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 calcareous sponge diversity of the Adriatic Sea, the type locality of some of the first described species of calcareous 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 calcareous species previously and currently known from the Adriatic Sea (39 species) is given. The Central Adriatic is indicated as the richest calcareous 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
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
Calcerean 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 Ruder 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′-TCATTTAGGAAAGTAAAAGTCG-3′) plus 5.8SR (5′-GCGTTCAAAGACTCGATGATTCT-3′) (Lôbo-Hajdu et al. 2004) and ITS2F (5′-CGGCTCGTGTCGATGATGAAACA-3′) plus ITS2R (5′-CGCCGTTACTGGGGAAATGCCCTGTG-3′) (Harcet et al. 2010). Partial 28SrDNA gene sequences were amplified with two pairs of primers, NL4F (5′-GACCCGAAAGATGTGATGA-3′) plus NL4R (5′-ACCTTGGAGACCTGATGAC-3′) (Nichols 2005), and primers CAL-28SFW (5′-GKCGGATCCCGAAYGAGCGCG-3′) plus CAL28SRV (5′-CCTCTATCACTCGGCTTTACC-3′) designed at the Laboratory of Molecular Genetics of the Ruder 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 MgCl2 (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-Blu repent cells and up to three clones were purified 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 | IT5S  | 28S   |
|--------------------------|--------------------------|----------------|--------------------------|-------|-------|
| CALCINEA                 |                          |                |                          |       |       |
| Arthuria hirsuta         | Cape Verde               | ZMAPOR07061    | KC843431                 | -     |       |
| Arthuria hirsuta         | Cape Verde               | ZMAPOR07103    | KC985143                 | -     |       |
| Arthuria spirallata      | Peru                     | MNJR 13652     | KC985140                 | -     |       |
| Arthuria spirallata      | Peru                     | MNJR 11414     | KC985142                 | -     |       |
| Ascaltis reticulatum     | Mediterranean Sea        | UFRJPOR6258    | HS885973                 | -     |       |
| Ascaltis reticulatum*    | Adriatic Sea             | PMR-13739 = UFRJPOR6870 | KP740022 | KP739998 |
| Ascandra corrupta        | Mediterranean Sea        | UFRJPOR6327    | HS885970                 | -     |       |
| Ascantra corallicola     | Norway                   | UFRJPOR6329    | HS885994                 | -     |       |
| Ascantra falcata         | Mediterranean Sea        | UFRJPOR5856    | HS885962                 | -     |       |
| Ascantra falcata         | Mediterranean Sea        | UFRJPOR6320    | HS885963                 | -     |       |
| Ascantra spalatensis sp. nov.* | Adriatic Sea            | PMR-17806 = UFRJPOR7540 | KP740024 | KP740003 |
| Borojevia cf. aspina     | Brazil                   | UFRJPOR5211    | HS885969                 | -     |       |
| Borojevia cf. aspina     | Brazil                   | UFRJPOR5245    | HS885998                 | -     |       |
| Borojevia brasiliensis   | Brazil                   | UFRJPOR5214    | HS885978                 | -     |       |
| Borojevia brasiliensis   | Brazil                   | UFRJPOR5230    | HS885999                 | -     |       |
| Borojevia cerebrum       | Mediterranean Sea        | UFRJPOR6322    | HS885964                 | -     |       |
| Borojevia cerebrum       | Mediterranean Sea        | UFRJPOR6323    | HS885971                 | -     |       |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CLB6       | KP740029 | KP740008 |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CLB32      | KP740031 | KP740010 |
| Borojevia cerebrum*      | Adriatic Sea             | PMR-17808      | KP740030 | KP740009 |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CLB33 = UFRJPOR7353 | KP740032 | KP740011 |
| Borojevia cerebrum*      | Adriatic Sea             | PMR-13740 = UFRJPOR6864 | KP740020 | KP739995 |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CL6        | KP740023 | KP740002 |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CLB18      | KP740027 | KP740006 |
| Borojevia cerebrum*      | Adriatic Sea             | IRB-CLB19      | KP740028 | KP740007 |
| Clathrina adusta         | GBR, Wistari Reef        | QMG313665      | -                        | JQ272288 |
| Clathrina aphrodita       | Peru                     | MNJR 14180     | KC985137                 | -     |       |
| Clathrina aphrodita       | Peru                     | MNJR 12994     | KC985138                 | -     |       |
| Clathrina aurea           | Brazil                   | MNJR 8998      | HQ885968                 | -     |       |
| Clathrina aurea           | Brazil                   | MNJR 8990      | HQ885958                 | -     |       |
| Clathrina antofagastensis| Chile                    | MNJR 9289      | HQ885985                 | -     |       |
| Clathrina antofagastensis| Peru                     | MNJR 11294     | KF002722                 | -     |       |
| Clathrina blanca          | Adriatic Sea             | PMR-14307      | KC479087 | KC479085 |
| Clathrina blanca*         | Adriatic Sea             | PMR-13744      | KP740017 | KP740000 |
| Clathrina clathrus        | Mediterranean Sea        | UFRJPOR6315    | HS885974                 | -     |       |
| Clathrina clathrus        | Mediterranean Sea        | UFRJPOR6325    | HS885965                 | -     |       |
| Clathrina clathrus        | Mediterranean Sea        | UFRJPOR6326    | HS885972                 | -     |       |
| Clathrina clathrus*       | Adriatic Sea             | IRB-CLB12      | KP740025 | KP740004 |
| Clathrina clathrus*       | Adriatic Sea             | PMR-13745      | KP740015 | KP740001 |
| Clathrina confera         | Brazil                   | MNJR 8997      | HQ885957                 | -     |       |
| Clathrina confera         | Brazil                   | MNJR 8991      | HQ885959                 | -     |       |
| Clathrina confera         | Adriatic Sea             | PMR-13738 = UFRJPOR6869 | KP740019 | KP739994 |
| Clathrina confera*        | Adriatic Sea             | PMR-17807      | KP740033 | KP740012 |
| Clathrina confera*        | Adriatic Sea             | IRB-S2 = UFRJPOR7541 | KP740034 | KP740013 |
| Clathrina confera*        | Adriatic Sea             | IRB-S3 = UFRJPOR7542 | KP740035 | KP740014 |
| Clathrina coriacea        | Norway                   | UFRJPOR6330    | HS885986                 | -     |       |
| Clathrina fiorida         | Chile                    | MNJR 8143      | HQ885984                 | -     |       |
| Clathrina helveola        | Australia                | QMG313680      | HS885988 | AM180987.1 |
| Clathrina hispanica       | Mediterranean Sea        | UFRJPOR6305    | KC843432                 | -     |       |
| Clathrina lucanosa        | Norway                   | UFRJPOR6334    | HS885991                 | -     |       |
| Clathrina lucanosa        | Norway                   | UFRJPOR6335    | HS885992                 | -     |       |
| Clathrina luteocucullitella| Australia                | QMG313684      | -                        | AM180988.1 |
| Clathrina peruana         | Peru                     | MNJR 13144     | KC985134                 | -     |       |
| Clathrina peruana         | Peru                     | MNJR 12839     | KC985135                 | -     |       |
| Clathrina primordialis    | Adriatic Sea             | PMR-14305      | KC479086 | KC479084 |
| Clathrina primordialis*   | Adriatic Sea             | IRB-CLB3 = UFRJPOR6863 | KP740016 | KP739996 |

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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://
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.
Type specimen
FRANCE: Banyuls-sur-Mer, Pyrénées, 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)

| Spicule | Actine | length (µm) | width (µm) |
|---------|--------|-------------|-------------|
| Diactine | - | 60.0 | 106.3 | 26.0 | 142.5 | 3.8 | 4.9 | 0.5 | 6.3 | 20 |
| Triactine | Basal | 57.6 | 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 |

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.
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)

| Spicule | Actine | length (µm) | width (µm) |
|---------|--------|-------------|-------------|
| Triactine | Basal | 43.2 | 9.5 | 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 |

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.”

Table 3. Spicule measurements of the holotype of *Ascandra spalatensis* sp. nov. (PMR-17806 = UFRJPOR 7540).
Our new species is a very typical *Ascandra*, with apically free, loosely anastomosed tubes. Its skeleton is very similar to that of *A. ascanroides*, 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. ascanroides* - 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. ascanroides* 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.](image)
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Table 4. Spicule measurements of *Borojevia cerebrum* (IRB-CLB33 = UFRJPOR 7539).

| Spicule | Actine | length (µm) | width (µm) |
|---------|--------|-------------|-------------|
|         |        | 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 officially 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.
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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      | 57.5 | 66.6 | 6.7 | 82.5 | 7.5 | 7.5 | 0.0 | 7.5 | 20 |
| Tetractine     | 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      | 50.0 | 66.1 | 8.6 | 80.0 | 7.5 | 7.8 | 0.7 | 10.0 | 20 |
| Tetractine     | 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 2/3 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.](image-url)
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)

|                | length (µm) | width (µm) |
|----------------|-------------|-------------|
| PMR-13738 = UFRJPOR6869 | 97.2 | 5.3 |
| IRB-S2 = UFRJPOR7541 | 102.6 | 6.8 |
| IRB-S3 = UFRJPOR7542 | 112.5 | 7.5 |

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

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?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.
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 (equiradiate and equiangular). 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

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  |
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 = UFRJ-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.

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

| Spicule Type                        | 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** |     |      |    |      |     |      |    |     |    |
| Paired                             | 324.0 | 624.5 | 192.3 | 1047.6 | 48.6 | 81.5 | 20.6 | 118.8 | 23  |
| Unpaired                           | 162.0 | 214.2 | 39.8 | 288.9 | 13.5 | 18.3 | 4.0 | 27.0 | 20  |
| **Choanosomal triactine**          |     |      |    |      |     |      |    |     |    |
| Paired                             | 108.0 | 189.7 | 58.9 | 351.0 | 13.5 | 19.8 | 4.1 | 29.7 | 20  |
| Unpaired                           | 99.9 | 154.0 | 26.4 | 199.8 | 8.1 | 12.4 | 2.4 | 16.2 | 19  |
| **Tetractine (canals)**            |     |      |    |      |     |      |    |     |    |
| Paired                             | 45.9 | 143.0 | 56.5 | 288.9 | 9.5 | 12.4 | 1.9 | 16.2 | 19  |
| Unpaired                           | 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 Ćiovo, 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).**

| Spicule          | 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)

Ocular 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. crambeasa Haeckel, 1872 has no microdiactines and has tetractines only in the atrium; L. nausicaeae (Schuffner, 1877) has no diactines and the atrial skeleton comprises only tetractines; L. riojai Ferrer-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.

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.](image-url)
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 *Leucandrella*.

Spicules (Table 10)

| Spicule Type | Description | Measurements |
|--------------|-------------|--------------|
| Ocular triactines | Strongly sagittal. Actines are conical and sharp. The unpaired actine is longer and thinner than the paired ones and basipetally directed. | Length: 1000.0–25.0 µm, Width: 25.0–50.0 µm |
| Diactines | Giant. They are present in the ocular 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. | Length: >330.0 µm, Width: 2.5–5.0 µm |
| Trichoxeas | Present in the ocular crown and cortex. They are thin, straight and most of them are broken. Size: >330.0/2.5–5.0 µm. | Length: >330.0 µm, Width: >2.5 µm |

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

| Spicule Type | Description | Measurements |
|--------------|-------------|--------------|
| Diactine | | Length (µm) | Width (µm) |
| Paired | 100.0 | 50.0 |
| Unpaired | 150.0 | 60.0 |
| Microdiactine | | Length (µm) | Width (µm) |
| Paired | 120.0 | 50.0 |
| Unpaired | 170.0 | 80.0 |
| Trichoxea | | Length (µm) | Width (µm) |
| Paired | 170.0 | 80.0 |
| Unpaired | 150.0 | 60.0 |
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 slightly conical with sharp tips (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-4AC4-84BF-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 suboscular 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

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**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.
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.

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

| Spicule Type                        | 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  |
| Atrial tetractine I                |      |      |      |       |      |      |      |     |     |
| Unpaired                           | 108.0| 212.4 | 36.5 | 264.6 | 4.1  | 6.0  | 1.5 | 8.1  | 21 |
| Paired                             | 94.5 | 153.5 | 31.7 | 202.5 | 7.6  | 10.8 | 2.1 | 16.2 | 17 |
| Atrial tetractine II               |      |      |      |       |      |      |      |     |     |
| 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 |

KLAUTAU M. et al., Adriatic calcarean sponges (Porifera, Calcarea)
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*

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**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 5 m 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 triaxines 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.](image-url)
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 Ascalitis 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. proboscidium (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 proboscidium 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. proboscidium. 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 conferta* 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 (according to Haeckel 1872); Heller 1864 (apud Haeckel 1872); Haeckel 1872 |
| *Ascaltis reticulum* (Schmidt, 1862) | 16.73         | 43.13        | Hvar = Lesina        | Lendenfeld 1891 (as *Ascetta spinosa*)                                |
| *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 | 15.50         | 42.12        | Tremiti Island       | Lendenfeld 1891                                                       |
| *Ascandra falcata* Haeckel, 1872 | 16.73         | 43.13        | Hvar = Lesina*       | Haeckel 1872; Lendenfeld 1891 (as *Homandra falcata*); 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 Čiovo      | Imešek et al. 2014                                                   |
| Species                      | Azimuth | Altitude | Location                          | Reference                                                                 |
|-----------------------------|---------|----------|-----------------------------------|---------------------------------------------------------------------------|
| *Clathrina lacunosa* (Johnston, 1842) | 13.63   | 45.08    | Rovinj                            | Lendenfeld 1891 (as *Ascanandra angulata*)                                |
| *Clathrina lacunosa* (Johnston, 1842) | 16.73   | 43.13    | Hvar = Lesina                     | Lendenfeld 1891 (as *Ascanandra 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); Heller 1864 (*apud* Haeckel 1872); 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  
Schmidt (according to 
Haeckel 1872); Lendenfeld 1891

**Leucetta solida** (Schmidt, 1862)  
16.87 42.73 Lastovo = Lagosta  
Schmidt (according to 
Haeckel 1872); 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 & Pron- 
zato 2011

**Leucetta solida** (Schmidt, 1862)  
16.87 41.13 Bari  
Longo & Pronzato 2011

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**Fig. 20.** Species richness in the Adriatic Sea divided by sectors. ▼: 1–9 species; ▼: 10–17 species; ▼: 18–26 species.
Table 13. Calcarean 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; Haeckel 1872; 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 Ćiovo | 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 Ćiovo | 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                     |
| Species                        | Coordinates | Location Details | Reference(s)                                                                 |
|-------------------------------|-------------|------------------|------------------------------------------------------------------------------|
| *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                                                            |
| *Sycteta 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 Haeckel 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 Haeckel 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)                                             |
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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