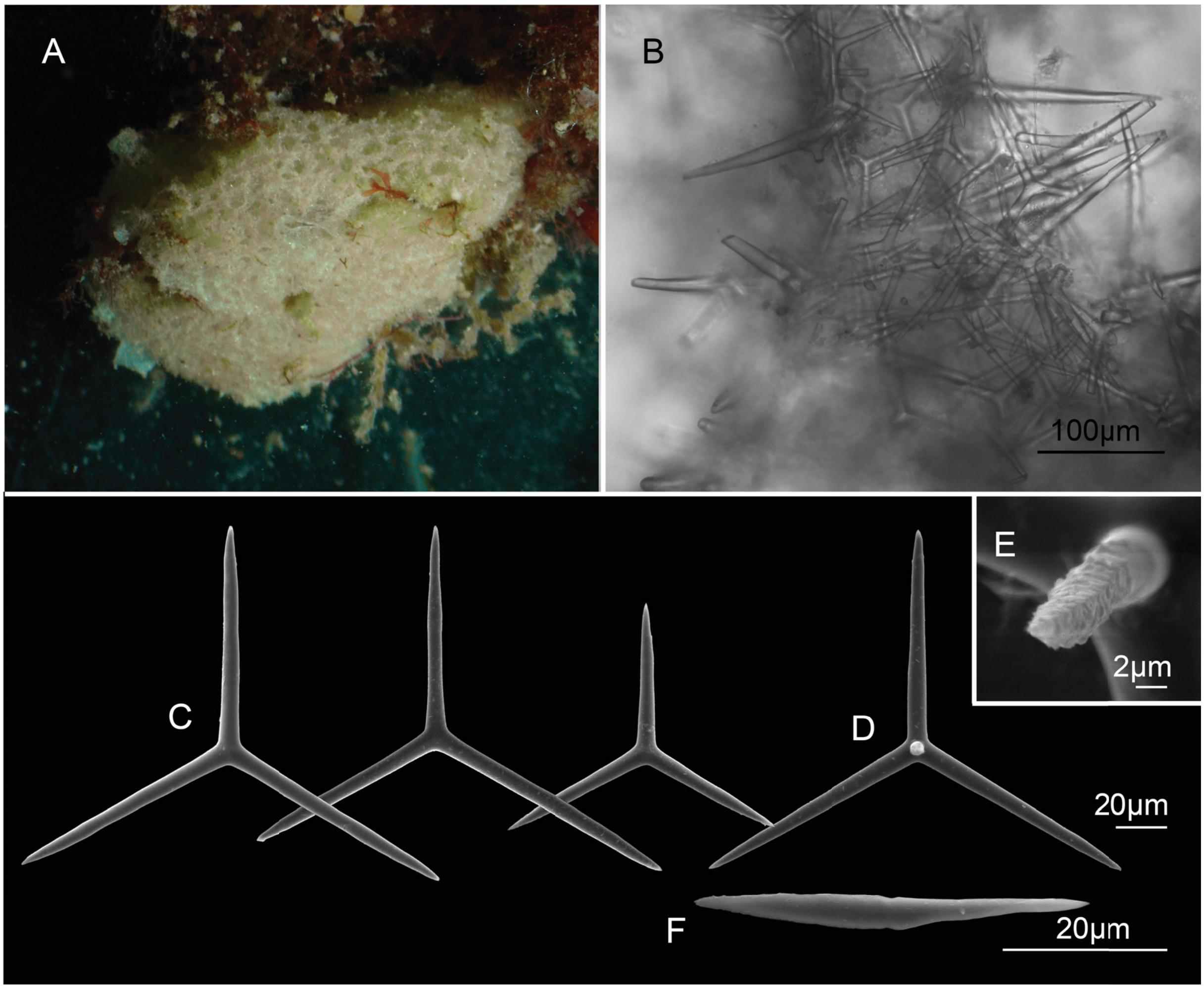

Fig. 2. *Ascaltis reticulum* (PMR 13739 = UFRJPOR 6870). **A**. Specimen *in situ*. **B**. Section showing the perpendicular arrangement of diactines. **C**. Regular triactines. **D**. Regular tetractine. **E**. Apical actine of a tetractine covered with short spines. **F**. Diactine.

Table 2. Spicule measurements of *Ascaltis reticulum* (Schmidt, 1862) (PMR-13739 = UFRJPOR 6870).

		length (µm)				**width (µm)**				
Spicule	Actine	min	mean	sd	max	min	mean	sd	max	n
Diactine	-	60.0	106.3	26.0	142.5	3.8	4.9	0.5	6.3	20
Triactine	Basal	56.7	88.2	11.7	108.0	6.8	9.5	1.5	10.8	21
Tetractine	Basal	60.0	79.1	12.4	107.5	7.5	8.8	1.3	10.0	30
	Apical	27.5	41.9	9.7	62.5	2.5	3.2	0.6	3.8	17

Type specimen

FRANCE: Banyuls-sur-Mer, Pyrenées, E.A. Minchin Collection (BMNH 1896.9.15.13, neotype proposed by Klautau & Valentine 2003).

Material examined

ADRIATIC SEA: near the Island of Čiovo, 43°28'58.5" N, 16°21'25.6" E, 5 m, 5 Nov. 2010, collected by B. Pleše and V. Nikolić (PMR-13739 = UFRJPOR 6870).

Colour

White in life and white in ethanol.

Description

Cormus is composed of regular and tightly anastomosed tubes. Water-collecting tubes are present (Fig. 2A). As the specimen was fragmented, it was not possible to observe the pseudoatrium. The skeleton is composed of one category of triactines, one of tetractines and diactines. Diactines are organised in tufts of two to five spicules, perpendicularly disposed in the tubes (Fig. 2B). Triactines are the most abundant spicules.

Spicules (Table 2)

TRIACTINES. Regular (equiangular and equiradiate). Actines are slightly conical to cylindrical, with sharp tips (Fig. 2C). Size: 88.2/9.5 µm.

TETRACTINES. Regular (equiangular and equiradiate). Actines are slightly conical to cylindrical, with sharp tips (Fig. 2D). The apical actine is very thin and shorter than the basal ones. It is cylindrical and blunt, covered by abundant tiny spines (Fig. 2E). Size: 79.1/8.8 µm (basal actine); 41.9/3.2 µm (apical actine).

DIACTINES. Slightly curved. The tip that protrudes through the surface is lanceolated (Fig. 2F). Trichoxeas are also present on the surface of the tubes. Size: 106.3/4.9 µm.

Ecology

Specimens were collected on a vertical, shaded hard limestone bottom.

Remarks

Klautau *et al.* (2013) proposed to transfer this species to the genus *Ascaltis* based mainly on morphological, but also on molecular data. Although the type species of this genus (*A. lamarcki* Haeckel, 1870) was not included in the molecular dataset, *A. reticulum* did not group with any of the included genera (Fig. 16). Besides, morphologically it is more similar to *Ascaltis* than to any other genus. Therefore, although the classification of *A. reticulum* in the genus *Ascaltis* must still be verified regarding the type species of the genus, it was morphologically and molecularly proved that it cannot be included in the genus *Clathrina*. Hence, we keep the proposition of Klautau *et al.* (2013) and name this species *A. reticulum*.

This is the first time that spines were observed on the apical actine of the tetractines of *A. reticulum*. For that reason, we examined the neotype of this species and detected spines as well. They are abundant and very small. We also observed a great variation in the size of the diactines, which are much larger in the neotype (102.0–212.2 (±54.1)–306.0 /14.3 (±5.1) µm).

Genus ***Ascandra*** Haeckel, 1872

Ascandra spalatensis sp. nov.

urn:lsid:zoobank.org:act:A5DC68F2-D856-4492-AF50-E5F6A1A7FD8A

Fig. 3; Table 3

Etymology

From the type locality. *Spalato* is an Italian name for Split, the largest city of the Dalmatian region.

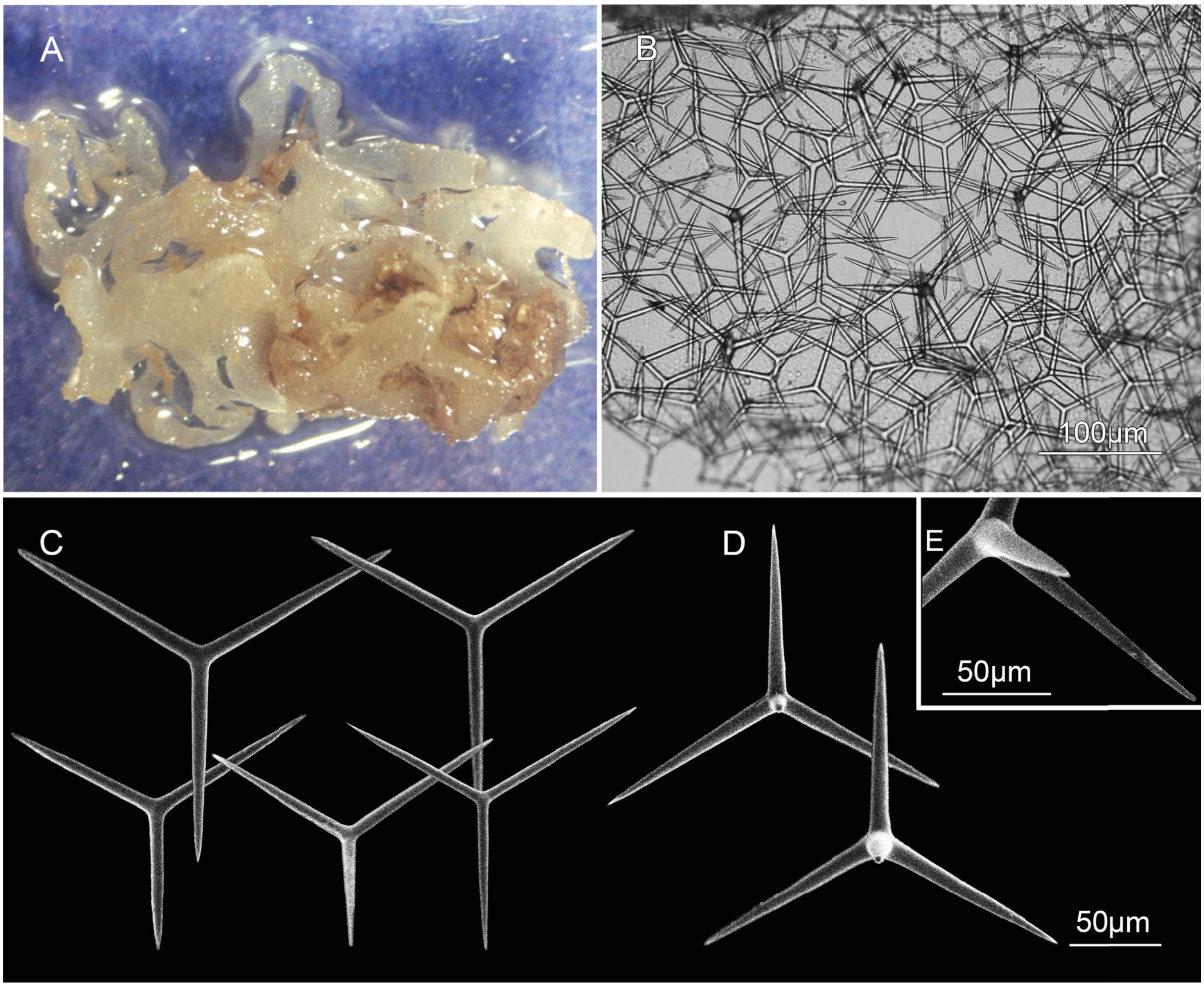

Fig. 3. *Ascandra spalatensis* sp. nov., holotype (PMR 17806 = UFRJPOR 7540). **A**. Specimen in ethanol. **B**. Tangential section. **C**. Triactines. **D**. Tetractines. **E**. Apical actine of a tetractine.

Table 3. Spicule measurements of the holotype of *Ascandra spalatensis* sp. nov. (PMR-17806 = UFRJPOR 7540).

		length (µm)				width (µm)				
Spicule	Actine	min	mean	sd	max	min	mean	sd	max	n
Triactine	Basal	43.2	90.5	17.2	113.4	6.8	8.0	0.8	9.5	20
Tetractine	Basal	51.3	99.4	16.9	135.0	8.1	12.0	1.6	14.9	21
	Apical	72.9	74.3	1.9	75.6	10.8	10.8	0	10.8	2

Material examined

Holotype

ADRIATIC SEA: near Zadar, 44°08'14.8" N, 15°12'38.2" E, 1 m, collected by V. Nikolić, 13 Feb. 2011 (PMR-17806 = UFRJPOR 7540, in ethanol).

Colour

White in ethanol.

Description

The sponge is small, only a fragment, but it is possible to recognise large and loosely anastomosed tubes, typical of *Ascandra* (Fig. 3A). The skeleton is composed of triactines and a few tetractines (Fig. 3B).

Spicules (Table 3)

Triactines. Regular (equiangular and equiradiate), but there are also subregular (sagittal) spicules. Actines are cylindrical to slightly conical with sharp tips (Fig. 3C). Size: 90.5/8.0 µm.

Tetractines. Regular (equiangular and equiradiate) or subregular. Actines are strongly conical with sharp tips (Fig. 3D). The apical actine is shorter than the basal ones, thick, conical, sharp and smooth (Fig. 3E). Size (basal actine): 99.4/12.0 µm; 74.3/10.8 µm (apical actine).

Ecology

The specimen was collected on a shaded, vertical hard bottom.

Remarks

The genus *Ascandra* is so far composed of 13 species: *A. falcata* Haeckel, 1872; *A. ascandroides* (Borojević, 1971); *A. atlantica* (Thacker, 1908); *A. biscayae* (Borojević & Boury-Esnault, 1987); *A. brandtae* (Rapp *et al.*, 2013); *A. contorta* (Bowerbank, 1866); *A. corallicola* (Rapp, 2006); *A. crewsi* Van Soest & De Voogd, 2015; *A. densa* Haeckel, 1872; *A. kakaban* Van Soest & De Voogd, 2015; *A. loculosa* (Dendy, 1891); *A. minchini* Borojević, 1966; and *A. sertularia* Haeckel, 1872.

In 2013, Klautau *et al.* proposed the following diagnosis for *Ascandra*: “Calcinea with loosely anastomosed tubes. Tubes are free, at least in the apical region. The skeleton contains regular (equiangular and equiradiate) or sagittal triactines and tetractines. Tetractines are the main spicules, occurring at least in the same proportion as the triactines. They have very thin (needle-like) apical actines. Diactines may be added. Asconoid aquiferous system.”

After the discovery of *A. spalatensis* sp. nov., we propose here an emendation to this diagnosis: “Calcinea with loosely anastomosed tubes. Tubes are free, at least in the apical region. The skeleton contains regular (equiangular and equiradiate) or sagittal triactines and tetractines. The apical actine is very thin (needle-like) or very thick at the base. Diactines may be added. Asconoid aquiferous system.”

Our new species is a very typical *Ascandra*, with apically free, loosely anastomosed tubes. Its skeleton is very similar to that of *A. ascandroides*, i.e., composed of triactines and tetractines, the former being more abundant than the latter and the apical actine of the tetractines being very thick at the base. Both species, however, can be differentiated by the size of the spicules (*A. ascandroides* - triactines: 90–130(±20)–163 / 13(±2); small tetractines: 107.5–164.5(±35)–260 / 16.5(±2.8); large tetractines: 193.8–313.1(±63.2)–418.2 / 39.8(±8.2)). Moreover, *A. ascandroides* has two categories of tetractines and *A. spalatensis* sp. nov. only one. In our ITS tree (Fig. 16) this species is well nested within the *Ascandra* clade, with high support values in both, Bayesian and ML analyses.

Genus ***Borojevia*** Klautau *et al.*, 2013

Borojevia cerebrum (Haeckel, 1872)
Fig. 4; Table 4

Ascaltis cerebrum Haeckel, 1872: 54.
Auloplegma cerebrum Haeckel, 1872: 55.
Ascaltis decipiens Haeckel, 1872: 55.
Ascaltis gyrosa Haeckel, 1872: 55.

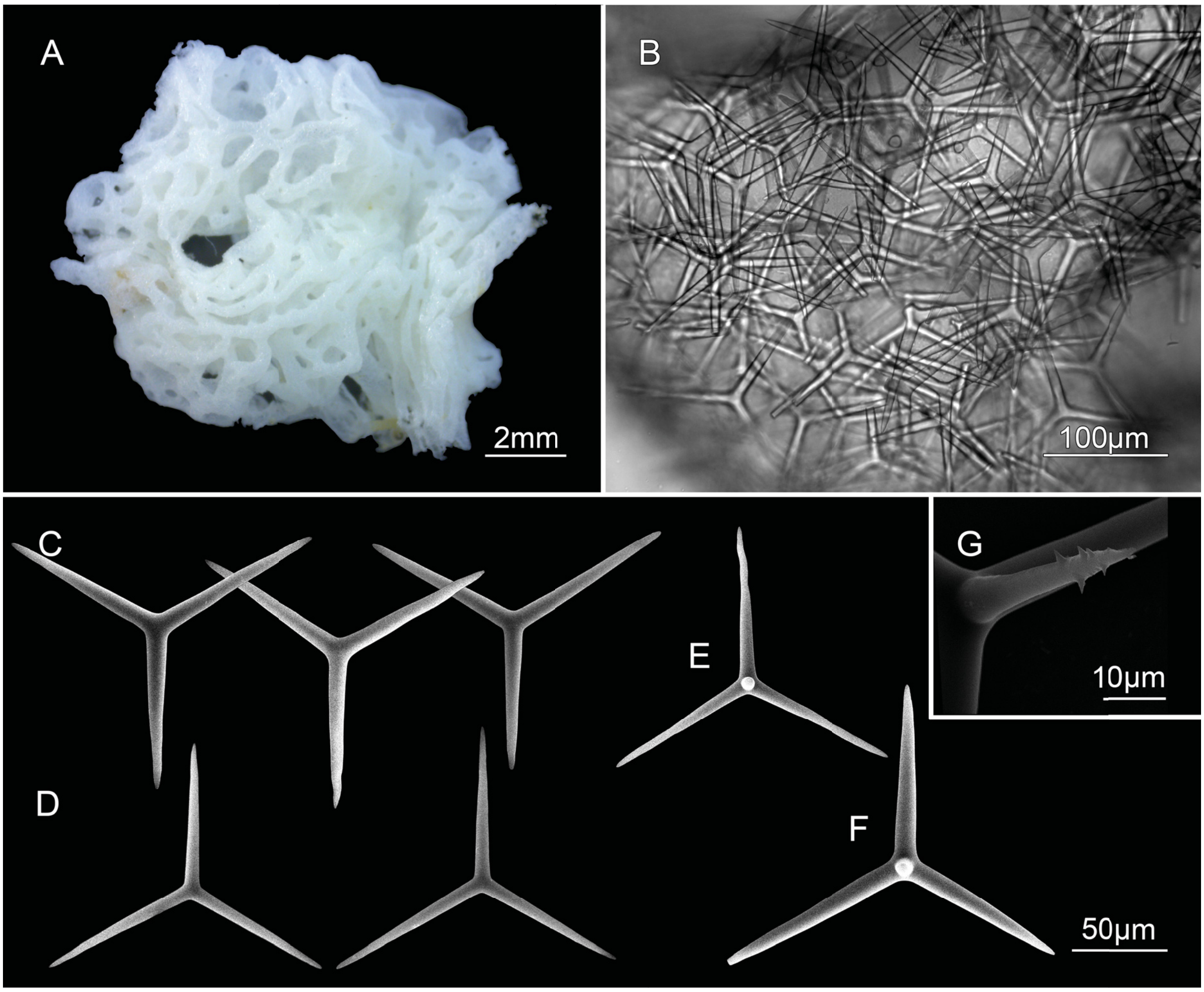

Fig. 4. *Borojevia cerebrum* (IRB-CLB33 = UFRJPOR 7539). **A**. Specimen in ethanol. **B**. Tangential section. **C**. Tripods. **D**. Triactines. **E**. Small tetractine. **F**. Large tetractine. **G**. Apical actine of a tetractine ornamented with spines.

Table 4. Spicule measurements of *Borojevia cerebrum* (IRB-CLB33 = UFRJPOR 7539).

		length (µm)				**width (µm)**				
Spicule	Actine	min	mean	sd	max	min	mean	sd	max	n
Tripod	Basal	72.9	91.8	9.9	108.0	8.1	11.2	1.5	13.5	20
Triactine	Basal	54.0	84.6	11.8	105.3	5.4	8.9	1.3	10.8	20
Tetractine	Basal	64.8	81.9	10.0	105.3	6.8	8.5	1.5	12.2	20
	Apical	35.1	46.8	8.4	64.8	5.4	5.4	0.0	5.4	20

Ascetta cerebrum – Bianco 1888: 386. — Lendenfeld 1891: 206 — Bidder 1891: 628.
Clathrina cerebrum – Minchin 1896: 359. — Borojević 1967: 192. — Borojević *et al.* 1968: 31. — Solé-Cava *et al.* 1991: 382. — Klautau & Valentine 2003: 14. — Longo & Pronzato 2011: 219.
Leucosolenia cerebrum – Kirk 1896: 207. — Breitfuss 1897b: 210; 1898c: 172; 1935: 8. — Dendy & Row 1913: 724. — Burton 1933: 236; 1963: 186. — Topsent 1934: 7; 1936: 17.
Leucosolenia decipiens – Dendy & Row 1913: 725. — Ferrer 1918: 9.— Breitfuss 1935: 9. — Tanita 1943: 78.
Borojevia cerebrum – Klautau *et al.* 2013: 452.

non Clathrina cerebrum – Borojević 1971: 526 (*non Clathrina cerebrum*).

Type specimen

ADRIATIC SEA: Lesina (Croatian: Island of Hvar), Haeckel collection (PMJ-Inv. Nr. Porif. 156, syntype/ethanol).

Material examined

ADRIATIC SEA: Vrulja Cove, 43°24'01.3" N, 16°53'10.9" E, 10 m, collected by V. Nikolić, 24 Aug. 2011 (PMR-17808; IRB-CLB33 = UFRJPOR 7539).

Colour

Light yellow in life and in ethanol.

Description

Cormus is composed of regular and tightly anastomosed tubes (Fig. 4A). Large water-collecting tubes are present. The skeleton consists of triactines, a few tetractines and tripods, which in fact are large triactines. It has no special organisation (Fig. 4B).

Spicules (Table 4)

Tripods. Regular (equiangular and equiradiate). The tripods of analysed specimens are more similar to large triactines than to true tripods with an elevated centre. Actines are conical, straight, with sharp tips (Fig. 4C). Size: 91.8/11.2 µm.

Triactines. Regular (equiangular and equiradiate). Actines are slightly conical to conical, straight, with sharp tips. Sometimes they are slightly undulated near the tips (Fig. 4D). Size: 84.6/8.9 µm.

Tetractines. Regular (equiangular and equiradiate). Actines are slightly conical to conical, straight, with sharp tips. Sometimes they are slightly undulated near the tips. It is possible to recognise two types of tetractines: small (Fig. 4E) and large (Fig. 4F). Large tetractines are the same size as tripods. The apical actine of the tetractines is shorter than the basal ones, slightly conical, sharp and frequently curved only at the tip. It is ornamented with few (*ca*. six) spines, which are large, conical and cover only the last third of the apical actine. (Fig. 4G). Size: 81.9/8.5 µm (basal actine); 46.8/5.4 µm (apical actine).

Ecology

The specimen was collected on a semi-vertical hard limestone bottom.

Remarks

Similar to other species of *Borojevia*, *B. cerebrum* has thin, regular and tightly anastomosed tubes forming the cormus. The oscula are present at the end of water-collecting tubes. The skeleton is composed of tripods (with the characteristic elevated centre or similar to large triactines), triactines and tetractines. Individuals of *B. cerebrum* always have spines on the apical actine of their tetractines; however, in the same individual some tetractines may be smooth. In *B. cerebrum*, the spines are not very abundant; they are large and scattered, only near the tip of the apical actine. The Adriatic and Mediterranean specimens of *B. cerebrum* formed a well supported clade in the ITS tree (Fig. 16), separated from the clade comprising *B. brasiliensis* (Solé-Cava, Klautau, Boury-Esnault, Borojević & Thorpe, 1991).

Borojevia cerebrum is the type species of the genus. Its type locality is Lesina (Island of Hvar) and it commonly occurs in the Mediterranean and the Adriatic Sea. The type specimen of *B. cerebrum* (PMJ-Inv. Nr. Porif. 156) is not very well preserved (Klautau & Valentine 2003); thus, we got a great opportunity to redescribe this species from near its type locality.

Analyses of other individuals of *B. cerebrum* from several sites in the Adriatic and Mediterranean Seas verify that the shape of the tripods is very variable. It varies from the characteristic shape of tripods, with stout actines and elevated centre, to only large triactines. This kind of variability may be assigned to polymorphism or plasticity. Indeed, Haeckel (1872) proposed two varieties of *B. cerebrum* (as *Ascaltis cerebrum*), based on the presence of either characteristic tripods or large triactines. The first variety he called *B. cerebrum* var. *gyrosa*, while the other one he considered *B. cerebrum* var. *decipiens*. Dendy & Row (1913) elevated *B. cerebrum* var. *decipiens* to species level (as *Leucosolenia decipiens*) and kept *B. cerebrum* (as *L. cerebrum*) as a valid species. The variety *gyrosa* had not been oficially elevated to the status of species; however, it was mentioned as *Ascaltis gyrosa* in a synonym list of *B. cerebrum* made by Burton (1963: 186).

Considering that both varieties were proposed only to differentiate specimens with characteristic tripods from those with only large triactines and that we found this morphological variation inside individuals and among specimens placed within the same species, we propose here the synonymisation of *B. decipiens* with *B. cerebrum*.

Borojevia croatica sp. nov.

urn:lsid:zoobank.org:act:A9F84084-E033-43A9-AB71-83670090C7C1

Fig. 5; Table 5

Etymology

From the type locality.

Material examined

Holotype

ADRIATIC SEA: near the Island of Čiovo, 43°28'58.5" N, 16°21'25.6" E, 5 m, collected by B. Pleše and V. Nikolić, 5 Nov. 2010 (PMR-13740 = UFRJPOR 6864, in ethanol).

Paratype

ADRIATIC SEA: same data as holotype (PMR-13741 = UFRJPOR 6865, in ethanol).

Colour

White in life and in ethanol.

Description

Cormus composed of regular and tightly anastomosed tubes (Fig. 5A). Water-collecting tubes are present and form a single apical osculum. The skeleton is composed of tripods, triactines and rare tetractines. It has no special organisation (Fig. 5B).

Spicules (Table 5)

TRIPODS. Regular (equiangular and equiradiate) or sagittal. Some of them have an elevated centre, but most appear like large regular triactines. Actines are conical, straight, with sharp tips (Fig. 5C). Size: 102.6/11.9 µm.

TRIACTINES. Regular (equiangular and equiradiate). Actines are conical, straight, with sharp tips (Fig. 5D). Size: 66.6/7.5 µm.

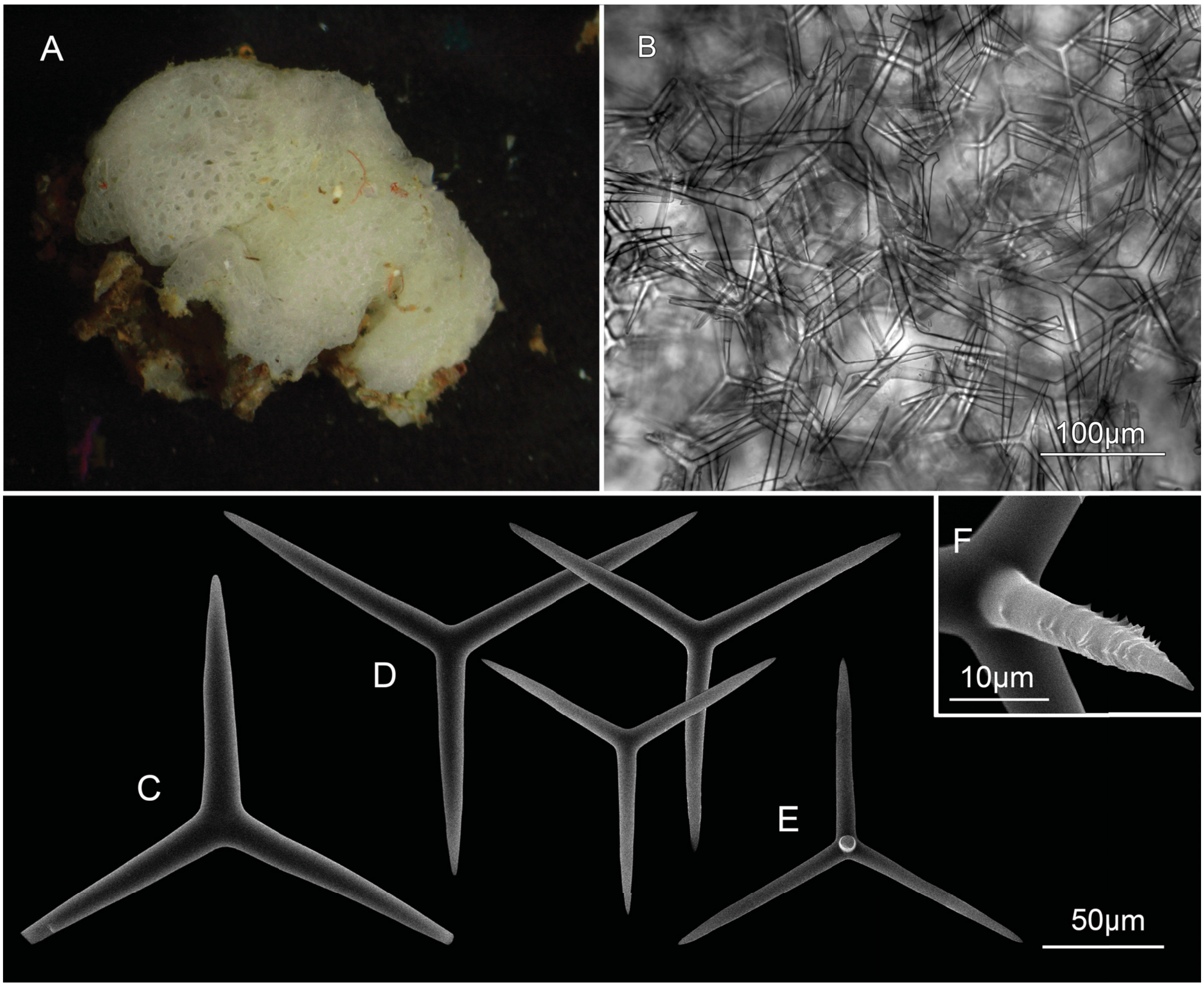

Fig. 5. *Borojevia croatica* sp. nov., holotype (PMR 13740 = UFRJPOR 6864). **A**. Specimen *in situ*. **B**. Tangential section. **C**. Tripod. **D**. Triactines. **E**. Tetractine. **F**. Apical actine of a tetractine ornamented with spines.

Table 5. Spicule measurements of *Borojevia croatica* sp. nov. Holotype (PMR-13740 = UFRJPOR 6864) and paratype (PMR-13741 = UFRJPOR 6865).

		length (µm)				**width (µm)**				
		min	mean	sd	max	min	mean	sd	max	n
Holotype										
Tripod	Basal	85.0	102.6	10.0	115.0	10.0	11.9	1.5	15.0	20
Triactine	Basal	57.5	66.6	6.7	82.5	7.5	7.5	0.0	7.5	20
Tetractine	Basal	60.0	70.0	6.3	77.5	7.5	8.3	1.2	11.3	10
	Apical	-	20	-	-	-	5.0	-	-	1
Paratype										
Tripod	Basal	50.0	78.8	19.1	115.0	7.5	10.8	1.8	15.0	13
Triactine	Basal	50.0	66.1	8.6	80.0	7.5	7.8	0.7	10.0	20
Tetractine	Basal	62.5	71.0	5.1	80.0	7.5	8.3	1.1	10.0	20

TETRACTINES. Regular (equiangular and equiradiate). Actines are conical, straight, with sharp tips (Fig. 5E). The apical actine has very short and abundant spines organised in parallel rows. These spines cover the first ⅔ of the apical actine (Fig. 5F). Size: 70.0/8.3 µm (basal actine); 20.0/5.0 µm (apical actine).

Ecology

Specimens were collected on a shaded, vertical, hard limestone bottom.

Remarks

The genus *Borojevia* is currently composed of five species: *B. aspina* (Klautau, Solé-Cava & Borojević, 1994), *B. brasiliensis*, *B. cerebrum*, *B. paracerebrum* (Austin, 1996) and *B. tetrapodifera* (Klautau & Valentine, 2003). All of them show a very well defined cormus, with regular and tightly anastomosed tubes and water-collecting tubes. The skeleton is always composed of tripods, triactines and tetractines with spines on the apical actines. Tetrapods may also be present (*B. tetrapodifera*). The sixth species of the genus, *B. croatica* sp. nov., is closer to *B.* cf. *aspina* in our ITS tree (Fig. 16). Both species have short spines; however, *B. croatica* sp. nov. has numerous spines, while in *B.* cf. *aspina* there are few.

Given that *B. cerebrum* is also present in the Adriatic Sea, the best way to differentiate it from *B. croatica* sp. nov. is by the shape and location of spines. They are shorter, more abundant and distributed along most of the actine length in *B. croatica* sp. nov., and larger, fewer and scattered only near the tip of the apical actine in *B. cerebrum*.

Genus ***Clathrina*** Gray, 1867

Clathrina conifera Klautau & Borojević, 2001

Fig. 6; Table 6

Clathrina conifera Klautau & Borojević, 2001: 404.

Clathrina primordialis (*non C. primordialis* (Haeckel, 1872)) – Borojević 1971: 527. — Borojević & Peixinho 1976: 992. — Mothes de Moraes 1985: 228. — Klautau *et al.* 1994: 372. — Muricy & Silva 1999: 160.

Clathrina conifera – Klautau & Valentine 2003: 18. — Monteiro & Muricy 2004: 682. — Muricy & Hajdu 2006: 86. — Lanna *et al.* 2007: 1554. — Custódio & Hajdu 2011: 4. — Muricy *et al.* 2011: 33. — Bouzon *et al.* 2012: 42.

Material examined

Holotype

BRAZIL: Arraial do Cabo, Rio de Janeiro (BMNH 1999.9.16.19, in ethanol).

Other material

ADRIATIC SEA: near the Island of Lokrum, 42°37’55.6” N, 18°06’49.4” E; 1–3 m deep, collected by V. Nikolić, 8 Oct. 2010 (PMR-13738 = UFRJPOR 6869); near Dubrovnik, 42°38'26.5" N, 18°06'14.2" E; 1 m, collected by V. Nikolić, 24 Sep. 2011 (PMR-17807, IRB-S2 = UFRJPOR 7541, IRB-S3 = UFRJPOR 7542).

Colour

White in life and white or brown in ethanol.

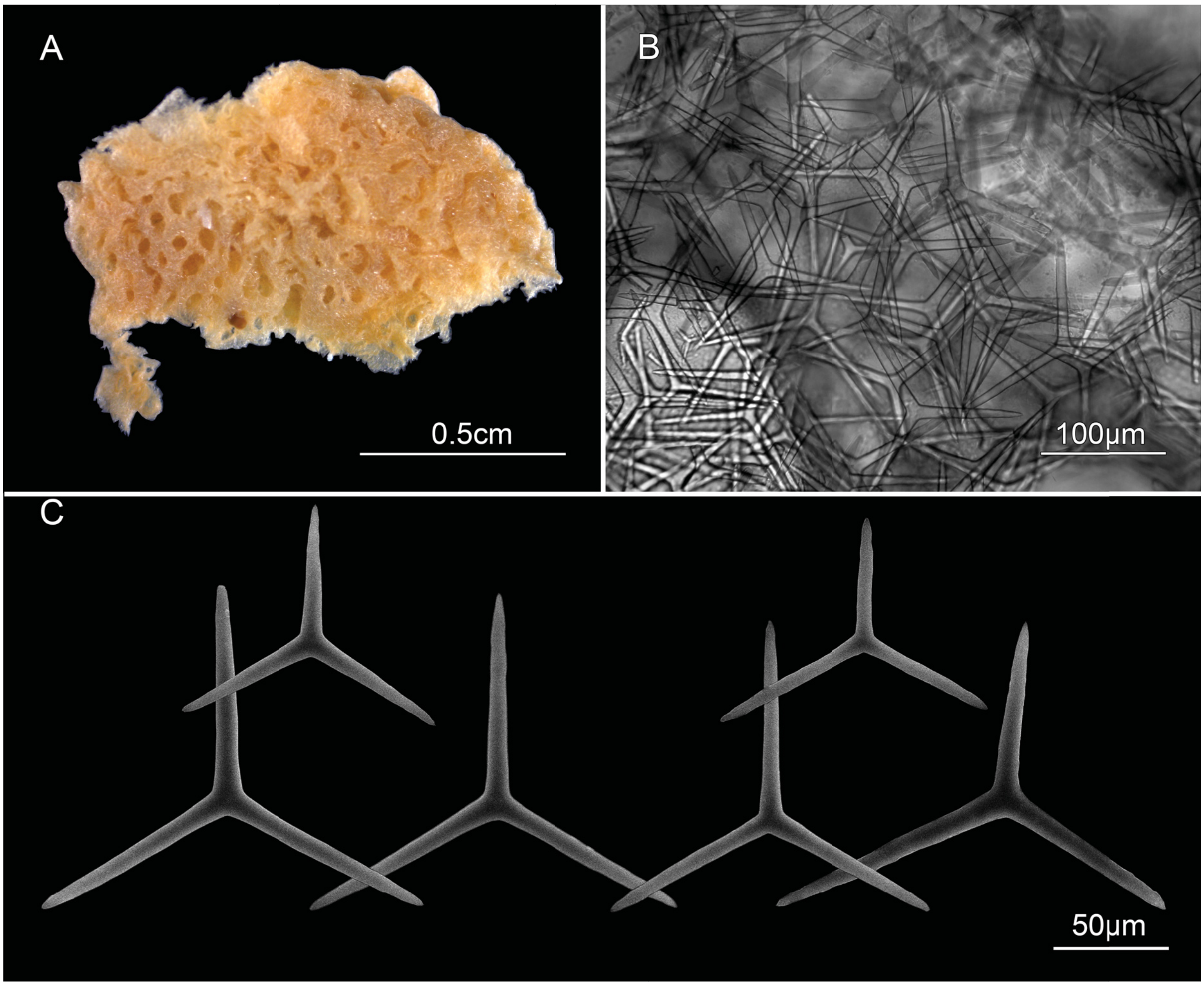

Fig. 6. *Clathrina conifera* (PMR 13738 = UFRJPOR 6869). **A**. Specimen in ethanol. **B**. Tangential section. **C**. Triactines with variable sizes and shapes.

Table 6. Spicule measurements (triactines) of *Clathrina conifera* Klautau & Borojević, 2001 (PMR-13738 = UFRJPOR 6869; IRB-S2 = UFRJPOR 7541; IRB-S3 = UFRJPOR 7542).

	length (µm)				**width (µm)**				
	min	mean	sd	max	min	mean	sd	max	n
PMR-13738 = UFRJPOR6869	57.5	88.5	11.8	122.5	7.5	10.2	1.6	15.0	30
IRB-S2 = UFRJPOR7541	59.4	86.3	9.6	97.2	8.1	9.5	1.4	10.8	20
IRB-S3 = UFRJPOR7542	64.8	82.5	8.9	102.6	6.8	8.8	1.3	10.8	20

Description

Cormus composed of irregular and loosely anastomosed tubes (Fig. 6A). Water-collecting tubes are not present. The skeleton consists of triactines without organisation (Fig. 6B).

Spicules (Table 6)

TRIACTINES. Regular (equiangular and equiradiate). Their size is very variable. Actines are slightly conical to conical, straight, with blunt tips. Sometimes they are slightly undulated near the tips (Fig. 6C). Size: 88.5/10.2 µm.

Ecology

Specimens were collected on a semi-shaded, vertical hard limestone bottom under overhangs. They were often found in association with the macroalga *Ellisolandia elongata* (J. Ellis & Solander, 1786).

Remarks

Until now, this species was considered endemic to Brazil (Borojević 1971; Mothes de Moraes 1985; Klautau *et al.* 1994; Monteiro & Muricy 2004; Muricy & Hajdu 2006; Lanna *et al.* 2007). Originally, it was identified as *C. primordialis* (Haeckel, 1872) (Borojević 1971; Mothes de Moraes 1985; Klautau *et al.* 1994). However, considering differences in the size of the actines (holotype of *C. conifera*: 62.5–77.3(±9.3)–97.5/9(±1.0) µm; Haeckel measurements of *C. primordialis*: 100–150/8–12 µm), it was described as a new species: *C. conifera*. In the present work, we confirmed by morphological and molecular analyses (Fig. 16) that *C. conifera* is really distinct from *C. primordialis* and that it occurs in the Adriatic Sea.

Clathrina primordialis (Haeckel, 1872)

Fig. 7; Table 7

?*Grantia pulchra* Schmidt, 1862:18.
?*Leucosolenia pulchra* Schmidt, 1866: 8.
Prosycum primordiale Haeckel, 1870: 237.
Olynthus simplex Haeckel, 1870: 237.
Nardoa arabica Miklucho *in* Haeckel, 1872: 16.
Ascetta primordialis Haeckel, 1872: 16.
Olynthus primordialis Haeckel, 1872: 16.
Clistolynthus primordialis Haeckel, 1872: 16.
Soleniscus primordialis Haeckel, 1872: 16.
Ascometra primordialis Haeckel, 1872: 16.
?*Ascaltis primordialis* Haeckel, 1872: 17.
?*Ascortis primordialis* Haeckel, 1872: 17.

?*Ascandra primordialis* Haeckel, 1872: 18.
Ascetta primordialis Lendenfeld, 1885: 897.
L. primordialis var. *apicalis* Brøndsted, 1931: 9.
Clathrina cf. *hondurensis* – Imesek *et al.* 2014: 25.

Ascetta primordialis – Lendenfeld 1891: 11. — Arnesen 1901: 12.
Clathrina primordialis – Carter 1886: 510. — Minchin 1896: 359. — Jenkin 1908: 436. — Row 1909: 184. — Klautau & Valentine 2003: 32. — Longo & Pronzato 2011: 229.
Leucosolenia primordialis – Lackschewitsch 1886: 299. — Breitfuss 1898a: 12; 1898b: 91. — Dendy & Row 1913: 726. — Ferrer Hernández 1918: 10. — Burton 1926: 71; 1963: 197. — Row & Hôzawa 1931: 736. —Breitfuss 1932: 242; 1935: 12. — Arndt 1941: 45. — Tanita 1942: 73; 1943: 370.

non C. primordialis – Borojević 1971: 527. — Borojević & Peixinho 1976: 992. — Mothes de Moraes 1985: 228. — Klautau *et al.* 1994: 372. — Muricy & Silva 1999: 160.

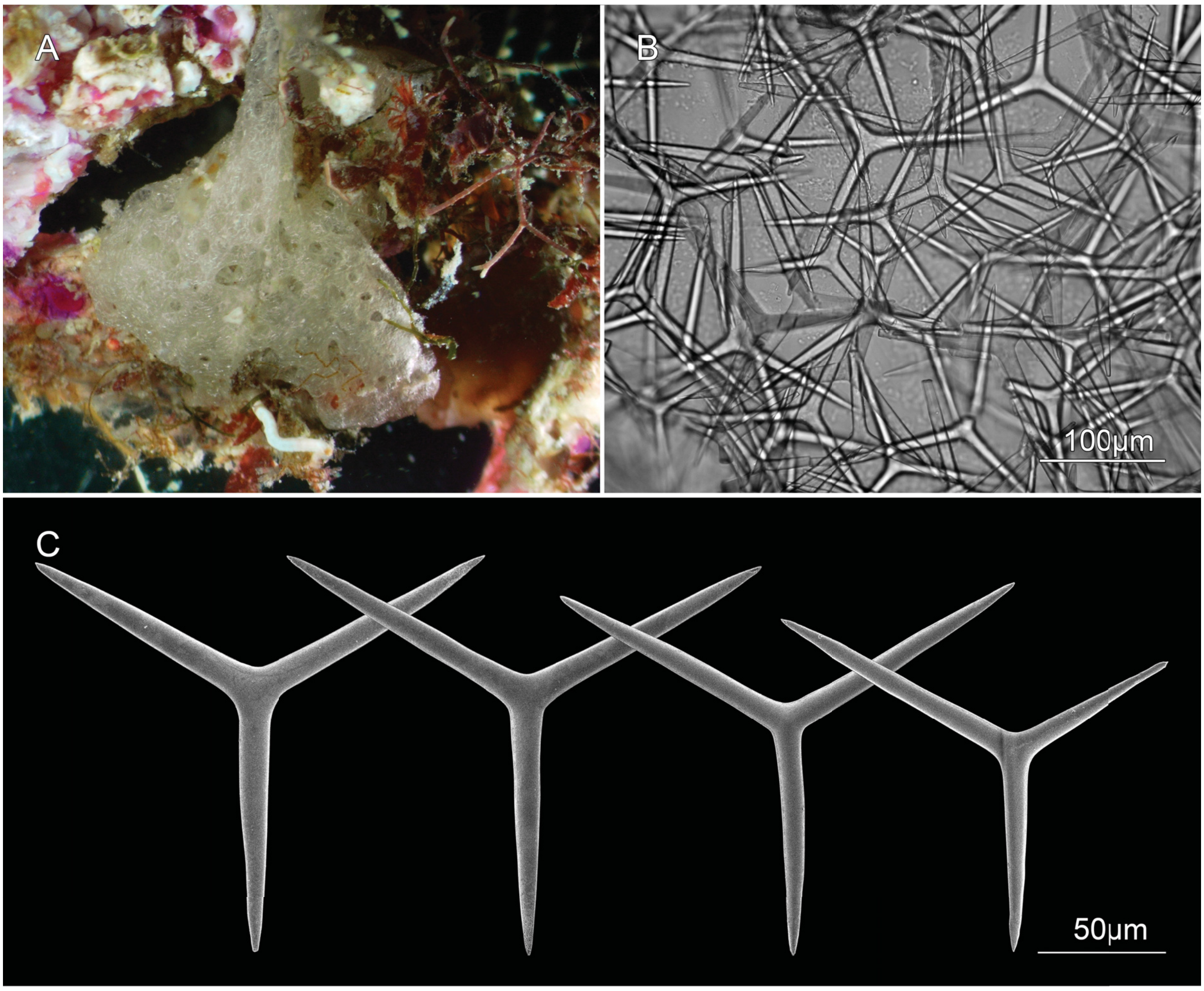

Fig. 7. *Clathrina primordialis* (IRB-CLB3 = UFRJPOR 6863). **A**. Specimen *in situ*. **B**. Tangential section. **C**. Triactines with variable sizes and shapes.

Table 7. Spicule (triactine) measurements of *Clathrina primordialis* (Haeckel, 1872). IRB-CLB3 = UFRJPOR 6863 (present work); PMJ 154 (suggested lectotype); PMR-14305 (*C.* cf. *hondurensis* in Imešek *et al.* 2014); BMNH 1938.3.28.4 (holotype of *C. hondurensis*).

	length (µm)				**width (µm)**				
	min	mean	sd	max	min	mean	sd	max	n
IRB-CLB3 = UFRJPOR 6863	47.5	121.5	27.9	157.5	7.5	12.2	2.4	15.0	20
Haeckel, 1872	100.0	-	-	150.0	8.0	-	-	12.0	-
PMJ154 (lectotype)	97.5	134.0	16.3	157.5	10.0	13.0	2.2	17.5	30
PMR-14305	101.8	128.0	9.6	151.5	13.3	15.2	1.3	19.0	33
BMNH 1938.3.28.4	105.6	133.4	17.0	156.0	12.0	15.6	1.7	19.2	20

Material examined

Syntype

ADRIATIC SEA: Lesina (Croatian: Island of Hvar), E. Haeckel collection (PMJ-Inv. Nr. Porif. 154, in ethanol).

Other material

ADRIATIC SEA: near the Island of Čiovo, Croatia, 43°28'58.5" N, 16°21'25.6" E; 5 m, collected by B. Pleše and V. Nikolić, 5 Nov. 2010 (IRB-CLB3 = UFRJPOR 6863).

Colour

White in life and in ethanol.

Description

Cormus is formed by large and loosely anastomosed tubes. Water-collecting tubes are absent (Fig. 7A). The skeleton is composed of one category of triactines (Fig. 7B). The size of the spicules is very variable and it is therefore not possible to categorize them.

Spicules (Table 7)

Triactines. Regular (equiangular and equiradiate). Actines are conical to slightly conical with sharp tips (Fig. 7C). Their size is very variable. Size: 121.5/12.2 µm.

Ecology

The specimen was collected on a shaded, vertical hard limestone bottom.

Remarks

Haeckel (1872) assigned the name *Ascetta primordialis* to a group of different species, and even genera, whose skeleton comprised only triactines, but, unfortunately, did not select a holotype. In 2003, Klautau & Valentine revised the genus *Clathrina* and analysed two specimens of *C. primordialis*, one from the Adriatic Sea (PMJ 154) and another one from Naples (ZMB 1306). Both specimens clearly represented different species and the authors suggested the specimen ZMB 1306 was the true *C. primordialis*, because *C. primordialis* (originally *Prosycum primordiale* Haeckel, 1870) was first described from Naples.

However, analysing the present specimen and re-analysing the slides of the specimens PMJ 154 and ZMB 1306 and the catalogue from the ZMB, we now have a different opinion. On the specimen's label and in the catalogue of the ZMB it is not noted that ZMB 1306 is a syntype of *C. primordialis*. Consequently, Klautau & Valentine (2003) should not have designated the specimen ZMB 1306 as a lectotype of *C. primordialis*. On the other hand, the label of the specimen PMJ 154 mentions it is a syntype of *C. primordialis*. Therefore, in our opinion, the specimen PMJ 154 is more reliably a true representative of this species and should be considered the lectotype of *C. primordialis*.

Considering the morphology of PMJ 154, the specimen IRB-CLB3 = UFRJPOR 6863 represents *C. primordialis*, as well as the specimen PMR 14305, recently published as *C.* cf. *hondurensis* Klautau & Valentine, 2003 (Imešek *et al.* 2014). The similarities between *C. primordialis* and *C. hondurensis* made us ponder on the possibility of synonymy between these two species. However, as we could not obtain DNA sequences of *C. hondurensis* from the type locality (Honduras) to verify this, we prefer to keep *C. hondurensis* as a valid species restricted to the Caribbean Sea, until further analyses are done.

Subclass Calcaronea Bidder, 1898
Genus ***Leucandra*** Haeckel, 1872

Leucandra falakra sp. nov.

urn:lsid:zoobank.org:act:3DEBFC2C-2E7E-4D8E-A7FA-7FE971CFEDB2

Figs 8–9; Table 8

Etymology

From the Greek *falákra* (φαλάκρα), meaning bald, for the absence of diactines.

Material examined

Holotype

ADRIATIC SEA: near the Island of Blitvenica, 43°37'31.96" N, 15°34'25.94" E; 5 m, collected by V. Nikolić, 10 Oct. 2012 (PMR-13748 = UFRJPOR 8349, in ethanol).

Colour

White in life and in ethanol.

Description

The sponge is massive and vase-shaped, with one apical osculum without crown. The atrium is central and large. The aquiferous system is leuconoid (Fig. 8A). The sponge surface is smooth, but harsh. The cortical skeleton is composed of small, tangentially arranged triactines. The choanosomal skeleton has no organisation (Fig. 8B). It is composed of two categories of triactines (giant triactines and triactines larger than those of the cortex) (Fig. 8C). There are also tetractines and some triactines surrounding the canals (Fig. 8D). The atrial skeleton is smooth, composed mainly of triactines, with a few tetractines also present (Fig. 8E).

Spicules (Table 8)

CORTICAL TRIACTINES. Subregular to sagittal, equiradiate and small. Actines are cylindrical, blunt and curved (Fig. 9A–B). Size: 136.4/11.1 µm (paired actine); 106.0/11.4 µm. (unpaired actine).

CHOANOSOMAL SMALL TRIACTINES. Subregular to sagittal. Actines are conical and sharp (Fig. 9C–D). Size: 214.2/18.3 µm (paired actine); 189.7/19.8 µm. (unpaired actine).

CHOANOSOMAL GIANT TRIACTINES. Subregular to sagittal, equiradiate. Actines are conical and sharp (Fig. 9E–F). Size: 624.5/81.5 µm.

CHOANOSOMAL TETRACTINES. Sagittal. These spicules are present only surrounding the canals. Actines are cylindrical, sharp and curved. The unpaired actine is a little shorter than the paired ones. The apical

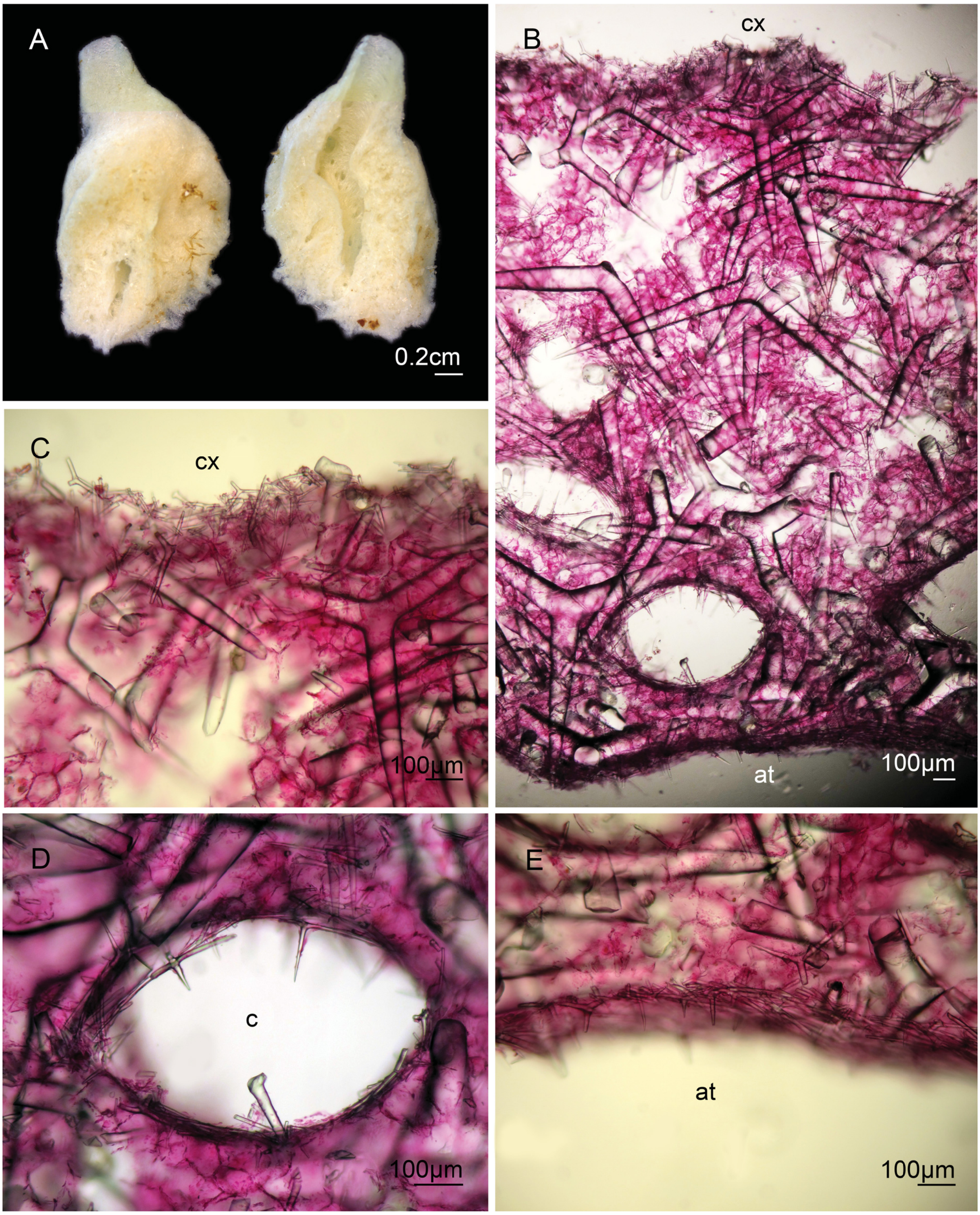

Fig. 8. *Leucandra falakra* sp. nov., holotype (PMR-13748 = UFRJPOR 8349). **A**. Specimen in ethanol. **B**. Cross section. **C**. Detail of the cortex. **D**. Detail of a canal in the choanosome. **E**. Atrial skeleton. Abbreviations: at = atrium; cx = cortex; c = canal.

actine is straight, short, conical and sharp (Fig. 9G–H). Size: 154.0/12.4 µm (paired actine); 143.0/12.4 µm (unpaired actine); 80.6/9.6 µm (apical actine).

Atrial triactines and tetractines. Strongly sagittal. Triactines are the most abundant spicules (Fig. 9I). Actines are cylindrical and blunt. The unpaired actine is shorter than the paired ones. The apical actine of the tetractines is conical, straight, sharp and short. Frequently they are longer and thicker than the apical actine of the choanosomal tetractines (Fig. 9J). Size (triactines): 222.7/15.1 µm (paired actine); 111.2/12.3 µm (unpaired actine). Size (tetractines): 191.4/14.9 µm (paired actine); 92.0/13.1 µm (unpaired actine); 110.3/11.9 µm (apical actine).

Ecology

The specimen was collected on a shaded, semi-vertical, hard limestone bottom.

Remarks

To our knowledge, there are only three described species of *Leucandra* without diactines and with triactines being the main atrial spicules: *L. consolida* Tanita, 1943, *L. glabra* Hôzawa, 1940 and

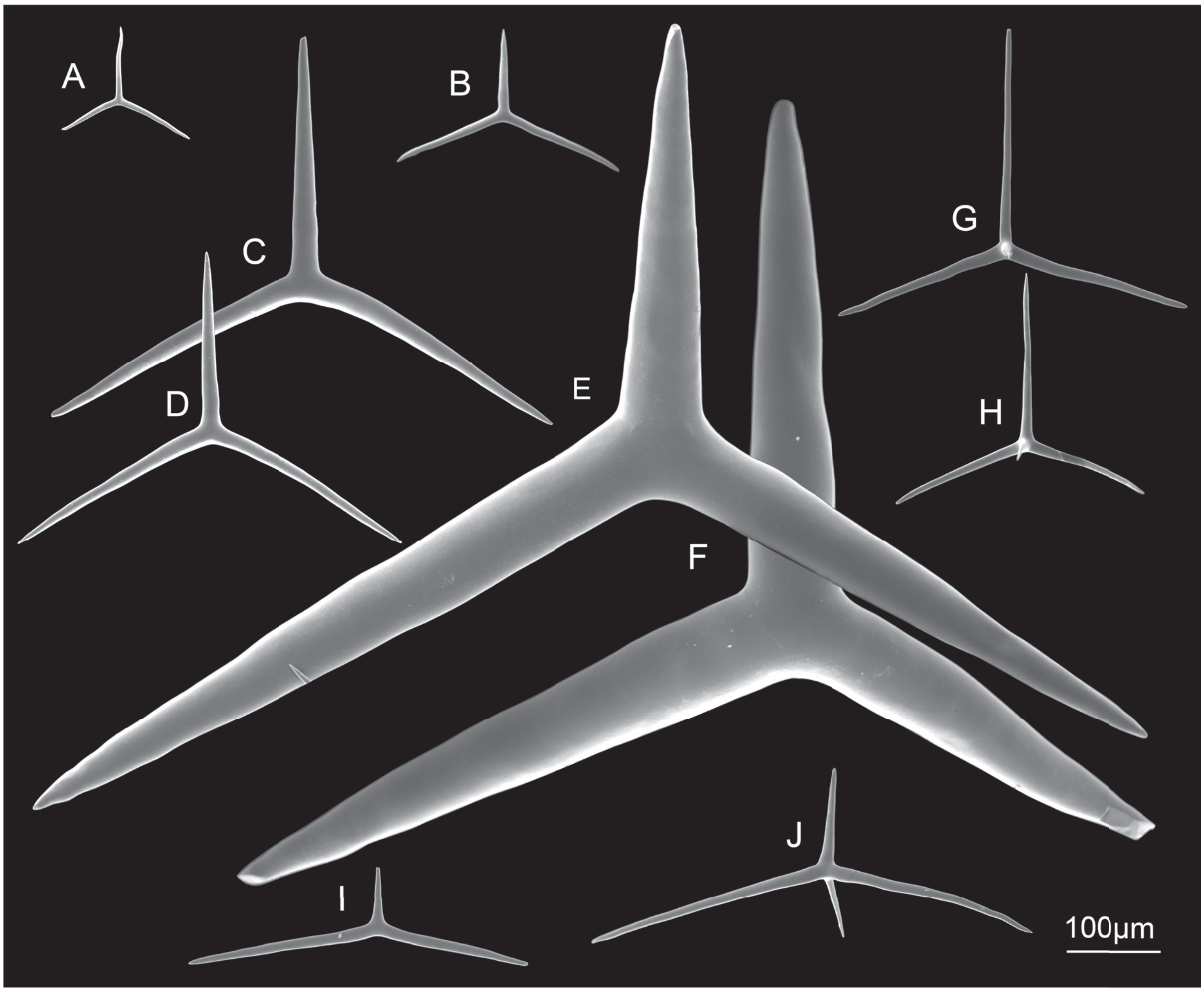

Fig. 9. *Leucandra falakra* sp. nov., holotype (PMR-13748 = UFRJPOR 8349). **A–B**. Cortical triactines. **C–D**. Small choanosomal triactines. **E–F**. Giant choanosomal triactines. **G–H**. Tetractines of the canals. **I**. Atrial triactine. **J**. Atrial tetractine. Scale bar A–J = 100 µm.

Table 8. Spicule measurements of *Leucandra falakra* sp. nov. (PMR-13748 = UFRJPOR 8349).

		length (µm)				**width (µm)**				
		min	mean	sd	max	min	mean	sd	max	n
Cortical triactine	Paired	94.5	136.4	24.0	180.9	8.1	11.1	1.9	13.5	20
	Unpaired	70.2	106.0	18.8	143.1	8.1	11.4	2.4	16.2	20
Cortical and choanosomal triactine		324.0	624.5	192.3	1047.6	48.6	81.5	20.6	118.8	23
Choanosomal triactine	Paired	162.0	214.2	39.8	288.9	13.5	18.3	4.0	27.0	20
	Unpaired	108.0	189.7	58.9	351.0	13.5	19.8	4.1	29.7	20
Tetractine (canals)	Paired	99.9	154.0	26.4	199.8	8.1	12.4	2.4	16.2	19
	Unpaired	45.9	143.0	56.5	288.9	9.5	12.4	1.9	16.2	19
	Apical	50.0	80.6	24.4	137.5	7.5	9.6	1.5	12.5	20
Atrial triactine	Paired	140.4	222.7	33.7	294.3	9.5	15.1	2.5	20.3	30
	Unpaired	78.3	111.2	24.4	159.3	8.1	12.3	1.7	16.2	30
Atrial tetractine	Paired	145.8	191.4	26.0	256.5	10.8	14.9	2.6	18.9	16
	Unpaired	59.4	92.0	22.1	126.9	10.8	13.1	1.7	16.2	16
	Apical	67.5	110.3	30.3	162.0	8.1	11.9	2.8	16.2	15

L. okinoseana Hôzawa, 1929, all three from Japan. *Leucandra falakra* sp. nov. can be differentiated from *L. consolida* by the presence of an oscular crown in the latter (although "feebly developed"), by the absence of tetractines in the choanosome, and by the absence of the large triactines in the cortex. Moreover, the size of some spicules is different (cortical triactines: 240–350/20–25 µm; choanosomal triactines - paired actines: 590–740/60–86 µm, unpaired actine: 550–720/60–86 µm; atrial triactines - paired actines: 220–270/15–18 µm, unpaired actine: 250–300/15–18 µm; atrial tetractines: same size of the atrial triactines but with an apical actine of 80/14 µm).

Leucandra glabra has a different external morphology, with several oscula in a single individual. Besides, the size of some spicules is different (cortical triactines: 120–240/14–28 µm; small choanosomal triactines: 100–200/10–20 µm; large choanosomal triactines: 400–950/42–110 µm; choanosomal tetractines: similar to the small choanosomal triactines but with an apical actine of 80/10 µm; atrial triactines: 90–200/12–20 µm).

Leucandra okinoseana can be differentiated from *L. falakra* sp. nov. by the presence of "small protuberances for attachment" in *L. okinoseana* and by the size of some spicules, which are larger in the Japanese species (cortical triactines - paired actines: 120–250/16–24 µm, unpaired actine: 150–350/14–16 µm; cortical and choanosomal large triactines: 400–1400/32–120 µm; tetractines of the canals - paired actines: 150–200/16–20 µm, unpaired actine: 120–570/12–16 µm, apical actine: 70–200/8–12 µm; atrial triactines - paired actines: 190–370/20–32 µm, unpaired actine: 70–270/16–24 µm; atrial tetractines - same size as the atrial triactines but with an apical actine of 50–110/8–16 µm).

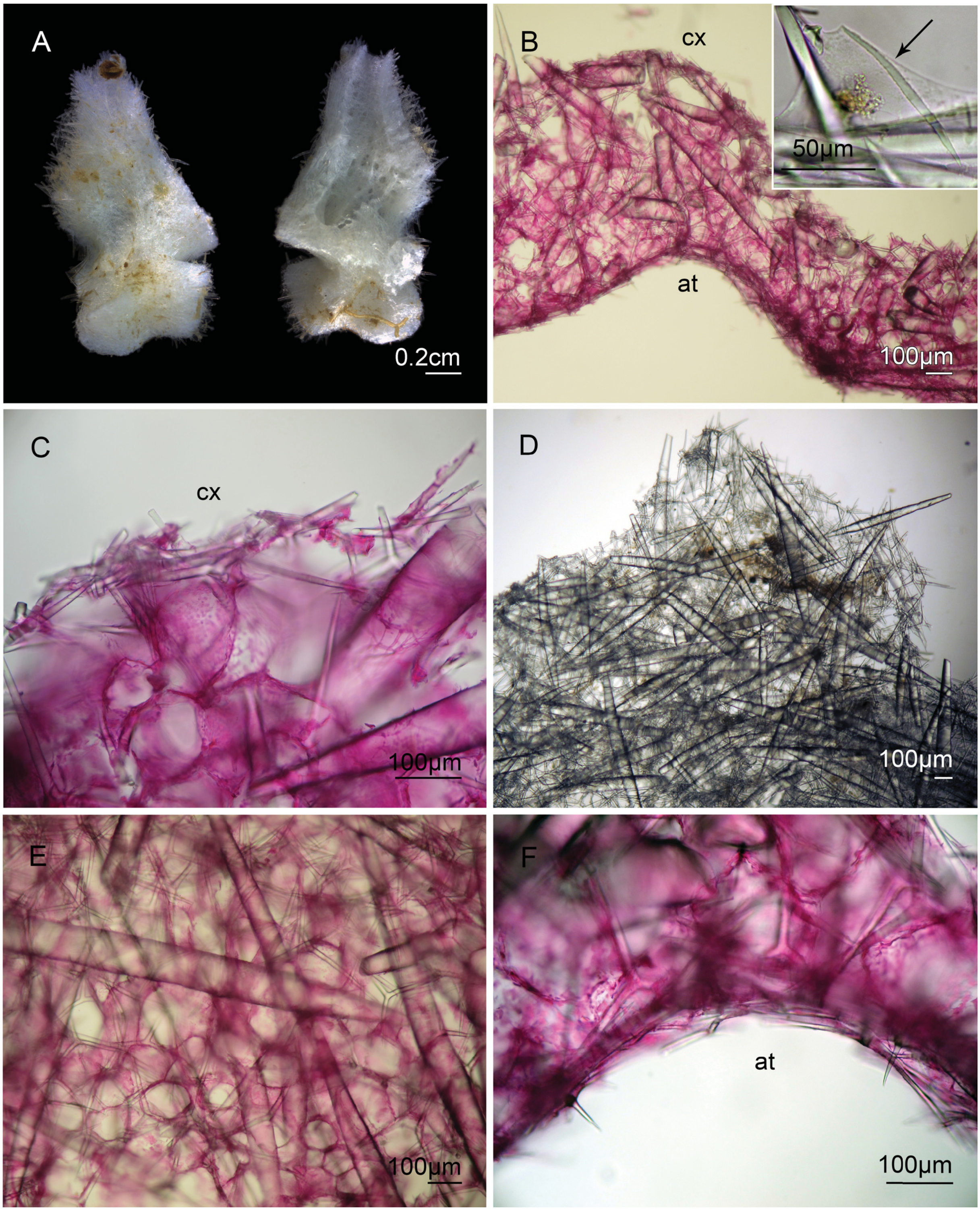

Fig. 10. *Leucandra spinifera* sp. nov., holotype (IRB-SG3 = UFRJPOR 8348). **A**. Specimen in ethanol. **B**. Cross section. Detail: cortical microdiactine (arrow). **C**. Detail of the cortex. **D**. Tangential section of the cortex. **E**. Choanosome. **F**. Atrial skeleton. Abbreviations: at = atrium; cx = cortex.

Leucandra spinifera sp. nov.

urn:lsid:zoobank.org:act:280369B2-48FF-4F3D-88E3-73317D5919A5

Figs 10–11; Table 9

Etymology

From the Latin *spinifer*, meaning prickly, for the presence of numerous diactines.

Material examined

Holotype

ADRIATIC SEA: Vrulja Cove, 43°24'01.3" N, 16°53'10.9" E, 10 m, collected by Vedran Nikolić, 24 Aug. 2011 (IRB-SG3 = UFRJPOR 8348, in ethanol).

Paratype

ADRIATIC SEA: Island of Čiovo, 43°28'58.5" N, 16°21'25.6" E, 5 m, collected by B. Pleše and V. Nikolić, 6 Nov. 2010 (PMR-13742 = UFRJPOR 6861, in ethanol).

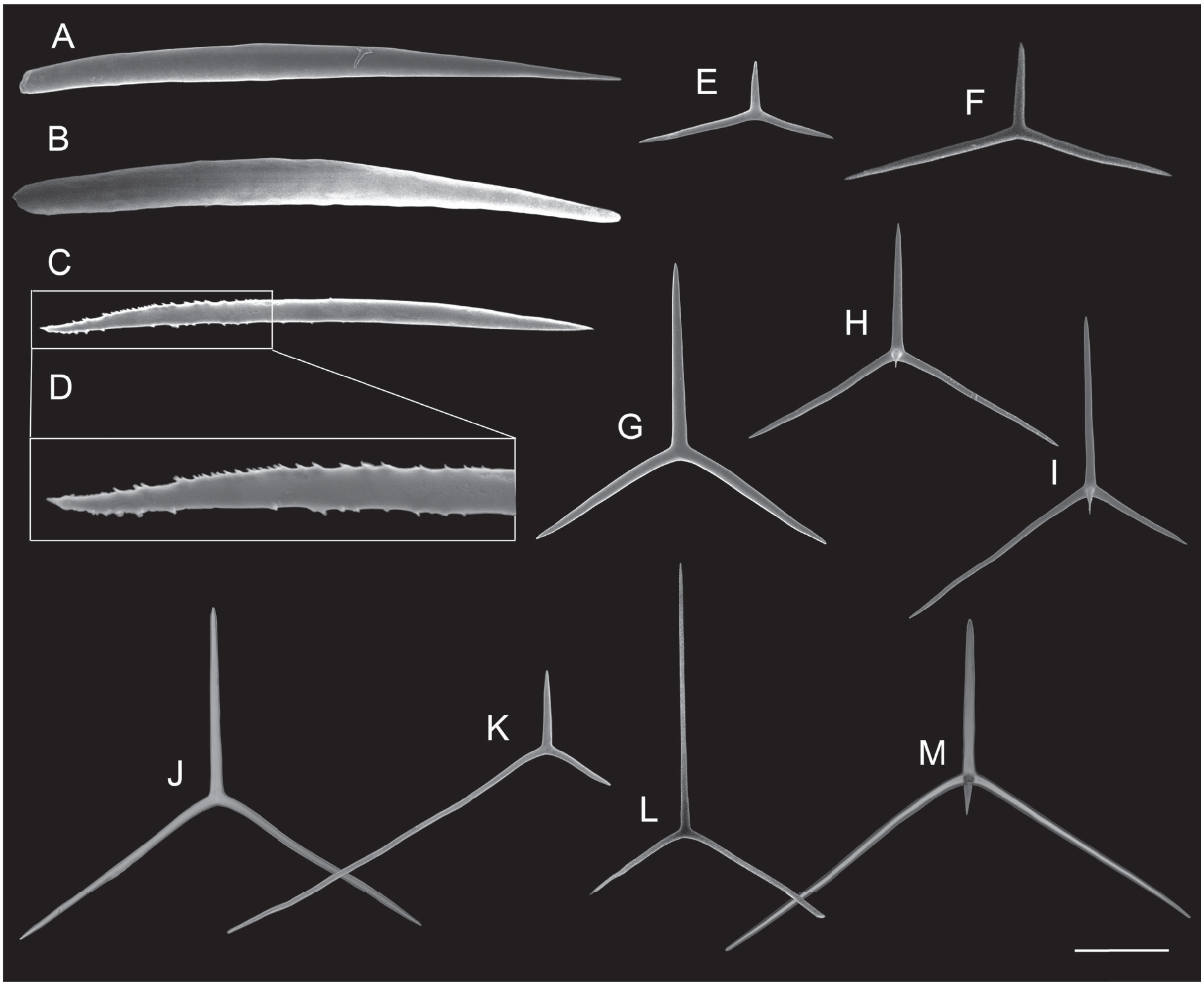

Fig. 11. *Leucandra spinifera* sp. nov., holotype (IRB-SG3 = UFRJPOR 8348). **A–B**. Cortical diactines (scale bar = 200 µm). **C**. Microdiactine (scale bar = 20 µm). **D**. Detail of the spines of a microdiactine (scale bar = 10 µm). **E–F**. Cortical triactines. **G**. Choanosomal triactine. **H–I**. Choanosomal tetractines. **J–L**. Atrial triactines. **M**. Atrial tetractine. Scale bar E–M = 100 µm.

Table 9. Spicule measurements of *Leucandra spinifera* sp. nov. (IRB-SG3 = UFRJPOR 8348).

		length (µm)				**width (µm)**				
		min	mean	sd	max	min	mean	sd	max	n
Diactine		430.0	866.5	217.6	1400.0	20.0	54.4	16.0	90.0	17
Microdiactine		70.0	100.4	29.9	180.0	2.5	4.2	1.2	7.5	20
Cortical triactine	Paired	110.0	189.5	45.2	260.0	7.5	12.9	2.8	17.5	20
	Unpaired	105.0	150.8	29.0	195.0	10.0	13.5	2.5	20.0	20
Choanosomal triactine	Paired	140.0	192.8	35.5	300.0	10.0	12.8	1.4	15.0	20
	Unpaired	115.0	188.8	36.6	260.0	10.0	14.4	1.6	17.5	20
Atrial triactine	Paired	230.0	305.3	69.8	500.0	7.5	7.9	0.9	10.0	14
	Unpaired	110.0	211.4	52.7	325.0	7.5	9.8	0.7	10.0	14
Atrial tetractine	Paired	165.0	276.8	63.3	362.5	7.5	8.4	1.1	10.0	15
	Unpaired	137.5	222.0	62.6	350.0	7.5	9.7	0.9	10.0	15
	Apical	32.5	42.5	16.8	67.5	5.0	6.9	1.6	8.8	4

Colour

White in life and in ethanol.

Description

The body has the shape of a vase (0.8 × 0.4 cm), with a single apical osculum surrounded by a membrane and a crown of a few, or even no trichoxeas (Fig. 10A). The osculum is supported by sagittal tetractines, but a few triactines are also present. They are organised in parallel and point their apical actines to the osculum. They become disorganized, smaller, thinner and less sagittal farther from the osculum. They are also substituted by triactines. Numerous diactines on the surface make it very hispid. The aquiferous system is leuconoid and the atrium is large (Fig. 10A). The cortical skeleton is composed of tangential triactines, perpendicular giant diactines, microdiactines and rare trichoxeas (Fig. 10B–E). The giant diactines frequently cross the entire choanosome (Fig. 10B). The choanosomal skeleton has no organisation. It is composed mainly of subregular triactines, with curved paired actines. Tetractines are also present, but only surrounding canals. The atrial skeleton has triactines and a few tetractines that project their apical actines into the atrium (Fig. 10F). Microdiactines are also present in the atrium.

Spicules (Table 9)

Oscular triactines (very few) and tetractines (abundant). Sagittal. Actines are cylindrical and blunt to sharp. The unpaired actine is thinner than the paired ones. The apical actine of the tetractines is conical, sharp, smooth and strongly curved towards the osculum aperture.

Trichoxeas. Very thin, long and straight. They are frequently broken. These spicules are rare, but can be found in the cortex and atrium.

Diactines. Almost fusiform. The tip that penetrates the choanosome is a little larger and more rounded (Fig. 11A–B). Size: 866.5/54.4 µm.

Microdiactines. Fusiform (Fig. 11C). They are present in the cortex and atrium. They frequently have microspines (Fig. 11D), but smooth spicules are also present. Size: 100.4/4.2 µm.

Cortical triactines. Sagittal. Actines are slightly conical, with blunt tips. The unpaired actine is shorter than the paired ones, which are curved. One of the paired actines is frequently shorter than the other (Fig. 11E–F). Size: 189.5/12.9 µm (paired actine); 150.8/13.5 µm (unpaired actine).

Choanosomal triactines. Subregular to sagittal. The paired actines are curved, consequently the unpaired angle is smaller than the paired angles. Actines are slightly conical with blunt tips. They are almost the same length (Fig. 11G). These spicules are spread in the choanosome and surrounding the canals. Size: 192.8/12.8 µm (paired actine); 188.8/14.4 µm (unpaired actine).

Choanosomal tetractines. Sagittal. The paired actines are curved, consequently the unpaired angle is smaller than the paired angles. Actines are slightly conical with blunt tips. The apical actine is straight or curved, conical, smooth and sharp (Fig. 11H–I). These spicules are present only surrounding the canals.

Atrial triactines and tetractines. Triactines are much more abundant. These spicules are strongly sagittal. The paired actines are curved and much longer than the unpaired one. Actines are slightly conical and blunt (Fig. 11J–L). The apical actine of the tetractines is straight or slightly curved near the end, conical, smooth and sharp (Fig. 11M). These tetractines are very similar to those of the choanosome. Size (triactine): 305.3/7.9 µm (paired actine); 211.4/9.8 µm (unpaired actine). Size (tetractine): 276.8/8.4 µm (paired actine); 222.0/9.7 µm (unpaired actine); 42.5/6.9 µm (apical actine).

Ecology

Specimens were collected on a cliff in a shaded area.

Remarks

This species differs from all other species of *Leucandra* mainly by the composition of the skeleton, particularly by the presence of mainly triactines in the atrial skeleton, with very long and slender paired actines and few spiny microdiactines in the cortex. The most similar species is the Californian *L. heathi* Urban, 1906. However, this species has no tetractines, while *L. spinifera* sp. nov. has a few tetractines. Besides, microdiactines are not abundant in *L. spinifera* sp. nov., while in *L. heathi* they form a continuous palisade in the cortex.

We found 10 species of *Leucandra* recorded from the Mediterranean until now, and *L. spinifera* sp. nov. can be differentiated from all of them: *L. aspera* (Schmidt, 1862) has no microdiactines; *L. balearica* (Lackschewitz, 1886) has only tetractines in the atrium and its microdiactines are much smaller (12-24/1 µm); *L. globosa* (Sarà, 1951) has different microdiactines; *L. bolivari* Ferrer-Hernandez, 1916 has no diactines; *L. crambessa* Haeckel, 1872 has no microdiactines and has tetractines only in the atrium; *L. nausicaae* (Schuffner, 1877) has no diactines and the atrial skeleton comprises only tetractines; *L. riojai* Ferrez-Hernandez, 1918 has only tetractines in the atrium; *L. rodriguezii* (Lackschewitz, 1886) has shorter microdiactines (12-14/1 µm) which occur only in the atrium and the atrium is also composed of only tetractines; *L. sulcata* Ferrer-Hernandez, 1918 has microdiactines of a different shape, which are present abundantly only in the cortex, while the atrium is composed mainly of tetractines.

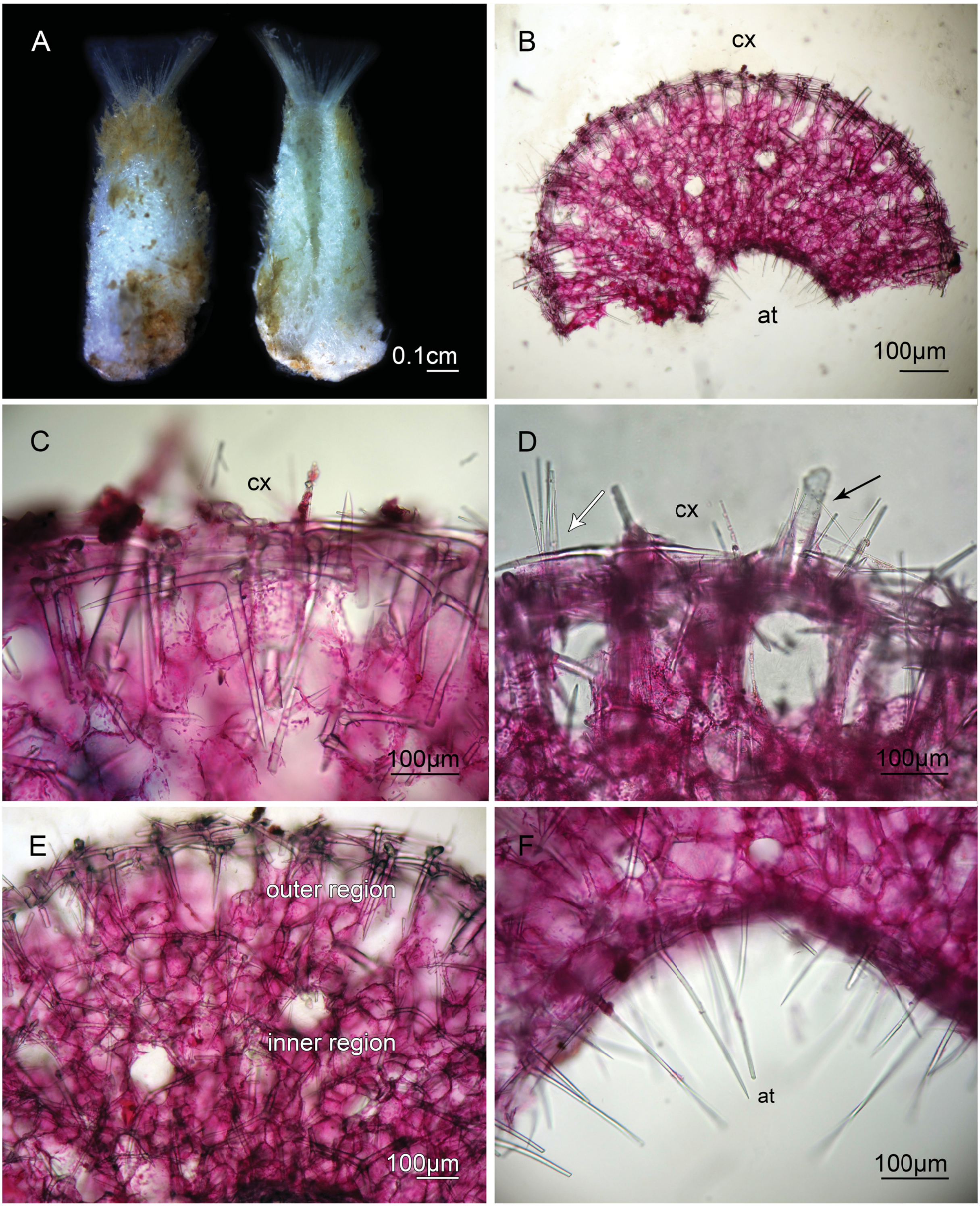

Fig. 12. *Paraleucilla dalmatica* sp. nov., holotype (IRB-SD5 = UFRJPOR 8346). **A**. Specimen in ethanol. **B**. Cross section. **C**. Cortex. **D**. Detail of the cortex showing the tufts of diactines (white arrow = trichoxeas; black arrow = diactine). **E**. Choanosome with the outer and inner regions. **F**. Atrial skeleton. Abbreviations: at = atrium; cx = cortex.

Genus **Paraleucilla** Dendy, 1892

Paraleucilla dalmatica sp. nov.

urn:lsid:zoobank.org:act:D066F56A-1FD7-4742-98E2-2CEE390D1E21
Figs 12–13; Table 10

Etymology

From the type locality. Dalmatia is one of the four historical regions of Croatia.

Material examined

Holotype

ADRIATIC SEA: near the Island of Čiovo, 43°29'02.0" N, 16°22'10.9" E, 5 m, collected by B. Pleše and V. Nikolić, 5 Nov. 2010 (IRB-SD5 = UFRJPOR 8346, in ethanol).

Paratype

ADRIATIC SEA: same data as holotype (PMR-13747, in ethanol).

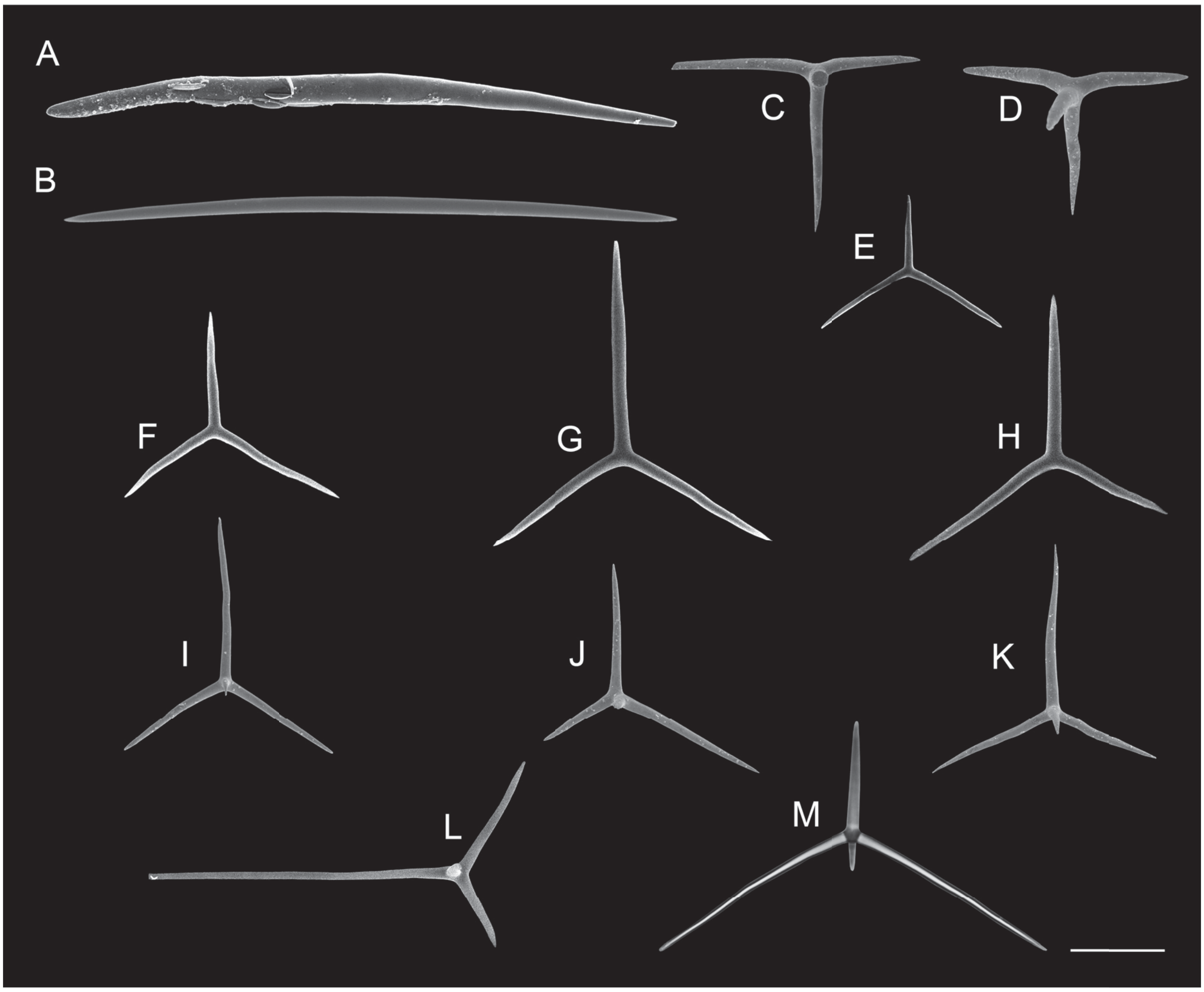

Fig. 13. *Paraleucilla dalmatica* sp. nov., holotype (IRB-SD5 = UFRJPOR 8346). **A**. Cortical diactine (scale bar = 50 µm). **B**. Cortical microdiactine (scale bar = 20 µm). **C–D**. Cortical tetractines. **E**. Cortical triactine. **F–H**. Subatrial triactines. **I–K**. Subatrial tetractines. **L–M**. Atrial tetractines. Scale bar C–M = 100 µm.

Table 10. Spicule measurements of *Paraleucilla dalmatica* sp. nov. (IRB-SD5 = UFRJPOR 8346).

		length (µm)				width (µm)				
		min	mean	sd	max	min	mean	sd	max	n
Diactine					1000.0	25.0			50.0	-
Trichoxea					>330.0	2.5			5.0	-
Microdiactine		57.5	95.0	23.5	142.5	2.5	2.5	0	2.5	15
Cortical triactine	Paired	85.0	142.8	35.8	190.0	5.0	12.4	4.3	20.0	20
	Unpaired	65.0	149.3	48.0	230.0	5.0	12.9	4.7	20.0	20
Cortical tetractine	Paired	120.0	159.1	19.2	195.0	10.0	13.4	2.0	17.5	16
	Apical	75.0	133.1	33.6	190.0	7.5	13.4	2.6	17.5	16
Subatrial tetractine and triactine	Paired	170.0	180.0	7.1	190.0	12.5	13.2	1.2	15.0	7
	Unpaired	155.0	205.8	26.7	245.0	10.0	12.7	1.1	15.0	15
	Apical	23.8	37.8	11.9	50.0	7.5	8.8	1.4	10.0	4
Atrial tetractine	Paired	105.0	157.9	32.0	197.5	5.0	10.5	3.0	17.5	21
	Unpaired	75.0	157.0	35.8	212.5	7.5	11.4	2.1	15.0	20
	Apical	57.5	115.7	55.4	245.0	5.0	7.3	0.6	7.5	25

Colour

Beige or light brown in life and white in ethanol.

Description

The body has the shape of a vase with a single apical osculum surrounded by a crown of trichoxeas (Fig. 12A). Surface is very hispid. The aquiferous system is leuconoid (Fig. 12B). The cortical skeleton is composed of the basal system of large tangential tetractines and few triactines (Fig. 12C). Giant diactines cross the surface, penetrating deeply into the choanosome. They are present from the osculum to the base of the sponge. Among these giant diactines there are also very thin and long trichoxeas, organised in tufts, and very few microdiactines (Fig. 12D). The choanosomal skeleton is characteristic of *Paraleucilla*, with an inarticulate region (outer region) and a zone without organisation (inner region) (Fig. 12E). The outer region is formed by the apical actine of the cortical tetractines, the unpaired actine of subatrial tetractines and very few triactines. The paired actines of these subatrial spicules are frequently curved, resembling a hook. The inner region is formed by scattered subatrial tetractines and very few triactines. The atrial skeleton is composed of tetractines only (Fig. 12F). In some parts of the sponge the inarticulate skeleton seems not to exist and it becomes more similar to *Leucandrilla*.

Spicules (Table 10)

Oscular triactines. Strongly sagittal. Actines are conical and sharp. The unpaired actine is longer and thinner than the paired ones and basipetally directed.

Diactines. Giant. They are present in the oscular crown and cortex. They are almost fusiform but slightly curved, with a thicker tip outside the sponge (Fig. 13A). The size is very variable. Many diatoms are attached to the diactines surrounding the osculum. Size: 1000.0/25.0–50.0 µm.

Trichoxeas. Present in the oscular crown and cortex. They are thin, straight and most of them are broken. Size: > 330.0/2.5–5.0 µm.

Microdiactines. Very rare, fusiform or arrow-headed. Sometimes one of the tips has small spines while the other one is thicker (Fig. 13B). They are present in the cortex. Size: 95.0/2.5 µm.

Cortical tetractines. Sagittal. Actines are conical with sharp tips. The apical actine is longer than the basal ones, conical, straight and sharp (Fig. 13C–D). Size: 159.1/13.4 µm (paired actine); 133.1/13.4 µm (apical actine).

Cortical triactines. There are very few, subregular to regular. Actines are slightly conical with sharp tips (Fig. 13E). Size: 142.8/12.4 µm (paired actine); 149.3/12.9 µm (unpaired actine).

Subatrial triactines and tetractines. The triactines are rare. Actines are conical and sharp. The unpaired actine is longer than the paired ones. The paired actines are frequently strongly curved. One of them is often shorter than the other. The apical actine of the tetractines is very short, thin, smooth and strongly curved (Fig. 13F–K). Size: 180.0/13.2 µm (paired actine); 205.8/12.7 µm (unpaired actine); 37.8/8.8 µm (apical actine).

Atrial tetractines. Sagittal. Actines are slightly conical and sharp. The apical actine is slightly conical, smooth, thinner than the basal ones and straight or only slightly curved (Fig. 13L–M). Size: 157.9/10.5 µm (paired actine); 157.0/11.4 µm (unpaired actine); 115.7/7.3 µm (apical actine).

Ecology

Specimens were collected on a cliff in a shaded area.

Remarks

Currently there are 11 known species of *Paraleucilla*, and *P. magna* Klautau *et al.*, 2004 is the only one that has been recorded in the Mediterranean Sea up to now. Both the external morphology and spicule composition differ in these two species. The most similar species to *P. dalmatica* sp. nov. are *P. perlucida* Azevedo & Klautau, 2007, from Brazil, and *P. princeps* (Row & Hôzawa, 1931), from Australia. Nonetheless, *P. dalmatica* sp. nov. can be differentiated from *P. perlucida* mainly by the absence of diactine I and trichoxea in the latter. *Paraleucilla princeps* also differs by the absence of diactine I and microdiactines. Therefore, *P. dalmatica* sp. nov. is the second species of *Paraleucilla* recorded from the Mediterranean Sea.

Genus ***Sycon*** Risso, 1826

Sycon ancora sp. nov.

urn:lsid:zoobank.org:act:F39F5C07-44BF-4AC4-822D-77AA1155B018

Figs 14–15; Table 11

Etymology

From the Latin *ancora*, meaning anchor, for the presence of anchor-like spicules for attachment.

Material examined

Holotype

ADRIATIC SEA: Island of Pag, 44°28'34.96" N, 15°02'39.74" E, 1 m, collected by V. Nikolić, 14 Feb. 2011 (PMR 17809 = UFRJPOR 8345, in ethanol).

Paratype

ADRIATIC SEA: near Split, 43°30'27.57" N, 16°23'20.55" E, 5–10 m, collected by V. Nikolić, 15 Aug. 2011 (IRB-SD12 = UFRJPOR 8347, in ethanol).

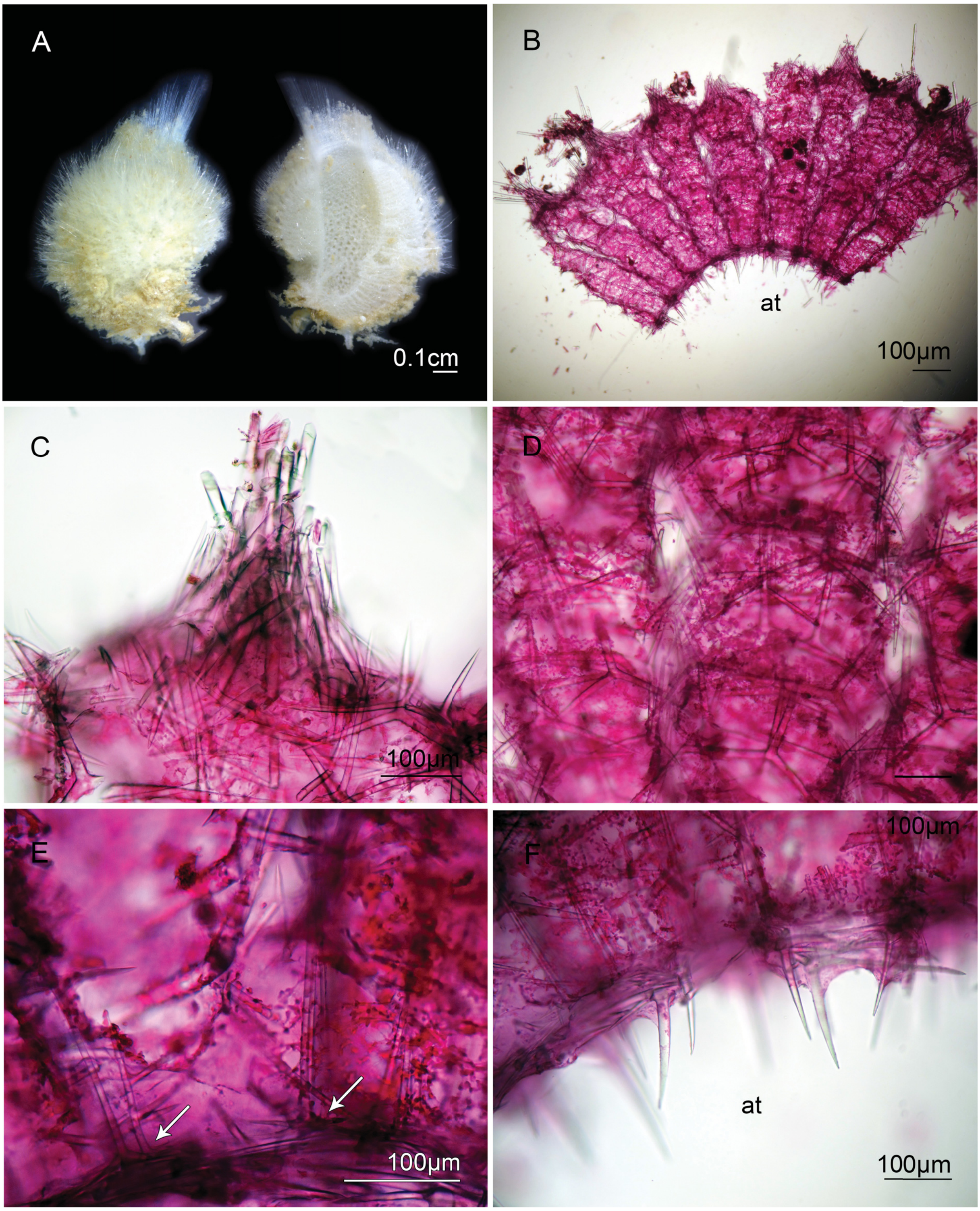

Fig. 14. *Sycon ancora* sp. nov., holotype (PMR 17809 = UFRJPOR 8345). **A**. Specimen in ethanol. **B**. Cross section. **C**. Detail of the distal cone. **D**. Tubar and subatrial skeletons. **E**. Atrial skeleton (white arrows = subatrial triactines). **F**. Tangential section of the atrial skeleton. Abbreviation: at = atrium.

Colour

White in life and in ethanol.

Description

The body is vase-shaped (1.1 × 0.8 cm), with a single apical osculum surrounded by a crown of trichoxeas (Fig. 14A) and diactines supported by sagittal tetractines. These tetractines are arranged parallel to each other and their unpaired actines are basipetally directed. The unpaired actine is longer and thinner than the paired ones and the apical actine is curved towards the osculum aperture. The paired actines are slightly curved. There is no subosculur region. The aquiferous system is syconoid and the atrium is central. The radial tubes are coalescent (Fig. 14B). Diactines and trichoxeas protrude through the distal cones; consequently, the surface is very hispid. These diactines (ca 10 to 15) penetrate only a little into the sponge surface (Fig. 14C). The unpaired actine of some triactines also protrudes through the cones.

The tubar skeleton is articulated, but not so well organised as in most sycons (Fig. 14D). It is composed of rows of sagittal triactines that point their unpaired actines to the surface. These tubar triactines are

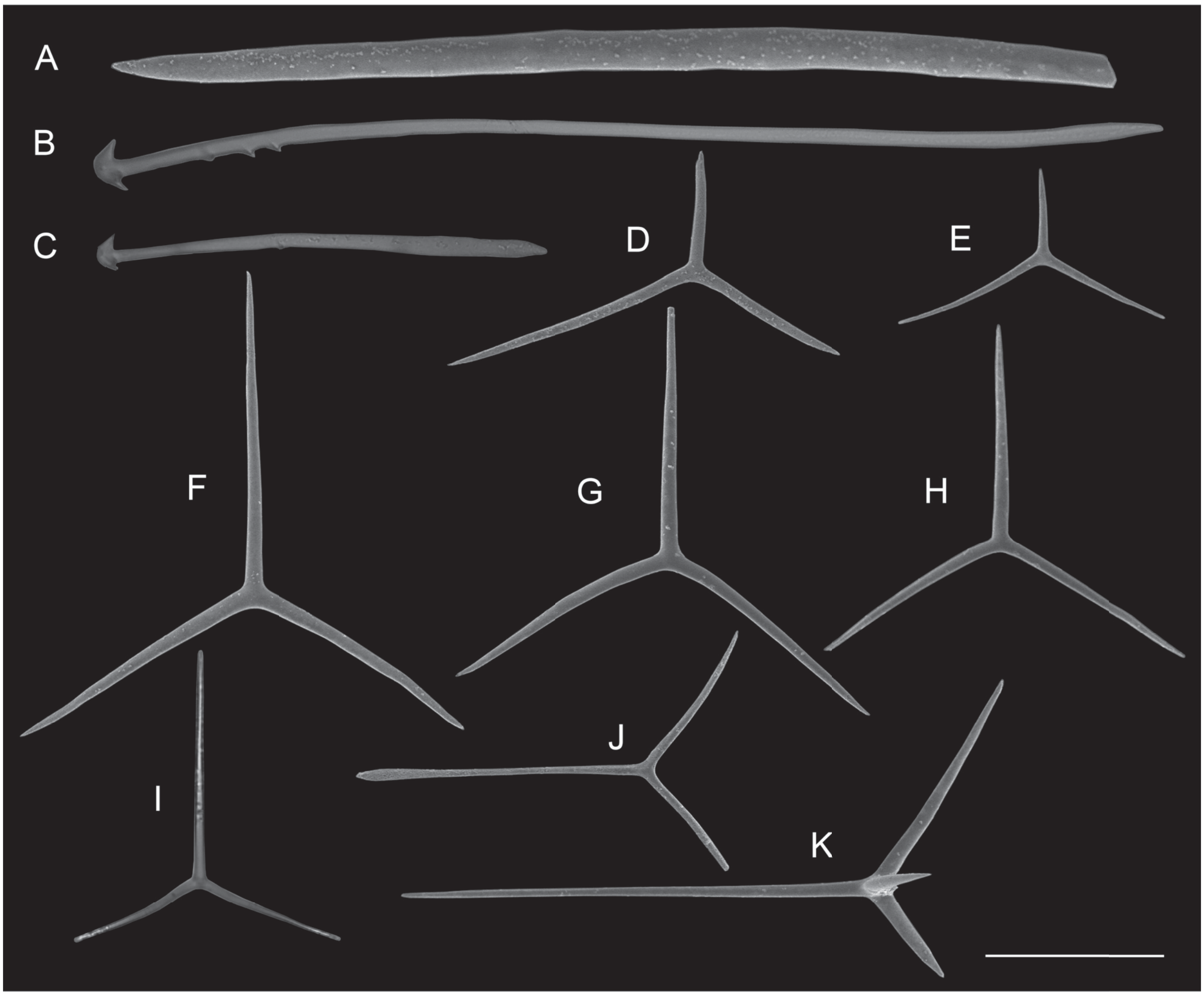

Fig. 15. *Sycon ancora* sp. nov., holotype (PMR 17809 = UFRJPOR 8345). **A**. Cortical diactine. **B–C**. Anchor-like tetractines. **D–E**. Triactines of the cones. **F–H**. Tubar triactines. **I**. Subatrial triactine. **J**. Atrial triactine. **K**. Atrial tetractine. Scale bar D–K = 100 µm.

Table 11. Spicule measurements of *Sycon ancora* sp. nov. (PMR 17809 = UFRJPOR 8345).

		length (µm)				width (µm)				
		min	mean	sd	max	min	mean	sd	max	n
Diactine		378.0	537.8	180.1	800.0	10.8	16.1	4.5	20	6
Anchor-like tetractine		-	-	-	>1000	25.0			37.5	2
Triactine (distal cone)	Paired	59.4	112.3	27.2	148.5	5.4	6.9	1.4	10.8	20
	Unpaired	54.0	78.6	18.7	124.2	5.4	7.0	1.5	10.8	20
Tubar triactine	Paired	116.1	168.2	25.8	216.0	8.1	13.0	2.7	16.2	20
	Unpaired	143.1	188.1	29.6	259.2	8.1	12.4	2.8	18.9	20
Subatrial triactine and tetractine	Paired	67.5	97.9	41.6	159.3	4.1	5.4	1.1	6.8	4
	Unpaired	108.0	212.4	36.5	264.6	4.1	6.0	1.5	8.1	21
Atrial tetractine I	Paired	94.5	153.5	31.7	202.5	7.6	10.8	2.1	16.2	17
	Unpaired	55.1	219.4	75.8	332.1	7.6	10.7	1.5	13.5	17
	Apical	97.5	123.8	21.9	177.5	8.8	11.4	1.4	12.5	20
Atrial tetractine II	Paired	-	162.5	-	-	-	6.3	-	-	1
	Unpaired	-	137.5	-	-	-	6.3	-	-	1
	Apical	50.0	77.1	14.5	112.5	5.0	5.6	1.0	7.5	20

larger than those of the distal cones and the paired actines are frequently curved. The subatrial skeleton is composed of sagittal triactines and tetractines (Fig. 14E) with very thin actines. The unpaired actine is much longer than the paired ones and the longest ones are frequently localized among the choanocyte chambers. They point their unpaired actines towards the distal cones. Some of the subatrial triactines are similar to pseudosagittal spicules. The atrial skeleton is composed of two categories of tetractines tangentially organized (Fig. 14E). They frequently have long, unpaired and short, paired actines. One of the paired actines is commonly shorter than the other; however, the three basal actines can have the same size (Fig. 14F). When one of the paired actines is shorter than the other, it frequently penetrates an exhalant canal. The main difference between the two categories of atrial tetractines is in the apical actine. Tetractines with thinner apical actines project these actines mainly into the canals, while thicker and curved apical actines penetrate into the atrium (Fig. 14E). Few anchor-like tetractines are present at the sponge base and project their basal actines into the substrate.

Spicules (Table 11)

Diactines. Almost fusiform, but the tip outside the sponge is a little thicker (Fig. 15A). Size: 537.8/16.1 µm.

Trichoxeas. Very thin, long and straight. They were always broken.

Anchor-like tetractines. The basal actines are very short and curved, while the apical one is very long. Frequently there are spines on the apical actine, but near the basal ones. They vary from four to seven, but seven spines are more common (Fig. 15B). Size: > 1000.0/25.0 µm.

Triactines of the cones. They are smaller than the tubar triactines. The unpaired actine protrudes through the cones and it is shorter than the paired ones, which are curved. Actines are slightly conical and sharp (Fig. 15C–D). Size: 112.3/6.9 µm (paired actine); 78.6/7.0 µm (unpaired actine).

Triactines of the tubes. Subregular to sagittal. The unpaired actine is a little longer or has the same length of the paired ones. The paired actines are straight or slightly curved. Actines are slightly conical and sharp (Fig. 15E–G). Size: 168.2/13.0 µm (paired actine); 188.1/12.4 µm (unpaired actine).

Subatrial triactines and tetractines. The subatrial spicules are very thin. They are sagittal or, sometimes, similar to pseudosagittal spicules. Actines are slightly conical and sharp. The unpaired actine is longer than the paired ones (Fig. 15H). The apical actine of the tetractines is conical, sharp, smooth, shorter than the basal ones and curved in the direction of the atrium. Size: 97.9/5.4 µm (paired actine); 212.4/6.0 µm (unpaired actine).

Atrial tetractines I and II and triactines. There are two categories of atrial tetractines and the triactines are very rare. They are sagittal or subregular. The unpaired actine is frequently longer than the paired ones (Fig. 15I). It is also common to find one of the paired actines shorter than the other (63.5–109.3(±64.7)–155.0/10–11.3(±1.8)–12.5 µm (n=2); Fig. 15J). This shorter, paired actine is frequently projected inside the exhalant canal. Actines are cylindrical and sharp. Sometimes, the tip of the unpaired actine is thicker (Fig. 15I). The main difference between the two categories of tetractines is in the shape and size of the apical actines, which are straight and thinner in one and curved and thicker in the other. Size (tetractine I): 153.5/10.8 µm (paired actine); 219.4/10.7 µm (unpaired actine); 123.8/11.4 µm (apical actine). Size (tetractine II): 162.5/6.3 µm (paired actine); 137.5/6.3 µm (unpaired actine); 77.1/5.6 µm (apical actine).

Ecology

Specimens were collected on a semi-vertical hard limestone bottom. They were found among *Cystoseira* macroalgae.

Remarks

Currently there are 12 accepted species of *Sycon* in the Mediterranean Sea, 10 of which have already been reported for the Adriatic. We compared our specimens to all known species of *Sycon* and even more carefully to the Mediterranean ones, yet we could not find a perfect match.

The main characteristic discerning *Sycon ancora* sp. nov. from other species is the shape of the atrial triactines and the presence of anchor-like tetractines at the base. If we exclude these characteristics, this species would be mostly comparable to *S. raphanus*; however, there are several important differences between them.

Sycon raphanus was originally described from the Adriatic Sea by Schmidt (1862). Unfortunately, his description was not detailed enough. According to him, *S. raphanus* has a bulb shape and a peduncle. He even considered these characteristics to distinguish *S. raphanus* from *S. ciliatum* (Fabricius, 1780), a species from the English Channel which he believed to be present in the Adriatic Sea.

Haeckel (1872) disagreed with the possibility of *S. ciliatum* occurring in the Mediterranean Sea and considered that all specimens called *S. ciliatum* were, in fact, *S. raphanus*. He also mentioned that he analysed all the specimens from Schmidt's collection identified as *S. raphanus* and found a potpourri of species, including *Leucandra aspera*, *Sycon humboldti*, *Sycon setosum* and "the real *S. raphanus*". Therefore, he made a detailed description of this species, which has since then been considered as the official description of *S. raphanus*. According to his description, *S. raphanus* is morphologically very variable, solitary or not, with or without peduncle. The skeleton is composed of tufts of 5–10 cylindrical diactines (var. *tergestina*) to 20–50 diactines (var. *procumbens*) and the size of the diactines varies from 400–800/20–30 µm up to 1000–2000/20–40 µm, rarely attaining 3000 µm. Analyzed specimens of *S. ancora* sp. nov. have tufts of 10–15 diactines measuring 378→1500/10.8–18.9 µm. *Sycon raphanus* has triactines with curved paired actines in the distal cones and in the tubar skeleton. The tubar triactines are 100–180/10–12 µm (paired) and 150–250/10–12 µm (unpaired), which are thinner than in *S. ancora* sp. nov. The subatrial skeleton of *S. raphanus* has triactines (paired: 100–180/5–8 µm; unpaired: 150–250/5–8 µm), while *S. ancora* sp. nov. has triactines and tetractines. The atrial skeleton of *S. raphanus* shows subregular to regular (rarely sagittal) triactines and tetractines (basal: 150–250/8–10 µm; apical: 60–120 µm), while our species has tetractines with two types of apical actines (there is a variation in the thickness and position), a long unpaired actine and paired actines with different sizes. Haeckel (1872) also mentioned the presence of only triactines supporting the oscular crown, while *S. ancora* sp. nov. has only tetractines.

Although we believe the entire genus *Sycon* is in urgent need of revision, the characteristics we found in our specimens strongly indicate the presence of a new species.

Other calcarean species from the Adriatic Sea

Apart from the species described here, we also recorded and molecularly analyzed specimens of *Clathrina blanca* (Miklucho-Maclay, 1868), *C. clathrus* (Schmidt, 1864), *C. rubra* Sarà, 1958 and *Paraleucilla*

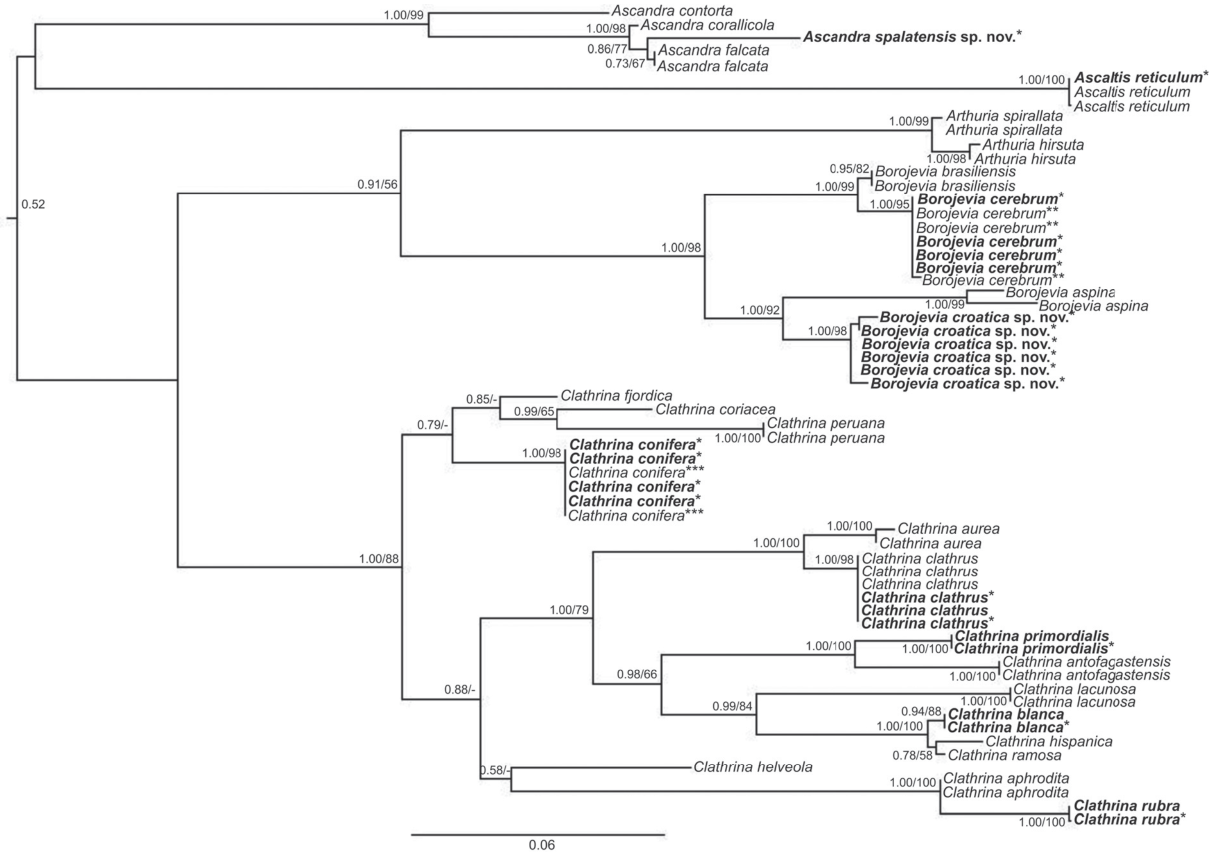

Fig. 16. Maximum likelihood (ML) tree based on ITS1-5.8S-ITS2 rDNA sequences of Calcinea. Bayesian posterior probabilities (PP) and bootstrap values (BS) are given near the branches (PP/BS; when >0.50). Adriatic specimens are written in bold; *Adriatic specimens obtained during this study; **Mediterranean specimens of *Borojevia cerebrum*; ***Brazilian specimens of *Clathrina conifera*.

magna Klautau, Monteiro & Borojević, 2004. These species are not redescribed here, since specimens from the Adriatic Sea have already been recorded and described in earlier works (Cvitković *et al.* 2013; Imešek *et al.* 2014). In the present study, *C. blanca* was recorded near Selce (45°09'07.8" N, 14°43'15.0" E), about 1 m deep and *C. rubra* was recorded near the Island of Čiovo (43°28'58.5" N, 16°21'25.6" E), about 5m deep on a shaded hard bottom. In August and November 2010 they were quite abundant, always only a few millimeters in size and often found on bryozoans. *C. clathrus* was found in numerous locations along the coast (e.g., Prapratno Cove, 42°48'36.8" N, 17°40'38.4" E; near the Island of Čiovo, 43°28'58.5" N, 16°21'25.6" E) and the cryptogenic species *P. magna* was found in large numbers in on aquaculture installations in Grška Cove on the Island of Brač and in the Port of Ploče.

Molecular analysis

The number of sites used for the final alignments (gaps included) was as follows: 513 for ITS Calcinea, 1434 for 28S Calcinea, 734 for ITS Calcaronea and 846 for 28S Calcaronea. Both markers revealed the same tree topology in both analyses (but see Fig. 19), yet the Bayesian analysis rendered much better support values than ML in all cases. However, the Adriatic species nested within the respective genera with high bootstrap (BS) and posterior probability (PP) values, thereby confirming the results of morphological analysis (Figs 16–19).

Once more the presence of diactines did not show any phylogenetic signal (Rossi *et al.* 2011; Klautau *et al.* 2013). Furthermore, we found former guanchas with only triactines reunited in a monophyletic clade in the ITS analysis, with high support values inside the *Clathrina* group (0.99 PP and 0.84 BS; Fig. 16). In the 28S calcinean tree (Fig. 17) we recovered a clade where *Levinella* represents a sister

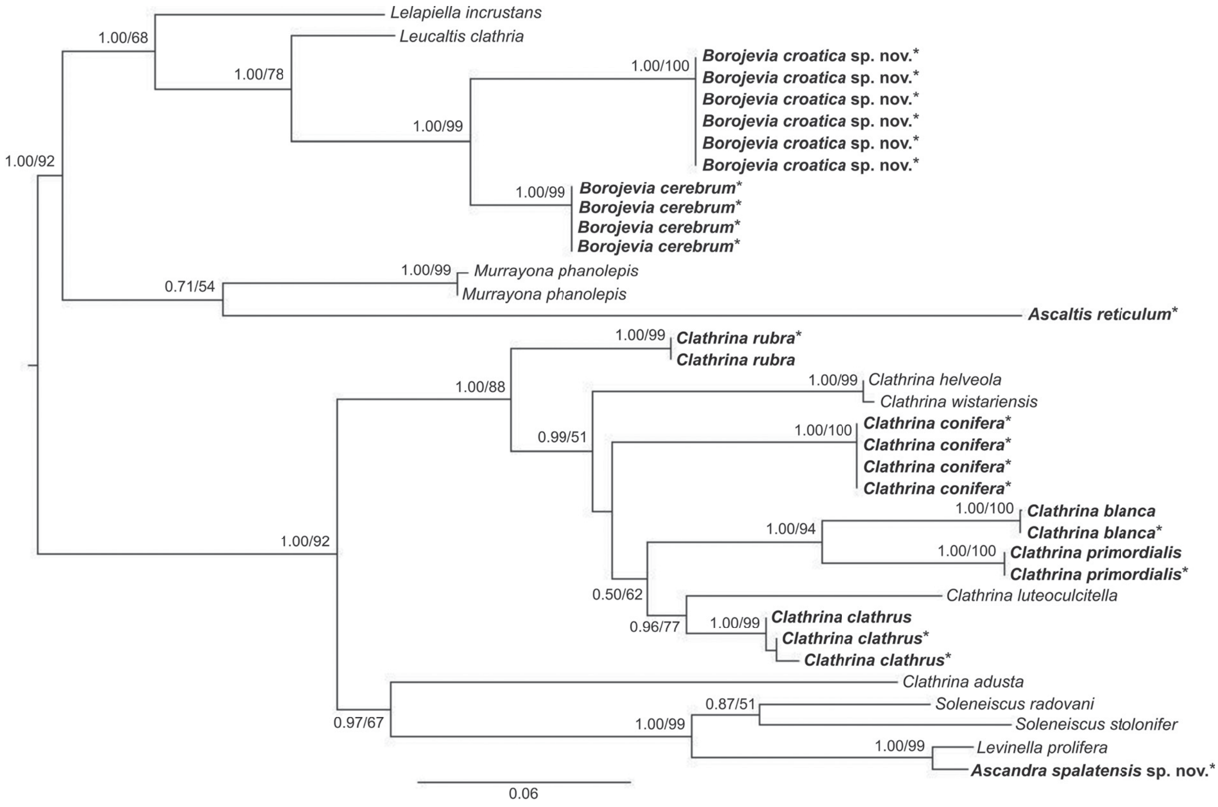

Fig. 17. Maximum likelihood tree (ML) based on partial 28S rDNA sequences of Calcinea. Bayesian posterior probabilities (PP) and bootstrap values (BS) are given near the branches (PP/BS; when >0.50). Adriatic species are written in bold. Adriatic specimens obtained in this study are marked with an asterisk.

group to *Ascandra* with high support values (1.00 PP and 0.99 BS), which confirms the results of Voigt *et al.* (2012). We also recovered a clade comprising the genera *Murrayona* and *Ascaltis* in both analyses; however, the support values were less good (0.71 PP and 0.54 BS). The molecular analyses also confirmed the presence of *P. magna* in the Adriatic Sea (Figs 18–19). Besides, we recovered a calcaronean clade with high support (1.00 PP and 0.99 BS in ITS analysis; 0.95 PP and 0.64 BS in 28S analysis) formed only by *Paraleucilla* species. The genus *Paraleucilla* formed a highly supported clade with *Leucandra nicolae*, while *Leucandra spinifera* sp. nov. is a sister species of *L. aspera* (Fig. 19). *Sycon ancora* sp. nov. represents a sister species of *S. raphanus* (Fig. 19). We confirmed the paraphyly of the genera *Sycon* and *Leucandra* (Voigt *et al.* 2012).

Species richness

Considering previous data, together with our present results based on morphological and molecular analyses, we found a total of 13 species of Calcinea (Table 12) and 26 of Calcaronea in the Adriatic Sea (Table 13). Taking into account the species richness by sectors (Fig. 20), the richest sector is the Central Adriatic, where 34 species were found, followed by the Northern Adriatic with 18, and the Southern Adriatic with only 5 species. Most of the species present in the Adriatic Sea are also present in other Mediterranean areas, yet, altogether we recorded six species provisionally endemic for the Adriatic, two calcinean and four calcaronean.

Discussion

Since some of the first studies on the class Calcarea were mainly done along the Dalmatian coast by Schmidt and Haeckel in the 19th century (e.g., Schmidt 1862, 1864; Haeckel 1870, 1872), the knowledge of the current species diversity and distribution certainly awakes taxonomic interest. Analysing previous

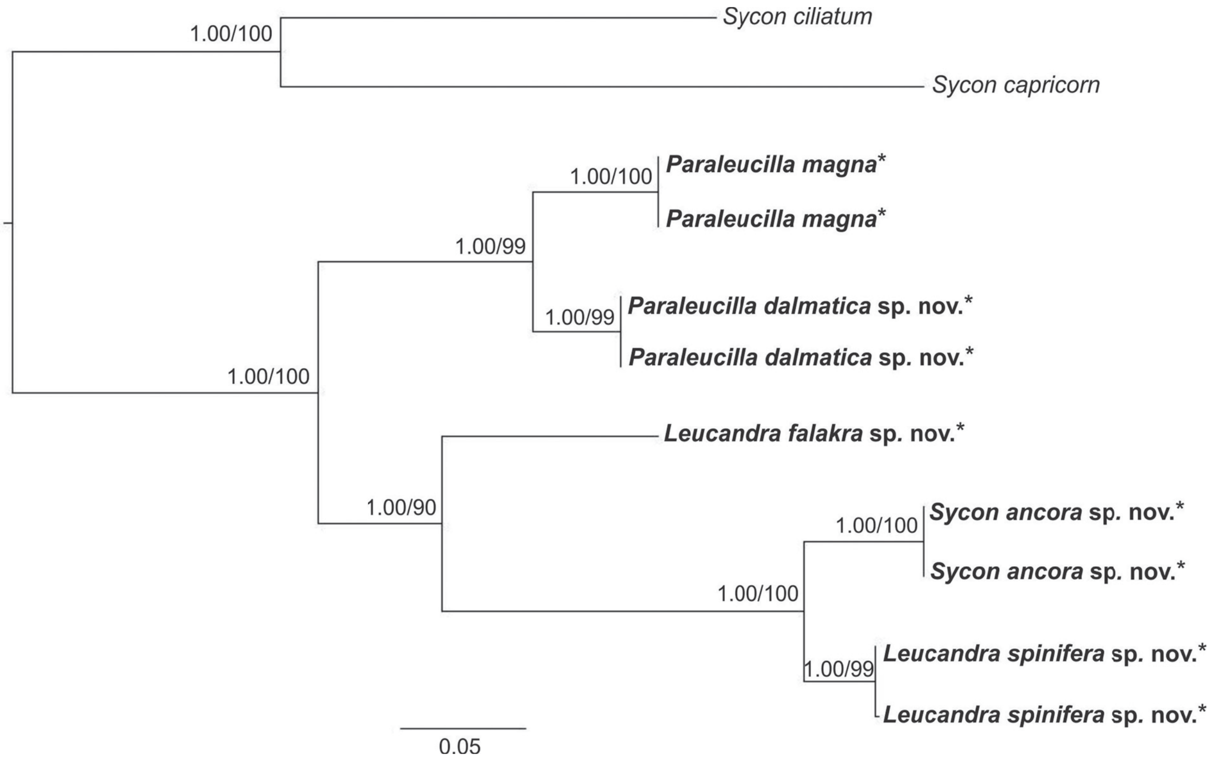

Fig. 18. Maximum likelihood tree based on ITS1-5.8S-ITS2 rDNA sequences of Calcaronea. Bayesian posterior probabilities (PP) and bootstrap values (BS) are given near the branches (PP/BS; when >0.50). Adriatic species are written in bold. Adriatic specimens obtained in this study are marked with an asterisk.

results with ours, we found a total of 39 species of calcarean sponges in the Adriatic Sea (Tables 12–13). In this species list we do not consider the records of *Clathrina coriacea* (Montagu, 1814), *Sycon ciliatum* (Fabricius, 1780) or *S. proboscideum* (Haeckel, 1870). The occurrence of *C. coriacea* was not considered because, analysing the descriptions of this species for the Adriatic Sea, we think that they most probably represent *C. conifera* or *C. primordialis*. *Sycon ciliatum* seems to be restricted to the North Atlantic and was probably mistaken for *S. raphanus* (Haeckel, 1872). *Sycon proboscideum* is a species from the Red Sea and its occurrence in the Adriatic Sea was mentioned only by Breitfuss (1935), which suggested that some specimens previously identified as *S. raphanus* could in fact be *S. proboscideum*. He did not give a description or any further clues. Therefore, the occurrence of this species in the Adriatic Sea has to be verified (Burton 1963; Longo & Pronzato 2011).

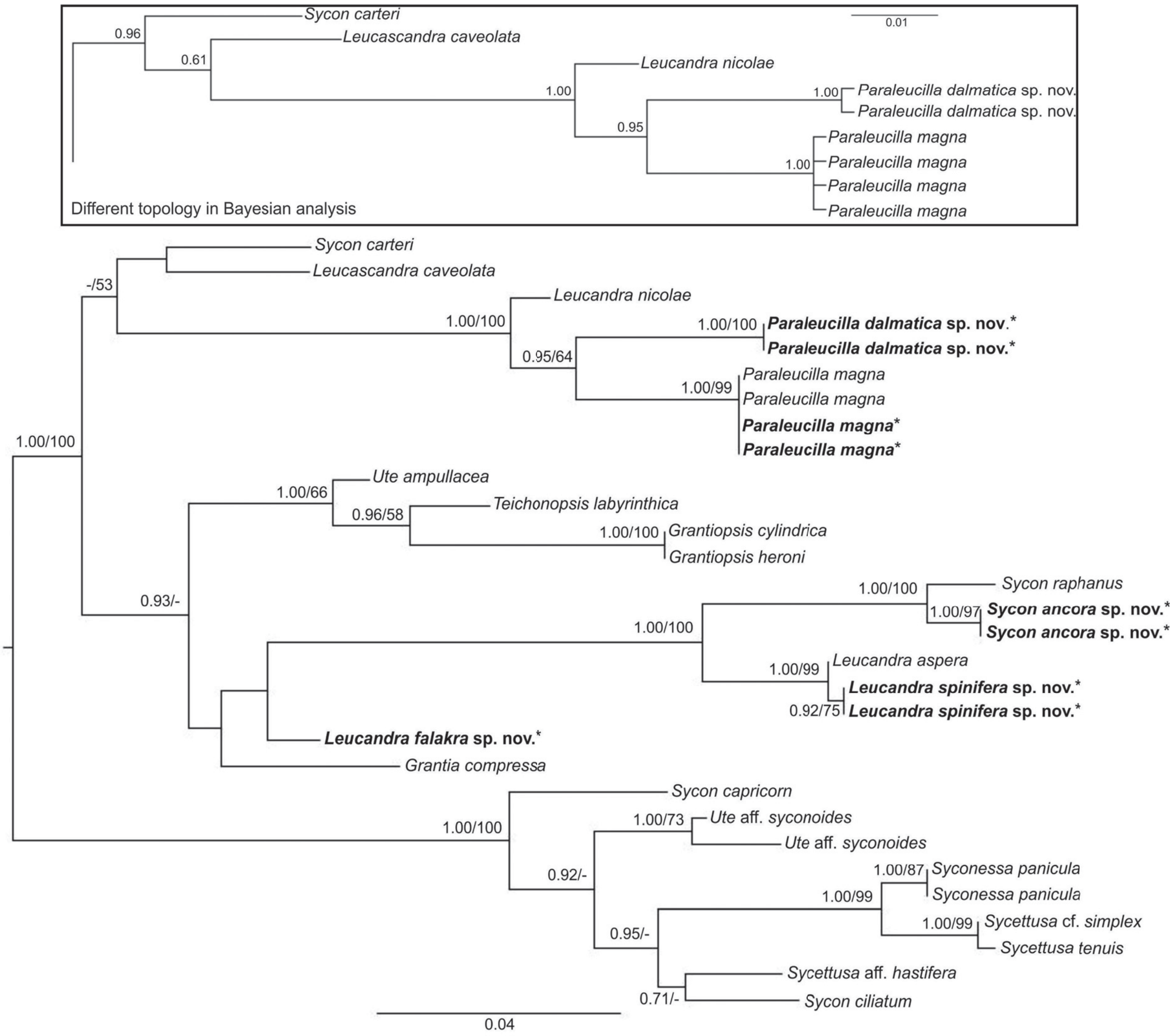

Fig. 19. Maximum likelihood tree based on partial 28S rDNA sequences of Calcaronea. Bayesian posterior probabilities (PP) and bootstrap values (BS) are given near the branches (PP/BS; when >0.50). Adriatic specimens are written in bold. Adriatic specimens obtained in this study are marked with an asterisk. The detached tree shows the only difference in the topology of the ML and Bayesian analyses.

Table 12. Calcinean species reported from the Adriatic Sea and their distribution. *Type locality. ** Probably *Clathrina conifera* or *C. primordialis*.

Species	Longitude (N)	Latitude (E)	Locality	Source
Ascaltis reticulum (Schmidt, 1862)	15.22	44.10	Zadar = Zara*	Schmidt 1862
Ascaltis reticulum (Schmidt, 1862)	15.92	43.73	Šibenik = Sebenico*	Schmidt 1862
Ascaltis reticulum (Schmidt, 1862)	16.73	43.13	Hvar = Lesina	Schmidt 1862 (according to Haeckel 1872); Heller 1864 (*apud* Haeckel 1872); Haeckel 1872
Ascaltis reticulum (Schmidt, 1862)	13.63	45.08	Rovinj	Longo & Pronzato 2011
Ascaltis reticulum (Schmidt, 1862)	16.36	43.48	Island of Čiovo	Present work
Ascandra contorta Bowerbank, 1866 (*sensu* Minchin 1905)	15.5	42.12	Tremiti Island	Lendenfeld 1891 (as *Ascetta spinosa*); Sarà 1961
Ascandra contorta Bowerbank, 1866 (*sensu* Minchin 1905)	13.80	45.63	Trieste	Lendenfeld 1891 (as *Ascetta spinosa*)
Ascandra contorta Bowerbank, 1866 (*sensu* Minchin 1905)	13.77	45.60	Muggio	Lendenfeld 1891 (as *Ascetta spinosa*)
Ascandra contorta Bowerbank, 1866 (*sensu* Minchin 1905)	16.22	43.01	Island of Vis = Lissa	Lendenfeld 1891 (as *Ascetta spinosa*)
Ascandra contorta Bowerbank, 1866 (*sensu* Minchin 1905)	16.73	43.13	Hvar = Lesina	Lendenfeld 1891 (as *Homandra falcata*)
Ascandra falcata Haeckel, 1872	13.63	45.08	Rovinj	Longo & Pronzato 2011
Ascandra falcata Haeckel, 1872	13.80	45.63	Trieste	Lendenfeld 1891
Ascandra falcata Haeckel, 1872	16.73	43.13	Hvar = Lesina*	Haeckel 1872; Lendenfeld 1891 (as *Homandra falcata*)
Ascandra falcata Haeckel, 1872	15.50	42.12	Tremiti Island	Sarà 1961; Longo & Pronzato 2011
Ascandra spalatensis sp. nov.	15.21	44.14	Zadar = Zara*	Present work
Borojevia cerebrum (Haeckel, 1872)	16.73	43.13	Hvar = Lesina*	Haeckel 1872; Lendenfeld 1891; Imešek *et al.* 2014
Borojevia cerebrum (Haeckel, 1872)	16.89	43.40	Vrulja Cove	Present work
Borojevia cerebrum (Haeckel, 1872)	13.63	45.08	Rovinj	Lendenfeld 1891
Borojevia croatica sp. nov.	16.37	43.48	Island of Čiovo*	Present work
Clathrina blanca (Miklucho-Maclay, 1868)	13.62	45.05	Island of St. Giovanni (near Rovinj)	Imešek *et al.* 2014
Clathrina blanca (Miklucho-Maclay, 1868)	13.63	45.08	Rovinj	Longo & Pronzato 2011
Clathrina blanca (Miklucho-Maclay, 1868)	16.73	43.13	Lesina=Hvar	Lendenfeld 1891
Clathrina blanca (Miklucho-Maclay, 1868)	14.72	45.15	Selce	Imešek *et al.* 2014
Clathrina blanca (Miklucho-Maclay, 1868)	16.37	43.48	Island of Ciovo	Imešek *et al.* 2014

Clathrina lacunosa (Johnston, 1842)	13.63	45.08	Rovinj	Lendenfeld 1891 (as *Ascandra angulata*)
Clathrina lacunosa (Johnston, 1842)	16.73	43.13	Hvar = Lesina	Lendenfeld 1891 (as *Ascandra angulata*)
Clathrina conifera Klautau & Borojević, 2001	18.1	42.64	Dubrovnik	Present work
Clathrina conifera Klautau & Borojević, 2001	18.11	42.63	Island of Lokrum	Present work
Clathrina clathrus (Schmidt, 1864)	15.92	43.73	Šibenik = Sebenico*	Schmidt 1864
Clathrina clathrus (Schmidt, 1864)	16.73	43.13	Hvar = Lesina	Schmidt (according to Haeckel 1872); Heller 1864 (*apud* Haeckel 1872); Haeckel 1872
Clathrina clathrus (Schmidt, 1864)	16.22	43.01	Island of Vis = Lissa*	Heller 1864 (*apud* Haeckel 1872)
Clathrina clathrus (Schmidt, 1864)	13.63	45.08	Rovinj	Imešek *et al.* 2014
Clathrina clathrus (Schmidt, 1864)	13.62	45.05	Island of St. Giovanni (near Rovinj)	Present work
Clathrina clathrus (Schmidt, 1864)	16.22	43.01	Island of Vis = Lissa*	Present work
Clathrina clathrus (Schmidt, 1864)	17.68	42.81	Prapratno Cove	Present work
Clathrina clathrus (Schmidt, 1864)	16.36	43.48	Island of Čiovo	Present work
Clathrina coriacea (Montagu, 1814)**	13.63	45.08	Rovinj	Longo & Pronzato 2011
Clathrina coriacea (Montagu, 1814)**	15.5	42.12	Tremiti Island	Sarà 1961; Longo & Pronzato 2011
Clathrina coriacea (Montagu, 1814)**	16.87	41.13	Bari	Longo & Pronzato 2011
Clathrina primordialis (Haeckel, 1872)	16.73	43.13	Hvar = Lesina*	Schmidt (according to Haeckel 1872); Haeckel 1872; Heller 1864 (*apud* Haeckel 1872); Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	13.80	45.63	Trieste	Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	13.77	45.60	Muggio	Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	15.22	44.10	Zadar = Zara	Schmidt (according to Haeckel 1872); Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	15.92	43.73	Šibenik = Sebenico	Schmidt (according to Haeckel 1872); Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	16.87	42.73	Lastovo = Lagosta	Schmidt (according to Haeckel 1872); Lendenfeld 1891
Clathrina primordialis (Haeckel, 1872)	16.22	43.01	Island of Vis = Lissa	Lendenfeld 1891

Clathrina primordialis (Haeckel, 1872)	13.63	45.08	Rovinj	Lendenfeld 1891; Imešek *et al.* 2014 (as *Clathrina* cf. *hondurensis*)
Clathrina primordialis (Haeckel, 1872)	16.36	43.48	Island of Čiovo	Present work
Clathrina rubra Sarà, 1958	13.63	45.08	Rovinj	Imešek *et al.* 2014
Clathrina rubra Sarà, 1958	16.36	43.48	Island of Čiovo	Present work
Leucetta solida (Schmidt, 1862)	15.92	43.73	Šibenik = Sebenico*	Schmidt 1862; Lendenfeld 1891
Leucetta solida (Schmidt, 1862)	16.87	42.73	Lastovo = Lagosta	Schmidt (according to Haeckel 1872); Lendenfeld 1891
Leucetta solida (Schmidt, 1862)	16.73	43.13	Hvar = Lesina	Schmidt (according to Haeckel 1872); Haeckel 1872; Lendenfeld 1891
Leucetta solida (Schmidt, 1862)	16.87	42.73	Lastovo = Lagosta (Zaklopatica = Porto Chiave)*	Schmidt 1864
Leucetta solida (Schmidt, 1862)	15.5	42.12	Tremiti Island	Sarà 1961; Longo & Pronzato 2011
Leucetta solida (Schmidt, 1862)	16.87	41.13	Bari	Longo & Pronzato 2011

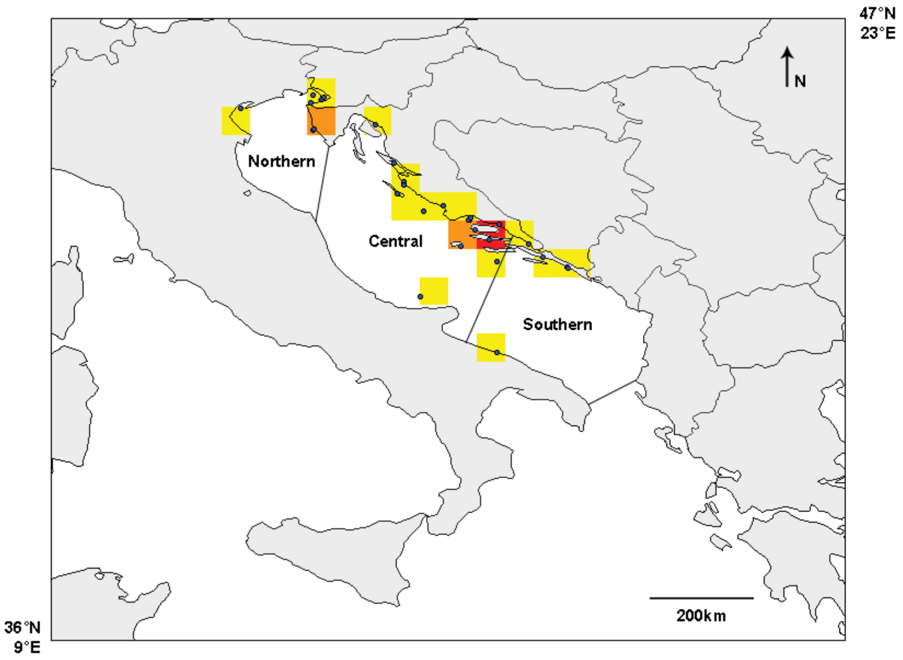

Fig. 20. Species richness in the Adriatic Sea divided by sectors. 🟨: 1–9 species; 🟧: 10–17 species; 🟥: 18–26 species.

Table 13. Calcaronean species reported from the Adriatic Sea and their distribution. * = Type locality.

Species	Longitude (N)	Latitude (E)	Locality	Source
Amphoriscus chrysalis (Schmidt, 1864)	16.73	43.13	Hvar = Lesina*	Schmidt 1864; Haeckel 1872
Amphoriscus chrysalis (Schmidt, 1864)	16.22	43.01	Island of Vis = Lissa*	Schmidt, 1864
Amphoriscus cylindrus (Haeckel, 1872)	16.73	43.13	Hvar = Lesina*	Haeckel 1872; Lendenfeld 1891
Amphoriscus gregori (Lendenfeld, 1891)	16.73	43.13	Hvar = Lesina*	Lendenfeld 1891
Aphroceras corticata (Lendenfeld, 1891)	16.73	43.13	Hvar = Lesina*	Lendenfeld 1891
Grantia capillosa (Schmidt, 1862)	15.92	43.73	Šibenik = Sebenico*	Schmidt 1862
Grantia capillosa (Schmidt, 1862)	13.63	45.08	Rovinj	Lendenfeld 1891
Grantia capillosa (Schmidt, 1862)	13.77	45.60	Muggio	Lendenfeld 1891
Grantia capillosa (Schmidt, 1862)	13.57	45.53	Pirano	Lendenfeld 1891
Grantia capillosa (Schmidt, 1862)	16.73	43.13	Hvar = Lesina	Schmidt 1864; Haeckel 1872; Lendenfeld 1891
Grantia capillosa (Schmidt, 1862)	15.92	43.73	Šibenik = Sebenico	Lendenfeld 1891
Leucandra aspera (Schmidt, 1862)	15.22	44.10	Zadar = Zara*	Schmidt 1862; Lendenfeld 1891
Leucandra aspera (Schmidt, 1862)	15.50	42.12	Tremiti Island	Sarà,1961; Longo & Pronzato 2011
Leucandra aspera (Schmidt, 1862)	16.22	43.01	Island of Vis = Lissa*	Heller 1864 (*apud* Haeckel 1872); Lendenfeld 1891
Leucandra aspera (Schmidt, 1862)	15.92	43.73	Šibenik = Sebenico	Schmidt 1862; Lendenfeld 1891
Leucandra aspera (Schmidt, 1862)	16.87	42.73	Lastovo = Lagosta	Schmidt 1862; Lendenfeld 1891
Leucandra aspera (Schmidt, 1862)	16.73	43.13	Hvar = Lesina	Schmidt 1862; Haeckel 1872; Lendenfeld 1891
Leucandra falakra sp. nov.	15.57	43.63	Blitvenica*	Present work
Leucandra spinifera sp. nov.	16.36	43.48	Island of Čiovo	Present work
Leucandra spinifera sp. nov.	16.89	43.40	Vrulja Cove*	Present work
Leucosolenia goethei Haeckel, 1870	13.63	45.08	Rovinj	Lendenfeld 1891
Leucosolenia variabilis Haeckel, 1870	13.63	45.08	Rovinj	Longo & Pronzato 2011
Paraleucilla dalmatica sp. nov.	16.36	43.48	Island of Čiovo	Present work
Paraleucilla magna Klautau, Monteiro & Borojević, 2004	17.43	43.05	Port of Ploče	Cvitković *et al.* 2013
Paraleucilla magna Klautau, Monteiro & Borojević, 2004	16.39	43.51	Port of Ploče	Present work

Paraleucilla magna Klautau, Monteiro & Borojević, 2004	16.48	43.29	Island of Brač	Present work
Polejaevia telum (Lendenfeld, 1891)	16.73	43.13	Hvar = Lesina	Lendenfeld 1891
Sycantha tenella Lendenfeld, 1891	13.8	45.63	Trieste	Lendenfeld 1891
Sycetta conifera (Haeckel, 1872)	16.73	43.13	Hvar = Lesina	Haeckel 1872; Lendenfeld 1891
Sycon ancora sp. nov.	15.04	44.48	Island of Pag*	Present work
Sycon ancora sp. nov.	16.39	43.51	Split	Present work
Sycon elegans (Bowerbank, 1845)	15.50	42.12	Tremiti Island	Sarà 1961
Sycon helleri (Lendenfeld, 1891)	16.73	43.13	Hvar = Lesina	Lendenfeld 1891
Sycon humboldti Risso, 1826	42.75	16.87	Lastovo = Lagosta	Schmidt 1862
Sycon humboldti Risso, 1826	16.22	43.01	Island of Vis = Lissa	Heller 1864 (*apud* Haeckel 1872); Schmidt 1862
Sycon humboldti Risso, 1826	16.73	43.13	Hvar = Lesina	Schmidt 1862; Haeckel 1872
Sycon humboldti Risso, 1826	12.34	45.44	Venice	Martens 1824 (*apud* Haeckel 1872)
Sycon humboldti Risso, 1826	15.10	43.93	Dugi otok = Isola Grossa	Martens 1824 (*apud* Haekkel 1872)
Sycon quadrangulatum (Schmidt, 1868)	13.61	45.68	Dalmatia, Gulf of Trieste	Schmidt,1868
Sycon quadrangulatum (Schmidt, 1868)	16.73	43.13	Hvar = Lesina	Haeckel 1872
Sycon quadrangulatum (Schmidt, 1868)	16.22	43.01	Island of Vis = Lissa	Heller 1864 (*apud* Haeckel 1872)
Sycon raphanus Schmidt, 1862	13.63	45.08	Rovinj	Longo & Pronzato 2011
Sycon raphanus Schmidt, 1862	16.73	43.13	Hvar = Lesina	Schmidt 1862; Haeckel 1872
Sycon raphanus Schmidt, 1862	16.87	41.13	Bari	Longo & Pronzato 2011
Sycon raphanus Schmidt, 1862	13.8	45.63	Trieste	Lieberkühn 1859 (*apud* Haeckel 1872); Schmidt,1862 (as *S. ciliatum*); Haeckel 1872
Sycon raphanus Schmidt, 1862	15.22	44.10	Zadar = Zara	Schmidt 1862
Sycon raphanus Schmidt, 1862	15.92	43.73	Šibenik = Sebenico	Schmidt 1862
Sycon schmidt (Haeckel, 1872)	42.75	16.87	Lastovo = Lagosta	Schmidt 1862 (*apud* Haekkel 1872); Haeckel 1872
Sycon schmidt (Haeckel, 1872)	16.73	43.13	Hvar = Lesina*	Haeckel 1872
Sycon setosum Schmidt, 1862	16.73	43.13	Hvar = Lesina	Heller 1864 (*apud* Haeckel 1872); Haeckel,1872
Sycon setosum Schmidt, 1862	16.22	43.01	Island of Vis = Lissa	Heller 1864 (*apud* Haeckel 1872)

Sycon setosum Schmidt, 1862	13.63	45.08	Rovinj	Longo & Pronzato 2011
Sycon tuba (Lendenfeld, 1891)	13.8	45.63	Trieste*	Lendenfeld 1891
Sycyssa huxleyi Haeckel, 1872	16.73	43.13	Hvar = Lesina*	Haeckel 1872
Ute glabra Schmidt, 1864	16.73	43.13	Hvar = Lesina	Haeckel 1872; Lendenfeld 1891
Ute glabra Schmidt, 1864	16.22	43.01	Island of Vis = Lissa	Heller 1864 (*apud* Haeckel 1872)
Ute glabra Schmidt, 1864	16.87	42.73	Lastovo = Lagosta (Zaklopatica = Porto Chiave)*	Schmidt 1864

Our results indicate *Sycon* to be the most diverse genus, with nine species, followed by *Clathrina* with six species. However, it is important to consider that *Sycon* is not a monophyletic genus. It is very difficult to identify *Sycon* species unequivocally, as most of them have a similar spicule composition - diactines, trichoxeas and triactines in the distal cones, tubar triactines, subatrial triactines and tetractines, and atrial tetractines. To date there have been no studies on the intraspecific morphological variability of *Sycon*. In addition, most species were poorly described and insufficiently analyzed on the molecular level, which also applies to the calcaronean genera *Paraleucilla*, *Leucandrilla*, *Leucandra* and *Leucilla.* Molecular phylogenetic studies including as many species as possible would be very desirable to evaluate the limits between these genera. Hence, new calcaronean species are welcome to facilitate more thorough revision of their systematics and to link the molecular traits to the phylogenetically important morphological traits.

We have also confirmed the presence of a few species known so far only from the Atlantic. It was unexpected to find *Clathrina conifera* in the Adriatic Sea, as this species was first described along the Brazilian coast and was considered endemic (Klautau *et al.* 1994). Our finding raises the question whether this species was ever truly endemic for Brazil. Since Adriatic calcarean sponges are vastly unexplored and *C. conifera* is part of the *C. primordialis* species complex, it is possible that it has been recorded previously as *C. primordialis* (or *C. coriacea*). In 2010, a specimen of *Clathrina conifera* was observed for the first time in the Southern Adriatic, near the Island of Lokrum, and a year later, more than 20 specimens were recorded near the city of Dubrovnik. As both locations are close to the area in Dubrovnik frequently visited by cruise ships, it is possible that this species has been introduced into the Adriatic. However, if this species arrived by anthropogenic means, we cannot state whether it arrived from the Western Atlantic to the Adriatic or vice-versa. It is important to mention that *Paraleucilla magna* is also present in the Southern Adriatic (Cvitković *et al.* 2013). It was first recorded in Brazil in the 1980's; however, the origin of this species is unknown. It seems to have been introduced by anthropogenic means into the Mediterranean (Longo *et al.* 2007) and to have spread into the Eastern Mediterranean, including the Adriatic Sea. Here, we molecularly confirm the presence of *P. magna* near the Port of Ploče and at a new location, near the Island of Brač (Table 13).

The molecular analyses revealed some interesting taxonomic traits. At the generic level, the monophyletic clade of former guanchas indicates that the development of a peduncle and of parasagittal spicules probably appeared only once in the evolution of *Clathrina*. *Clathrina hispanica* was nested within this group, although in the original description of this species neither peduncle nor parasagittal spicules were mentioned (Klautau & Valentine 2003). The type specimen of this species is fragmented, resulting in the impossibility of confirming if a peduncle was present or not; however, we re-analysed the slides of the holotype and found some parasagittal spicules. Another interesting result indicated the close relationship among *Ascandra*, *Soleneiscus* and *Levinella* revealed in the 28S analysis. Voigt *et al.* (2012) showed that the genus *Ascandra* is closely related to *Soleneiscus* and *Levinella*, which is now confirmed by our results. This implies that in the future the genera *Levinella* and *Soleneiscus* might be synonymised with the genus *Ascandra*; nonetheless, more detailed molecular and morphological analyses on a larger number of specimens and species are needed to confirm this action. At the species level, the molecularly confirmed presence of *Clathrina conifera* in the Adriatic raises a doubt of the earlier identification of the *C. primordialis* syntype, allowing the selection of a true lectotype of this species. Additionally, the re-description of *Borojevia cerebrum*, based on a molecular analysis of specimens discovered near its type locality (Lesina - Island of Hvar), confirmed the presence of this species in the Mediterranean Sea (Table 1; Fig. 16). Observing the morphological variations within a single, molecularly verified species, enabled the synonymization of two "*cerebrum*" varieties. Step by step, the "*cerebrum* complex" is being solved. All this again confirms that molecular verification of morphological traits is very important for a proper species assignment. It goes hand in hand with morphological confirmation relying on the type specimens, which often become deteriorated or even lost, without detailed descriptions. This review of some of the first species of calcarean sponges, that were last recorded and described by Haeckel in the 19th century, allowed validation of their taxonomic status at the molecular and morphological levels. It bears a significant weight in reviving museum collections, which would be of a great help for systematics research of calcarean sponges in the future.

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