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A new species of *Ernstia* (Porifera: Calcarea) described from marine aquarium

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Abstract

A new species of calcareous sponge, *Ernstia pyrum* **sp. nov.** (Calcarea, Calcinea, Clathrinida, Clathrinidae) is described from marine reef aquarium. It is assumed that its type locality is Indonesia. Molecular data render this species as a member of the genus *Ernstia*, although its morphology deviates in significant points from the original definition of the genus. In particular *Ernstia pyrum* **sp. nov.** has a clear atrial cavity not lined with choanocytes (true atrium), a feature not reported before for this genus.

Key words: Porifera, Calcarea, *Ernstia,* Indonesia, Aquarium

Introduction

Marine reef aquariums stocked with tropical corals and invertebrates became rather popular during the last decade. An important component of such reef aquariums are so called "living stones", highly porous dead coral fragments populated densely by various little metazoans (Borneman 2001; Sprung & Delbeek 2005). They provide an easy opportunity to study small marine invertebrates, which may be rare or hard to find in the natural habitats, but which, for some, often not clearly understood reasons, proliferate and become numerous and common in reef aquariums. A number of species of various taxonomic groups, from vertebrates (fishes) to protists, originally described from aquariums, is rather high. Some of these species were subsequently recorded in the nature, others are known only from aquariums and so far not recorded in the natural habitats. Probably the most known examples of such aquarium-based species are *Amakusaplana acroporae* Rawlinson, Gillis, Billings & Borneman, 2011, a parasitic flatworm known for most marine aquarists as AEFW (Acropora Eating Flat Worm), which has global distribution in marine aquariums, but whose origin is not known (Rawlinson *et al.* 2011), and recently described *Ancoracysta twista* Janouškovec, Tikhonenkov, Burki, Howe, Rohwer, Mylnikov & Keeling, 2017, an unusual protist representing "a new lineage of Eukaryotes" (Janouškovec *et al.* 2017: 3717) which is also known only from marine aquarium.

Sponges constitute a significant part of the fauna of "living stones" in marine aquariums, many species are recognized by marine aquarists but almost all of them are not identified (Sprung & Delbeek 2005). In the present paper we describe a calcareous sponge belonging to a species which appear to be rather common in marine reef aquariums. The species described in the present paper was found in abundance and proliferates in marine aquarium maintained by the first two authors of this paper. The sponge first appeared on "living stones" imported from Indonesia, therefore we assume that this is an Indonesian species. Morphological and molecular analyses showed that it is an undescribed species of the genus *Ernstia*, family Clathrinidae.

In the recent years a considerable progress has been made on the systematics and taxonomy of Calcarea and especially on clathrinid sponges (*e.g.* Klautau &Valentine 2003, Klautau *et al.* 2013). It was shown that most of the species have limited distribution, the so-called cosmopolitan taxa are actually represented by several morphologically and genetically distinct species, and the general number of species and genera considerably exceeds previous estimations (Klautau &Valentine 2003; Klautau *et al.* 2013). Many new species were recently described, most of

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them coming from Brazil (*e.g.* Azevedo *et al.* 2017), tropical Pacific and Indian Ocean (Van Soest & De Voogd 2015; 2018), Australia (Wörheide & Hooper 1999), Norway (Rapp 2006), Adriatic Sea (Klautau *et al.* 2016) and Caribbean Sea (Cóndor-Luján *et al.* 2018; Fontana *et al.* 2018; López *et al.* 2018). Van Soest & De Voogd (2015), who described 16 new species in their monograph and thereby almost doubled the number of known species from Indonesia, suspected that many more species are to be discovered in this region. In our paper we add another new species to this list.

Material and methods

Numerous specimens were collected in a reef aquarium stocked with "living stones" imported from Indonesia. Most specimens were fixed in 96% ethanol. For histology, several specimens were fixed in seawater formalin (about 24 hours), then decalcified in 5% solution of Na_2EDTA (about 6 hours), embedded in paraffin using isopropanol-mineral oil method (Sanamyan *et al.* 2013), cut into 2.5 μm sections and stained with acid fuchsin and methyl blue. Spicule preparations and measuring followed standard procedures (Wörheide & Hooper 1999; Klautau & Valentine 2003). The type material is stored in the Zoological Institute of the Russian Academy of Sciences, St. Petersbourg (ZIN) and in the Zoological Museum of the Moscow State University, Moscow (ZMMU).

For molecular analysis a small piece of tissue of one specimen (ZMMU G-259) was used for DNA extraction with Syntol S-Sorb kit by Syntol Company, according to the producer's protocols. Extracted DNA was used as a template for the amplification of partial sequences of the ribosomal internal transcribed spacer (ITS). DNA was amplified by PCR with the following primers: RA2 (GTCCCTGCCCTTTGTACACA) and ITS2.2 (CCTGGTTAGTTTCTTTTCCTCCGC) (Wörheide 1998). Polymerase chain reaction (PCR) amplifications were carried out in a 20 μL reaction volume, which included 4 μL of 5x Screen Mix (Eurogen Lab), 0.5 μL of each primer (10 μM stock), 1 μL of genomic DNA (concentration not measured), and 14 μL of sterile water. The amplification was performed with an initial denaturation for 3 min at 96°C, followed by 30 cycles of 60 sec at 95°C (denaturation), 30 sec at 55°C (annealing), and 60 sec at 72°C (extension), with a final extension of 7 min at 72°C. DNA sequences of both strands were obtained using the ABI PRISM1Big-Dye™ Terminator v. 3.1. on an automated DNA sequencer (Applied Biosystems Prism 3700). Sequence was deposited in GenBank (accession number MK617946). Additional molecular data for *Ernstia* species were obtained from GenBank. ITS sequences were aligned using the Mafft v7.409 with E-INS-i algorithm and "--maxiterate 1000" option (Katoh & Standley 2013). Alignment was inspected and edited manually in AliView v.1.24 (Larsson 2014). Final alignment contained 859 bp including gaps. A ML tree was generated by RAxML v.8.2.9 (Stamatakis 2014) running on local machine and with the following options: "-GTRGAMMA" (GTR+G model of substitution), "-f a" (conduct rapid bootstrap analysis and search for the best-scoring ML tree in one single program run) and "autoMRE" to let RAxML halt bootstrapping automatically using MRE-based bootstopping criterion. To view, edit and print final tree MEGA7 (Kumar *et al.* 2016) was used. Species names and GenBank accession numbers of the DNA sequences used for the phylogenetic analysis are listed in Table 1.

TABLE 1. Species included in the phylogenetic analyses of this study. *Species with newly generated DNA sequences.

Species	GenBank accession number
Ernstia pyrum sp. nov.*	MK617946
Ernstia citrea	KC843433
Ernstia citrea	KX548927
Ernstia arabica	KY366406
Ernstia klautauae	KC843451
Ernstia rocasensis	KX548929
Ernstia solaris	KX548915
Ernstia tetractina	HQ589000
Ascandra contorta	HQ588970
Ascandra spalatensis	KP740024
Ascandra falcata	HQ588963

Results

Class Calcarea Bowerbank, 1864

Subclass Calcinea Bidder, 1898

Order Clathrinida Hartman, 1958

Family Clathrinidae Minchin, 1900

***Ernstia pyrum* sp. nov.**
(Figures 1–3, Table 2)
urn:lsid:zoobank.org:act:1B70AF30-D9DB-42CF-98AE-173D1D0776C4

Material examined. Holotype: ZIN 11879, marine aquarium, on "live stones" imported from Indonesia (more accurate location is not available), 17 January 2019. Paratypes: ZIN 11880, same data, 1 specimen; ZMMU G-259, same data, 1 specimen; ZIN 11881, same data, 24 specimens.

Etymology. The specific epithet *pyrum* (pear in Latin) refers to the shape of most specimens of this sponge.

General morphology. The cormus is yellow, oval, pear-shaped or, occasionally somewhat less regular compact mass made up of tightly anastomosed thin tubuli (Fig. 1A, B). It is attached to the substratum by a wider basal part or by a side, usually upright. The consistence is not especially soft, compressible. Overall size is up to 2 cm in its greatest dimension, but usually smaller, most specimens 10–12 mm in height and 5–8 mm in diameter. All tubuli are of about the same diameter (0.2–0.3 mm), no water collecting tubes are recognizable on the surface or in the sections. Usually one terminal osculum is present, up to 6 mm long and 2–3 mm in diameter, rarely several oscular tubes are developed. There is a rather voluminous atrial cavity delimited by clear atrial membrane without choanocytes (Fig. 1A, C). No cells with granules are present.

Skeleton. The skeleton has no special organization and is composed of triactines, tetractines and often, but not always, trichoxeas. Trichoxeas are present only on the surface, some of them protrude perpendicularly or obliquely to the surface of the sponge but most are parallel to it (Fig. 2C). The walls of the tubuli are built by a few layers of triactines and tetractines. The apical actines of the tetractines protrude into the tubule lumen (Fig. 2D). Triactines are the most numerous spicules, the ratio of triactines to tetractines on the surface and internal layers (choanosome) is rather stable and varied from 5:1 to 5.7:1 in several measured specimens. Tetractines occur in somewhat higher frequency in the wall of the atrial cavity but still are two times less numerous here than the triactines. The size of triactines and tetractines differs in different regions of the sponge: the spicules in the wall of the atrial cavity and, especially, in the oscular membrane and are noticeably larger than the spicules in the other parts of the body.

Spicules (Table 2, Fig. 2).

Triactines. Equiangular and mostly equiradiate. They possibly may be divided into two categories: the spicules with the short and wide conical actines and sharp tips, and the spicules with the long narrow cylindrical, sometimes slightly undulating actines and usually blunt tips. However there are all transitions between these extremities and we failed to delimit these categories clearly either by size or by the shape (Fig. 2A) and measured them together as a single category.

Tetractines. Similar to the triactines and show the same size and shape variations. Apical actine is needle-like, smooth, long (80–120 μm), thin, straight and sharp.

Trichoxeas. Thin, long and straight.

Habitat. Numerous specimens of this species were found on so called "live stones" (lime stones consisting mostly of dead parts of stony corals collected at coral reefs), imported from Indonesia for marine aquarium trade. They rapidly proliferate in the so called "reef aquarium" with stony corals and marine fishes at the conditions required to maintain stony corals: water temperature 25–27ºC, salinity 35‰, low level of inorganic nutrients (concentration of inorganic nitrate is about 2 ppm, inorganic phosphate less than 0.05 ppm). These sponges prefer dim places but may occur on well-lit areas too.

Type locality. Article 76.1.1. of the International Code of Zoological Nomenclature (ICZN, 1999) says for the species based on the specimens transported by humans: "the type locality is the place from which the name-bearing type [...] began its unnatural journey". The type locality for *Ernstia pyrum* sp. nov. is therefore assigned to Indonesia from where the "living stones" with this species were imported.

TABLE 2. Spicule measurements of *Ernstia pyrum* **sp. nov.**

Specimen	Spicule	Body region	Actine	Length (µm) min–mean–max, SD	Width (µm) min–mean–max, SD	n
ZIN 11879	Triactines	Surface and choanosome	Basal	40.8–**74.8**–105.5 SD=14.4	4.3–**6.9**–9.7	101
		Atrial membrane	Basal	38.3–**83.7**–135.3, SD=22.1	5.4–**7.9**–10.3	130
		Osculum	Basal	38.9–**103.9**–184.5, SD=30.5	4.8–**6.9**–9.7, SD=1.0	123
	Tetractines	Surface and choanosome	Basal	52.3–**73.6**–95.1 SD=13.7	5.0–**6.6**–8.9	20
			Apical	38.2–**93.5**–143.9, SD=25.4	1.8–**3.1**–4.3, SD=0.7	20
		Atrial membrane	Basal	50.6–**94.1**–142.1 SD=24.5	5.3–**7.4**–9.2	71
			Apical	not measured	not measured	–
		Osculum	Basal	49.0–**89.1**–174.1 SD=28.8	5.1–**6.7**–9.0	34
			Apical	not measured	not measured	–
	Trichoxeas	Surface		up to 900	0.5 or less	–
ZIN 11880	Triactines	Surface and choanosome	Basal	50.0–**76.0**–95.7 SD=9.1	5.6–**7.4**–10.1, SD=1.1	30
		Atrial membrane	Basal	70.3–**83.4**–115.8, SD=8.6	5.4–**7.9**–9.8, SD=1.1	30
		Osculum	Basal	60.7–**94.3**–135.0, SD=22.7	4.5–**6.8**–9.9, SD=1.0	33
	Tetractines	Surface and choanosome	Basal	52.9–**72.9**–104.7, SD=10.5	4.8–**7.2**–9.9, SD=1.2	30
			Apical	26.5–**46.3**–90.1, SD=15.2	1.8–**3.3**–4.7, SD=0.8	18
		Atrial membrane	Basal	64.2–**85.3**–114.3, SD=11.1	5.9–**8.2**–9.9, SD=1.0	32
			Apical	not measured	not measured	–
		Osculum	Basal	62.8–**84.4**–132.2, SD=14.9	5.3–**7.0**–8.9, SD=0.9	30
			Apical	22–56	not measured	2
ZIN 1181	Triactines	Surface and choanosome	Basal	45.6–**73.9**–96.4, SD=10.8	5.0–**8.0**–10.0, SD=1.2	32
	Tetractines	Surface and choanosome	Basal	53.0–**76.2**–96.5, SD=10.8	5.5–**7.6**–9.9, SD=1.1	32
			Apical	42.3–**69.3**–98.0, SD=19.6	2.5–**4.3**–8.5, SD=1.6	12

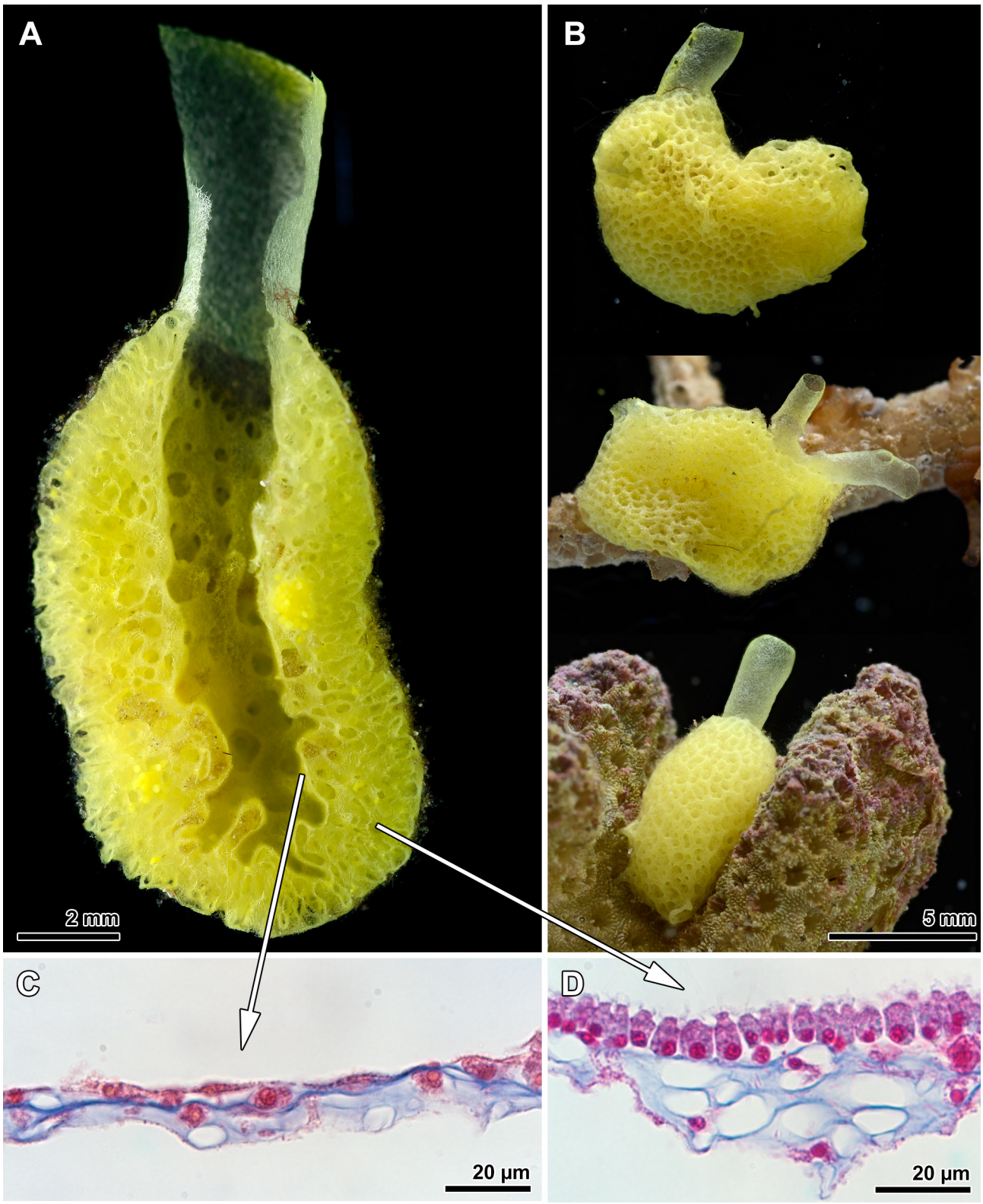

FIGURE 1. *Ernstia pyrum* **sp. nov.** A, holotype sectioned longitudinally, note well developed atrium; B, three paratypes, in live; C, histological section of the atrial wall, note the absence of choanocytes; D, histological section of a tubule lined by choanocytes.

Remarks. Molecular sequence data render this species as belonging to the genus *Ernstia* and being closely related to *E. citrea* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017 described from Rocas Atoll, Brazil (Fig. 3). Indeed, in life these two species appear to be very similar, both have compact yellow cormus composed of tightly anastomosed tubuli and a long terminal osculum (see Azevedo *et al.* 2017, Figure 16E). As perceived from

the published photographs (Azevedo *et al.* 2017, Figures 10A, B and 16E), *E. citrea* has less tightly anastomosed tubuli forming larger meshes on the surface of the sponge than in *E. pyrum* **sp. nov.** The diameter of the tubuli in *E. citrea* is said to be 3 mm, but according to scale bars on the above mentioned photographs this is an obvious mistype, probably should be 0.3 mm i.e. about the same as in *E. pyrum* **sp. nov.** The triactines and tetractines of *E. pyrum* **sp. nov.** show significantly greater range of size variations (38–185 µm) than those of *E. citrea* (67–108 µm). Further, the spicules with long cylindrical actines, occurring in the oscular and atrial wall of *E. pyrum* **sp. nov.**, are not reported for *E. citrea*.

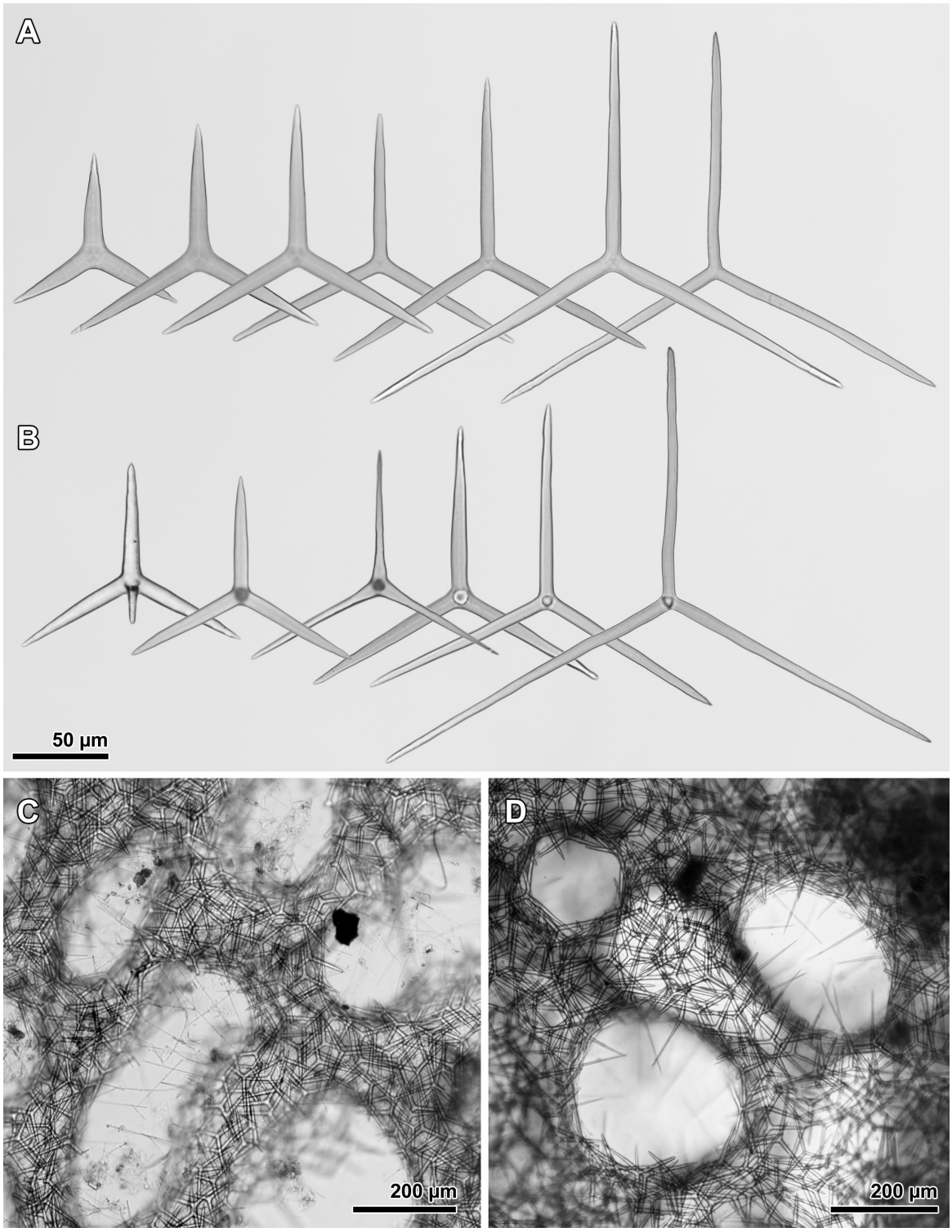

FIGURE 2. *Ernstia pyrum* **sp. nov.** A, triactines; B, tetractines; C, surface of the sponge, note numerous trichoxeas; D, inner wall of the atrium, note long apical actines of the tetractines projecting into the lumen of tubuli.

Other similar yellow *Ernstia* species with tightly anastomosed tubuli are *E. arabica* Voigt, Erpenbeck & Wörheide, 2017 from the Red Sea, *E. solaris* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017 from the SW Atlantic off Brazil, and *E. klautauae* Van Soest & De Voogd, 2015 from Indonesia. According to molecular data they all group together but are less related to *E. pyrum* **sp. nov.** than *E. citrea* (Fig. 3). Morphological differences are as follows: *E. solaris* has smaller and significantly less variable spicules than *E. pyrum* **sp. nov.**, triactines and tetractines are in almost the same proportion (Azevedo *et al.* 2017); *E. arabica* and *E. klautauae* have tri- and tetractines with conical actines, no spicules with long cylindrical actines are mentioned, tetractines either dominate or are in the same proportion as triactines (Voigt *et al.* 2017; Van Soest & De Voogd 2015; 2018).

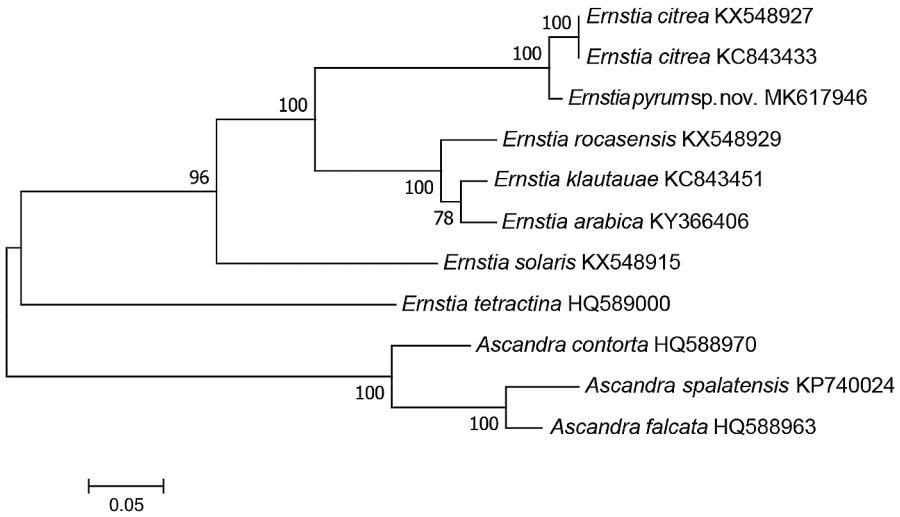

FIGURE 3. Maximum Likelihood tree inferred from the ITS rDNA sequences under the GTR model of *Ernstia* species (available in GenBank and generated in the present study) and three *Ascandra* species (as an outgroup). Bootstrap values are indicated.

Discussion

Ernstia pyrum **sp. nov.** has two features not expected for the genus *Ernstia*. The first feature is the skeleton composed of significantly more numerous triactines than tetractines. In most other species of *Ernstia*, for which this feature is reported, the tetractines either dominate (*e.g.* in *E. tetractina*, *E. arabica*) or occur in about the same proportion as triactines (*e.g.* in *E. crysops*, *E. citrea*). The only exception is one specimen of *E. naturalis* with more numerous triactines reported by Van Soest & De Voogd (2015), while all other specimens of this species either have similar proportion of tri- and tetractines (Van Soest & De Voogd 2015) or the tetractines are more numerous (Van Soest & De Voogd 2018). Another species with predominated triactines once assigned to *Ernstia* was *E. adusta* (Wörheide & Hooper, 1999), but this species was reassigned to *Arturia* based on morphological (Voigt & Wörheide 2016) and molecular data (Van Soest & De Voogd 2018).

The second unusual feature is the presence of clear atrial cavity delimited by a membrane not lined by choanocytes. We sectioned several specimens to ensure that this is not an anomaly and the interpretation is correct. Atrial cavity was quite obvious in all of them. Histological sections clearly show that in contrast with the regular tubuli forming the cormus, the inner surface of the atrial cavity is devoid of choanocytes (compare Figs 1C and 1D), therefore it should be regarded as a true atrium, and an aquiferous system is solenoid, a feature not reported previously for *Ernstia*. In the light of these findings it would be interesting to study other species of this genus to see whether they may have a true atrium overlooked previously. The true atrium is present in the related genera *Leucascus* or *Bidderia*, however these genera have well developed cortex which is not present in *Ernstia pyrum* **sp. nov**.

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