



SYMPOSIUM

A Molecular Phylogeny for the Order Clathrinida Rekindles and Refines Haeckel's Taxonomic Proposal for Calcareous Sponges

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Synopsis Most biological groups are still longing for a phylogenetically sound taxonomic organization. In this article, we aimed to verify the consistency of morphological characters in calcarean sponges of the well-known non-monophyletic order Clathrinida using a molecular phylogeny. For this we included 50 species, including six type species, currently assigned to eight different genera. A maximum likelihood topology was generated for the nuclear ITS marker using the General Time Reversible model and the bootstrap reliability test. Our topology indicated 10 clathrinid clades that included species with consistent morphological characters. In the present study, we defined nine of these clades as clathrinid genera, including four newly described and two newly diagnosed genera. Recent studies have indicated that not much phylogenetic information may be found in morphology, but our findings contradict this general assertion. Our study confirms the suitability of skeleton and body anastomosis as valid characters in a phylogenetically sound taxonomy for the order. Interestingly, we have also found that, apart from the Calcinea/Calcaronea split and a few minor details, Haeckel's original proposal is remarkably similar to our own, which was based on a molecular phylogeny 140 years later.

Introduction

The central biological tenet of evolution may be finely tuned with classification to compose a phylogenetically sound taxonomy (de Queiroz and Gaultier 1992). The first step towards a phylogenetic proposal for the taxonomy of calcareous sponges was tailored by Ernst Haeckel (1872). His detailed taxonomic scheme for the group was based on the composition of spicules and on the aquiferous system. Nevertheless, his proposal met strong criticism and was deemed unnatural by fellow taxonomists (Poléjaeff 1883; Dendy 1891, 1893; Minchin 1896). After Haeckel's pioneering study, other researchers came forward with different suggestions for the taxonomy of Calcarea.

Among those, Poléjaeff (1883) suggested that a more natural taxonomy would take the aquiferous system into account but not the composition of spicules as proposed by Haeckel. A decade later, Minchin (1896) proposed that the first major distinction of Calcarea should be between two large groups, later named Calcinea and Calcaronea (Bidder 1898). His proposal was strongly supported by several cytological features and it was a crucial step towards a phylogenetic taxonomy for the calcareans.

By the turn of the 20th century, the first formal phylogenetic tree for the Calcarea became available (Dendy and Row 1913). In that study, the authors acknowledged the clear-cut division between Calcinea and Calcaronea, as evidenced by several cytological

observations, but claimed that it would be impractical to use such laborious techniques in taxonomy. Hence, their phylogenetic proposal was based on aspects of the architecture of the skeleton.

Half a century later, [Hartman \(1958\)](#) confirmed division into the two subclasses Calcinea and Calcaronea ([Bidder 1898](#)), and these have been in use ever since. Furthermore, he added features related to the body cortex (corticalization) as key characters for lower taxonomical levels, proposing the orders Leucettida and Clathrinida for Calcinea. In the most recent review, [Borojevic et al. \(1990, 2002\)](#) claimed that corticalization and the aquiferous systems evolved in several lineages and extinguished the order Leucettida *sensu* [Hartman \(1958\)](#).

When the results of the first molecular studies became available, they unquestionably supported the division of the class Calcarea into Calcinea and Calcaronea ([Manuel et al. 2003, 2004](#)), but not of the lower taxonomic ranks ([Dohrmann et al. 2006; Voigt et al. 2012](#)). Most of these molecular studies indicated that the aquiferous system might not be phylogenetically informative ([Manuel et al. 2003, 2004; Dohrmann et al. 2006](#)), but a more recent analysis showed otherwise ([Voigt et al. 2012](#)). Due to poor taxon sampling, however, other morphological characters have never been properly tested in a molecular phylogenetic study and the taxonomy of Calcarea remains mainly typological.

In a recent study, our research group found a surprisingly strong phylogenetic signal for spicule composition and body anastomosis when many species of *Clathrina* were analyzed ([Rossi et al. 2011](#)). Hence, a phylogenetic systematics for this

group might be within reach if such characters are considered. It remains to be tested, however, whether the consistency of these characters remains in a broader taxonomic perspective.

In this study, our aim was to propose a phylogenetically sound scenario for the classification of the order Clathrinida, the most speciose order in the subclass Calcinea. For this, we have gathered an unprecedented taxon sampling with 50 clathrinid species, including six type species, currently assigned to eight genera, so as to evaluate the consistency of morphological characters with a well-resolved molecular phylogeny. Furthermore, we included samples from different geographical regions in order to test the consistency of current diagnoses of species ([Klautau et al. 1999; Manuel et al. 2003](#)).

Materials and methods

Specimens

The subclass Calcinea is monophyletic and it is currently divided into two orders, Clathrinida and Murrayonida. Nevertheless, it has been shown that genera of Murrayonida cluster within Clathrinida ([Voigt et al. 2012](#)), revealing that the presence of a hyper-calcified skeleton, as in Murrayonida, is not a valid taxonomic character. For this study, we are considering order Clathrinida *sensu* [Borojevic et al. \(2002\)](#), since we were unable to include species of Murrayonida in our dataset.

Our dataset comprises 50 species currently assigned to eight genera of Clathrinida, making this the most extensive dataset analyzed to date in the order ([Table 1](#)). Furthermore, due to a reported plasticity of the morphological characters of sponges ([Cavalcanti et al.](#)

Table 1 Analyzed specimens with collection sites, voucher numbers, and GenBank accession numbers

Species	Collection site	Voucher number	GenBank (ITS)
<i>Calcinea</i>			
<i>Ascandra falcata</i>	Mediterranean Sea	UFRJPOR 5856	HQ588962
<i>Clathrina antofagastensis</i>	Chile	MNRJ 9289	HQ588985
<i>Clathrina aspina</i>	Brazil	UFRJPor 5245	HQ588998
<i>Clathrina aurea</i>	Brazil	MNRJ 8998	HQ588968
<i>Clathrina brasiliensis</i>	Brazil	UFRJPor 5214	HQ588978
<i>Clathrina cerebrum</i>	Mediterranean Sea	UFRJPor 6322	HQ588964
<i>Clathrina clathrus</i>	Mediterranean Sea	UFRJPOR 6315	HQ588974
<i>Clathrina conifera</i>	Brazil	MNRJ 8991	HQ588959
<i>Clathrina contorta</i>	Mediterranean Sea	UFRJPor 6327	HQ588970
<i>Clathrina corallicola</i>	Norway	UFRJPor 6329	HQ588994
<i>Clathrina coriacea</i>	Norway	UFRJPor 6330	HQ588986
<i>Clathrina cylindractina</i>	Brazil	UFRJPor 5206	HQ588979

(continued)

Table 1 Continued

Species	Collection site	Voucher number	GenBank (ITS)
<i>Clathrina fjordica</i>	Chile	MNRJ 8143	HQ588984
<i>Clathrina helveola</i>	Australia	QMG313680	HQ588988
<i>Clathrina hirsuta</i>	Cabo Verde	ZMAPOR07061	KC843431
<i>Clathrina hispanica</i>	Mediterranean Sea	UFRJPOR6305	KC843432
<i>Clathrina luteoculcitella</i>	Australia	QMG313684	HQ588989
<i>Clathrina nansenii</i>	Greenland	UFRJPor 6332	HQ588982
<i>Clathrina reticulum</i>	Mediterranean Sea	UFRJPOR 6258	HQ588973
<i>Clathrina tetractina</i>	Brazil	UFRJPor 5183	HQ589000
<i>Clathrina wistariensis</i>	Australia	QMG313663	HQ588987
<i>Clathrina</i> sp. nov. 1	Brazil	UFRJPOR6621	KC843433
<i>Clathrina</i> sp. nov. 2	Brazil	UFRJPOR6617	KC843434
<i>Clathrina</i> sp. nov. 3	Caribbean, Curaçao	UFJPOR6737a	KC843435
<i>Clathrina</i> sp. nov. 4	Caribbean, Curaçao	UFRJPor 6733	KC843436
<i>Clathrina</i> sp. nov. 4	Caribbean, Curaçao	UFRJPor 6741	KC843437
<i>Clathrina</i> sp. nov. 5	French Polynesia, Moorea	UF:Porifera:1600	KC843438
<i>Clathrina</i> sp. nov. 5	French Polynesia	UFRJPOR6461	KC843439
<i>Clathrina</i> sp. nov. 6	New Zealand	UFRJPOR6839	KC843440
<i>Clathrina</i> sp. nov. 7	New Zealand	UFRJPOR6843	KC843441
<i>Clathrina</i> sp. nov. 8	Brazil	UFRJPOR6545	KC843442
<i>Clathrina</i> sp. nov. 8	USA, Florida	UFRJPOR5818	KC843443
<i>Clathrina</i> sp. nov. 8	Caribbean, Virgin Islands	ZMAPOR08344	KC843444
<i>Clathrina</i> sp. nov. 8	Caribbean, Curaçao	UFRJPOR6761	KC843445
<i>Clathrina</i> sp. nov. 9	French Polynesia	BMOO16290	KC843446
<i>Clathrina</i> sp. nov. 10	Caribbean	UFRJPOR6945	KC843447
<i>Clathrina</i> sp. nov. 11	Brazil	UFRJPOR6084	KC843448
<i>Clathrina</i> sp. nov. 11	Caribbean	P10x13	KC843449
<i>Clathrina</i> sp. nov. 12	Azores	UFRJPOR5627	KC843450
<i>Clathrina</i> sp. nov. 13	Indonesia	ZMAPOR08390	KC843451
<i>Clathrina</i> sp. nov. 14	Antarctica, Weddell Sea	SMF11866	KC874655
<i>Guancha lacunosa</i>	Norway	UFRJPor 6334	HQ588991
<i>Guancha ramosa</i>	Chile	MNRJ 10313	HQ588990
<i>Guancha</i> aff. <i>blanca</i>	Norwegian Sea	ZMBN90440	KC874656
<i>Leucaltis clathria</i>	Australia, DJ's Reef	QMG316022	AJ633861
<i>Leucaltis clathria</i>	Caribbean, Panama	P10x28T	KC843452
<i>Leucaltis nuda</i>	Chile	MNRJ 10804	KC843453
<i>Leucascus simplex</i>	French Polynesia, Moorea	BMOO16283	KC843454
<i>Leucetta chagosensis</i>	French Polynesia, Moorea	BMOO16210	KC843455
<i>Leucetta floridana</i>	Caribbean, Panama	PTL09.P100	KC843456
<i>Leucetta microraphis</i>	Australia, Wistari Reef	QMG313659	AJ633874
<i>Leucetta potiguar</i>	Brazil	UFPEPor547	EU781986
<i>Leucetta</i> cf. <i>pyriformis</i>	Antarctic	MNRJ13843	KC843457
<i>Leucetta</i> sp.	Antarctic, Weddell Sea	SMF 11868	KC874654
<i>Leucetta</i> sp.	Antarctic, Weddell Sea	MNRJ 13798	KC849700
<i>Leucettusa</i> sp.	New Zealand	OCDN6676-Q	KC843458
<i>Pericharax heteroraphis</i>	Australia	QMG313657	AF479062.1

2007), we decided to include samples from different localities to provide a consistency check for taxonomic assignments at the species level. Species, collection sites, and voucher and GenBank accession numbers for all sequences are provided in Table 1.

DNA sequencing

In our dataset, we included sequences of the internal transcribed spacer (ITS) since it appears to be suitable for *Calcarea* phylogeny (Wörheide et al. 2004; Rossi et al. 2011). Genomic DNA was extracted from ethanol-preserved specimens with the guanidine/phenol-chloroform protocol (Lôbo-Hajdu et al. 2004) or with a QIAamp[®] DNA MiniKit (Qiagen).

The entire region comprising the two spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA was amplified by PCR with the following primers: 18S (5'-TCATTTAGAGGAAGTAAAAGTCG-3') and 28S (5'-GTTAGTTTCTTTTCCTCCGCTT-3') (Lôbo-Hajdu et al. 2004). PCR mixes contained buffer (75 mM Tris-HCl, pH 8.8, 20 mM (NH₄)₂SO₄, 0.01% Tween 20), 50 µg/mL bovine serum albumin, 0.4 mM dNTPs, 0.5 pmol mL⁻¹ of each primer, 1 mM MgCl₂, and one unit of Taq DNA-polymerase (Fermentas or Bionline).

PCR steps included 5 min at 95°C, 35 cycles of 1 min at 92°C, 1 min at 50–55°C, and 1 min at 72°C, followed by 5 min at 72°C. Forward and reverse strands were automatically sequenced in ABI 3500 (Applied Biosystems). The sequences obtained were edited using the programs Chromas Lite 2.01, DNASTAR (SeqMan) or Geneious, and BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/>) were performed to confirm their biological source.

Alignment and phylogenetic analyses

ITS sequences were aligned using the Q-INS-i option of the MAFFT program (Katoh and Standley 2013), with Scoring matrix 200 PAM/ $k=2$, gap penalty 1.53 and offset value = 0. This step was critical for obtaining a reliable alignment for the ingroup sequences, because the option takes the secondary structure into consideration. Final alignments were 1407 bp for ITS1, 5.8S, and ITS2 and were visually inspected. Furthermore, due to their high variability, most of the ITS sequences from calcarean species did not align properly with the ingroup sequences and a suitable outgroup is not available. Therefore, we decided to root our tree using the mid-point rooting method that has been shown to be remarkably efficient in obtaining the root (see Hess and Russo 2007).

A maximum likelihood tree was generated using the MEGA 5.0 platform (Tamura et al. 2011). The

substitution model was selected by that option in MEGA, which indicated general time reversal (GTR) with four gamma categories. The ML algorithm also requires an input tree, and a BIONJ tree (Gascuel 1997) was used. A heuristic tree bisection and reconnection algorithm was applied on the BIONJ tree to find the ML tree. Gap sites were maintained for the phylogenetic analyses. One thousand bootstrap pseudo-replicates (Felsenstein 1985; Russo 1997) were performed on the ML tree.

Results and discussion

Since sequences from geographically distant sponges identified by morphological characters as a nominal species clustered in our tree, our results demonstrate that these morphological characters are reliable for determining actual biological entities in the order Clathrinida. In our tree, 10 distinct lineages are evident (Fig. 1). Of those, nine may be clearly defined with morphological characters (Table 2) and a high bootstrap support and, thus, we are formally designating them as distinct calcarean genera.

The first lineage includes the major cluster of 20 clathroid species that we designate as the genus *Clathrina* (100 BP). The *Clathrina* cluster is distinct from all remaining lineages since it includes *Clathrina clathrus*, the type species, and other species all devoid of tetractines. This lineage has been recovered previously (Rossi et al. 2011) but in the present article we have included many additional species and the same morphological pattern remained. Based on our tree, we are now formally proposing a new diagnosis for the genus *Clathrina* (see Diagnoses section).

Apart from the skeleton, a large clade, formed by yellow sponges with only triactines, has been previously reported (Rossi et al. 2011). In that article, the authors showed a second lineage of yellow sponges with tetractines, indicating that the yellow color appeared at least twice in Clathrinida. In the present study, we have included more yellow *Clathrina* species to our analysis and they also clustered. Nevertheless, two yellow *Clathrina* species from New Zealand grouped separately from the yellow *Clathrina* clade. Curiously, these yellow species from New Zealand are the only true clathrinids that also possess tripods. This result shows that the yellow color appeared twice in the genus *Clathrina* and that the presence of tripods marks the latter clade.

Furthermore, some species of *Guanacha*, with no tetractines, are grouped within the clade *Clathrina* and must be transferred to *Clathrina*, as previously indicated (Rossi et al. 2011). In the present article, however, we have also included a specimen for which

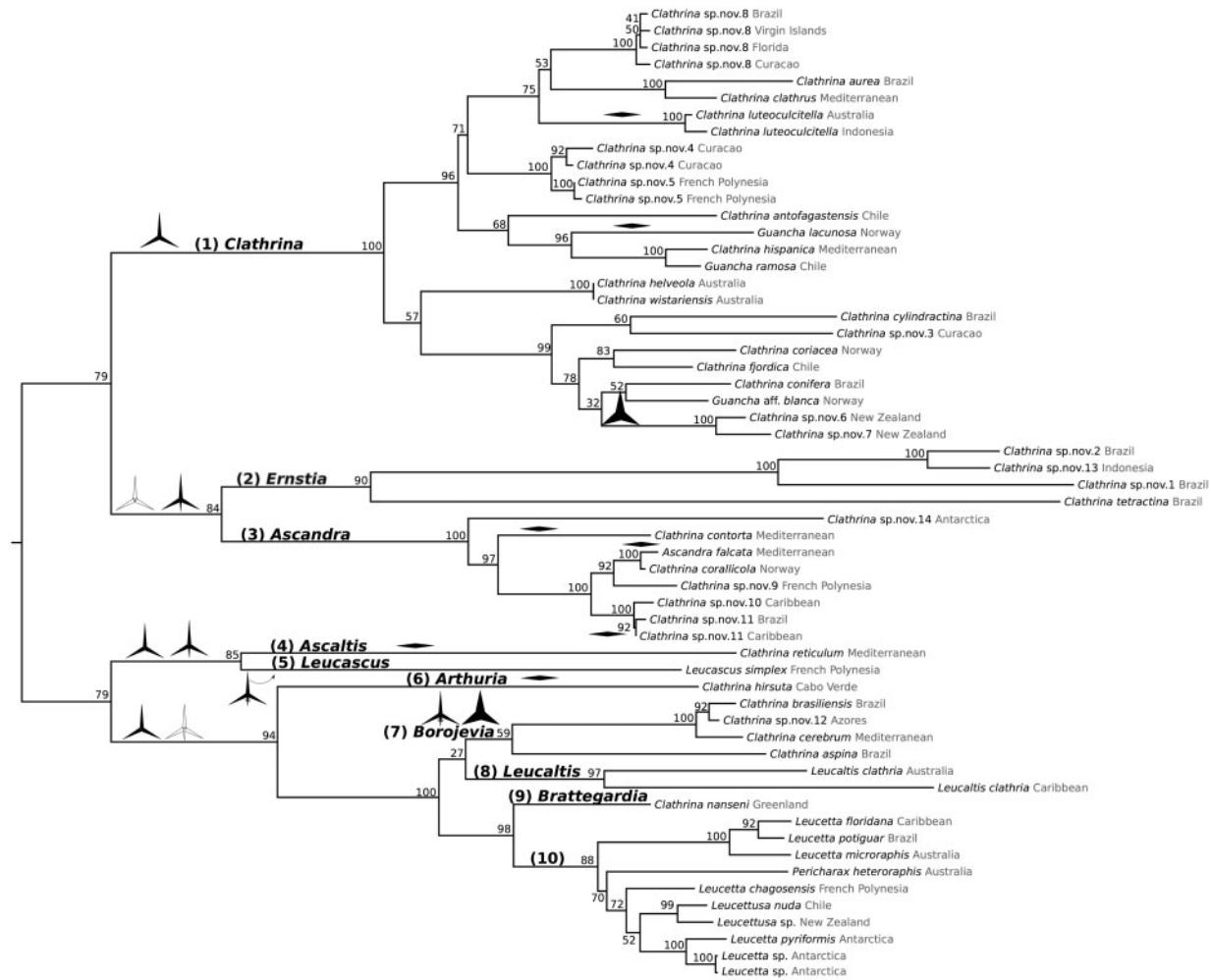


Fig. 1 Maximum likelihood tree built with the GTR plus gamma correction, with 1407 bp of the nuclear ITS marker for 50 clathrinid species, assigned to 12 genera. Black spicules represent the most abundant categories.

the morphological pattern confers with that of the type species of *Guancha*, *G. aff. blanca*. Since *G. blanca* has triactines only, we are formally synonymizing *Guancha* with *Clathrina*. The morphological distinction between *Guancha* and *Clathrina* is limited to the presence of peduncle and the presence of parasagittal spicules in *Guancha* and species of this genus appear scattered in the *Clathrina* portion of the tree. Therefore, all *Guancha* species with triactine spicules only must be assigned from now on to *Clathrina*. On the other hand, *Guancha* species with tetractines must be assigned to the other genera we are proposing, according to the composition of their skeleton.

The second lineage (90 BP) comprises four clathroid species with triactines and tetractines. In this group, tetractines are, at least, as abundant as triactines but frequently surpass their proportion. We are ranking this lineage as a new genus, named *Ernstia*. The apical actine of the tetractines of *Ernstia* gen.

nov. is remarkably long, thin, and needle-like, a feature that is also found in the sister group, the genus *Ascandra*. The third clade, *Ascandra* (100 BP), presents seven species, including the type species, *Ascandra falcata*. Based on their morphology, we provide a new diagnosis for this genus.

The main difference between the two genera is that *Ernstia* gen. nov. has a regular clathroid body quite similar to that of *Clathrina*, but in *Ascandra* the body anastomosis is loose with free tubes at least at the apical region of the cornus. The similarity between species formerly known as *Clathrina* and *Ascandra* has been reported earlier in morphological analyses (Borojevic 1971). In that study, the author discussed this point when he originally described *Clathrina ascandroides*. Indeed, according to the present study, this particular species must be transferred to the genus *Ascandra* along with other species that conform to the diagnostic features of free tubes, abundant tetractines, and very thin apical actines.

Table 2 Morphology table

Species	Spicules	Apical actine Spines/shape	Body	Cortex	Aquiferous system	Color
<i>Arthuria hirsuta</i>	Tri ^a , tetra (rare), di	No/conical	Clathroid, irreg, loose	No	Asconoid	?
<i>Ascalitis reticulum</i>	Tri ^a , tetra, di	No/conical	Clathroid, reg, tight	No	Asconoid, pseud.	White
<i>Ascandra</i> sp. nov. 14	Tri, tetra ^a	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	Beige
<i>Ascandra contorta</i>	Tri, tetra ^a , di, trich	No/needle	Clathroid, irreg, tight (no apical anastomosis)	No	Asconoid	White
<i>Ascandra corallicola</i>	Tri I, tri II, tetra I ^a , tetra II ^a	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	White (transp)
<i>Ascandra falcata</i>	Tri, tetra ^a , di	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	White
<i>Ascandra</i> sp. nov. 10	Tri (r), tetra I ^a (r), tetra II ^a (r/s), di	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	White
<i>Ascandra</i> sp. nov. 11	Tri (r), tetra I ^a (r), tetra II ^a (r/s), di	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	White
<i>Ascandra</i> sp. nov. 9	Tri, tetra I ^a (r/s), tetra II ^a (r/s)	No/needle	Clathroid, irreg, loose (no apical anastomosis)	No	Asconoid	White
<i>Borojevia aspina</i>	Tri ^a , tetra, trip	Yes/conical	Clathroid, reg, tight	Rud	Asconoid	White
<i>Borojevia brasiliensis</i>	Tri ^a , tetra, trip	Yes/conical	Clathroid, reg, tight	Rud	Asconoid	White
<i>Borojevia cerebrum</i>	Tri ^a , tetra, trip	Yes/conical	Clathroid, reg, tight	Rud	Asconoid	White
<i>Borojevia</i> sp. nov. 12	Tri ^a , tetra, trip	Yes/conical	Clathroid, irreg, tight	Rud	Asconoid	White
<i>Brattgardia nansenii</i>	Tri, tetra I, tetra II	No/conical	Clathroid, reg, tight	Rud	Asconoid	White
<i>Clathrina</i> aff. <i>blanca</i>	Tri, tri (p)	No	Clathroid, irreg, loose, peduncle	No	Asconoid	White
<i>Clathrina antofagastensis</i>	Tri I, tri II	No	Clathroid, irreg, tight	No	Asconoid	White
<i>Clathrina aurea</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	Yellow
<i>Clathrina clathrus</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	Yellow
<i>Clathrina conifera</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina coriacea</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina cylindractina</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina fordica</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina helveola</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina hispanica</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	?
<i>Clathrina lacunosa</i>	Tri, tri (p), di	No	Clathroid, irreg, tight, peduncle	No	Asconoid	White
<i>Clathrina luteoculcitella</i>	Tri, di	No	Clathroid, irreg, tight	No	Asconoid	Yellow
<i>Clathrina ramosa</i>	Tri, tri (p)	No	Clathroid, irreg, loose, peduncle	No	Asconoid	White
<i>Clathrina</i> sp. nov. 3	Tri I, tri II	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Clathrina</i> sp. nov. 4	Tri I, tri II, tri III	No	Clathroid, irreg, loose	No	Asconoid	Yellow

(continued)

Table 2 Continued

Species	Spicules	Apical actine Spines/shape	Body	Cortex	Aquiferous system	Color
<i>Clathrina</i> sp. nov. 5	Tri	No	Clathroid, irreg, loose	No	Asconoid	Yellow
<i>Clathrina</i> sp. nov. 6	Tri, trip	No	Clathroid, reg, tigh	No	Asconoid	Yellow
<i>Clathrina</i> sp. nov. 7	Tri, trip	No	Clathroid, reg, tigh	No	Asconoid	Yellow
<i>Clathrina</i> sp. nov. 8	Tri	No	Clathroid, reg, tight	No	Asconoid	Yellow
<i>Clathrina wistariensis</i>	Tri	No	Clathroid, irreg, loose	No	Asconoid	White
<i>Ernstia</i> sp. nov. 1	Tri ^a , tetra, trich	No/needle	Clathroid, reg, tight, globose	No	Asconoid	Yellow
<i>Ernstia</i> sp. nov. 13	Tri ^a , tri II ^a , tetra	No/needle	Clathroid, irreg, loose	No	Asconoid	?
<i>Ernstia</i> sp. nov. 2	Tri, tetra ^a	No/needle	Clathroid, irreg, loose	No	Asconoid	Yellow
<i>Ernstia tetractina</i>	Tri, tetra ^a	No/needle	Clathroid, irreg, loose	No	Asconoid	White
<i>Leucalis clathria</i>	Tri I, tri II (r/s), tetra I, tetra II (r/s)	No/conical	Anastomosed tubes	Yes	Leuconoid	Pink
<i>Leucascus simplex</i>	Tri, tetra	No/conical	Clathroid, reg, tight, globose	Yes	Solenoid	Beige
<i>Leucetta</i> cf. <i>pyriformis</i>	Tri I, tri II, tetra (rare)	No/conical	Massive, globose, surface smooth, no oscular crown	Yes/very thin	Leuconoid, reduced atrium	Bright yellow
<i>Leucetta chagosensis</i>	Tri I (r/s), tri II, tetra	No/conical	Massive, globose, surface smooth, subdermal cavities, no oscular crown	Yes/thin	Leuconoid, large atrium	Bright yellow
<i>Leucetta floridana</i>	Tri I, tri II, tetra I, tetra II	No/conical	Massive, lobate, surface with ridges, no oscular crown	Yes	Leuconoid, large atrium	Light blue
<i>Leucetta microraphis</i>	Tri I (r), tri II (r/s), tetra (r/s)	No/conical	Massive, lobate, surface smooth, subdermal cavities, no oscular crown	Yes	Leuconoid, reduced atrium	Dark yellow
<i>Leucetta potiguar</i>	Tri I, tri II, tetra I, tetra II	No/conical	Massive, lobate, surface smooth, no oscular crown	Yes	Leuconoid, reduced atrium	Light pink
<i>Leucetta</i> sp.	Tri, tetra (rare)	No/conical	Massive and ovoid tube, no oscular crown	Yes/very thin	Leuconoid	Beige
<i>Leucettusa</i> sp.	Tri I, tri II, tetra (rare)	No/conical	Massive, tubular	Yes	Leuconoid	?
<i>Leucettusa nuda</i>	Tri I, tri II, tetra I, tetra II	No/conical	Ramified tubes	Yes	Leuconoid	White
<i>Pericharax heteroraphis</i>	Tri I, tri II, tetra, trip (r/s)	No/conical	Massive, folded, no oscular crown, subdermal cavities	Yes	Leuconoid, large atrium	Dark yellow

^aMost abundant spicule. tri, triactines; tetra, tetractines; trip, tripods; di, diactines; trich, trichoxeas; r, regular (equiangular and equiradiate); p, parasagittal; s, sagittal; reg, regularly anastomosed; irreg, irregularly anastomosed; tight, tightly anastomosed; rud: rudimentary; pseud, pseudoatrium; trans, transparent.

The next clade includes the fourth and fifth lineages. *Leucascus* is represented by the type species, *Leucascus simplex*, and the species *Clathrina reticulum*, which is neither a *Clathrina* nor a *Leucascus*. The cormi of *C. reticulum* and *L. simplex* are well-defined, with tightly anastomosed tubes and their sequences form a fairly well-supported clade (85 BP), but our tree indicates a large distance between them. Additionally, a long array of morphological characters would easily permit their clear-cut distinction into two different genera. For instance, *C. reticulum* possesses a pseudo-atrium, a distinct cavity with no pinacoderm, while *L. simplex* has a true atrium, with a pinacoderm, apical actines with spines, and a solenoid aquiferous system (Cavalcanti and Klautau 2011; Cavalcanti et al. 2013). Therefore, we consider that these two species should be assigned to two different genera.

In fact, Haeckel (1872) originally described *C. reticulum* as an *Ascaltis*. Nevertheless, he based his assertion on the asconoid aquiferous system of this species and on the presence of triactines, tetractines, and diactines in the skeleton, which were formerly diagnostic characters for *Ascaltis*. The current diagnosis for this genus is quite different as it is characterized by the presence of a pseudoatrium and a thin cortex. *Clathrina reticulum* does not exhibit a cortex, but a well-defined cormus is evident. The sponge body is composed of tightly anastomosed tubes forming an external structure that does resemble a cortex. Therefore, species such as *C. reticulum*, *Clathrina gardineri*, and *Clathrina panis*, with a well-defined cormus and a pseudoatrium, should be temporarily transferred to *Ascaltis* until the type species, *Ascaltis lamarcki*, is analyzed under an integrative molecular framework.

The sixth lineage has a single species, *Clathrina hirsuta*. This species is characterized by the clathroid body and by the presence of diactines, triactines, and tetractines, the latter being very rare. We are proposing here that species with triactines and rare tetractines should be included in the new genus *Arthuria*. Our tree includes only one species of *Arthuria* but the species (*C. hirsuta*) is well separated from all the others and it easily may be characterized in morphological terms. We believe that other species that bear such characteristics, previously assigned to *Clathrina*, will group under this new genus such as *Clathrina africana*.

The seventh lineage (59 BP) included four clathroid sponges with tightly anastomosed tubes and a skeleton composed of triactines, tripods, and tetractines with spines. We are calling this new genus *Borojevia*. Tripods and tetractines with spines also

appeared in other clades of our tree. Tripods, for instance, are present in a *Clathrina* clade, whereas all *Leucascus* also present spines on the apical actine of tetractines (Cavalcanti et al. 2013). Therefore, the new genus *Borojevia* is characterized by the well-defined cormus with tripods on the external tubes, triactines, and tetractines with spines on the apical actines. The clade that reunites *Clathrina brasiliensis*, *Clathrina* sp. nov. 12, and *Clathrina cerebrum* had a strong support (100 BP), but *Clathrina aspina* joined this clade with a low support. We are including *C. aspina* in *Borojevia* gen. nov. since the group is well defined on the basis of morphological characters although spines in *C. aspina* have a different shape.

The eighth lineage (97 BP) contained two *Leucaltis* specimens that fit the current diagnosis for *Leucaltis clathria*, one from Australia and another from the Caribbean. The genus *Leucaltis* comprises sponges with a body of very large anastomosed tubes. Differently from *Clathrina*, however, in *Leucaltis* each tube has a distinct cortex with large spicules. Also, the aquiferous system is considered leuconoid, but may be composed of elongated and ramified choanocyte chambers. A true atrium is present and the choanosome is full of small triactines and tetractines. *Leucaltis* was previously assigned to the family Leucaltidae, along with *Leucettusa*. In fact, the only difference between these genera is that *Leucaltis* has anastomosed tubes, but *Leucettusa* does not. According to our results, *Leucaltis* is a valid genus, but it is more closely related to *Borojevia* gen. nov. than to *Leucettusa* (see also Voigt et al. 2012 for the same result). Since *L. clathria* is the type species of the genus and the type locality is the Caribbean Sea (Florida), we suggest that *L. clathria* from Australia is a distinct species and must receive a new name. In this sense, the diagnosis for type species of *Leucaltis* must be revised to avoid lumping of distinct biological species into a single name.

Our ninth lineage includes a single species, *Clathrina nanseni*. This is a clathroid species with a single osculum and a cormus surrounded by a membrane, at least in the young forms. A stalk may be present. The skeleton is composed of triactines and two categories of tetractines, one with normal apical actine and the other with a rudimentary knob-like apical actine. Parasagittal spicules may be found at the base of some specimens (Rapp 2006). We propose a new genus for this lineage: *Brattegardia* gen. nov.

The tenth lineage includes nine species assigned to *Leucetta*, *Pericharax*, *Leucaltis*, and *Leucettusa* (88 BP). It must be noted, however, that the species originally described as *Leucaltis nuda* (Azevedo et al.

2009) is present in this clade as *Leucettusa nuda*. The species was later found to be a *Leucettusa* after further detailed morphological examination and we are formally assigning the species to *Leucettusa* with a new name, *Leucettusa nuda*.

In a recent article, Voigt et al. (2012) suggested that *Leucetta* is not monophyletic, which is consistent with our results. The monophyletic status of *Leucettusa* and *Pericharax* was not broken and their diagnostic characters remain consistent after our molecular analyses. In order to maintain phylogenetic consistency, however, the former genus *Leucetta* should be split into three genera: (1) *Leucetta floridana*, *Leucetta potiguar*, and *Leucetta microraphis* (100 BP); (2) *Leucetta chagosensis*; and (3) *Leucetta* cf. *pyriformis* and *Leucetta* sp. (99 BP). Unfortunately, however, we do not have the type species, *Leucetta primigenia*, in our tree. Thus, it would be unclear at this point which lineage would retain the generic name. Additionally, our preliminary morphological analysis showed no obvious diagnostic characters for the three lineages. Hence, a formal revision (Alencar, Rapp, and Klautau, unpublished results) and a detailed morphological analysis are required before the split of *Leucetta*.

Final remarks

Recent publications have stated that not much phylogenetic information is contained in the morphological characters of calcareous sponges (Manuel et al. 2003, 2004; Dohrmann et al. 2006; Voigt et al. 2012). Our results contradict this general assertion and, instead, revealed a rather strong phylogenetic signal in carefully selected morphological characters within the order Clathrinida. Our systematic proposal for Clathrinida is based on spicule composition, body anastomosis, and aquiferous system. Using these morphological characters, we were able to recognize 11 genera that agree with our molecular phylogenetic pattern. Considering our dataset, only the genus *Leucetta* still requires further analyses.

It is surprising to perceive how our new systematic proposal is similar to that proposed by Haeckel in the 19th century (Haeckel 1872). Haeckel's taxonomic proposal was based mainly on the aquiferous system and on composition of spicules. According to him, Calcarea should be divided into three families according to the aquiferous system: Ascones, Sycones, and Leucones. The genera in those families would all use the prefixes Asc, Syc, and Leuc, respectively. In order to complete the generic name, each prefix would receive a suffix that would make reference to the presence of spicule types. Thus, asconoid species with only triactines would be included in

genus *Ascetta*, whereas asconoid species with only tetractines would be *Ascilla*, and so on.

Comparing Haeckel's system to our proposal, it becomes obvious that he selected, more than 140 years ago, the same morphological characters that are disclosed as clade markers in our tree. In fact, he would be surprisingly close to a phylogenetic classification of Calcarea apart from three points. The most important is that the major split between Calcinea and Calcaronea was not clear to him. Additionally, he used diactines as markers and he also neglected the importance of the relative abundance of spicule types. Still, many of Haeckel's genera may be well compared with those revealed in our tree. The exclusive presence of triactines, for instance, would be diagnostic for his genus *Ascetta*, as it is to our *Clathrina*, a genus of asconoid calcinean sponges. Species with only tetractines were associated with the *Ascilla* in his monograph. His taxon *Ascilla* would be comparable to our *Ernstia* and *Ascandra* clade, asconoid calcinean with a much larger proportion of tetractine spicules.

In the past few years, a more detailed and finer picture of the evolution of morphological characters is beginning to unfold in calcareous sponges. It is our expectation that the availability of additional variable molecular markers (Lavrov et al. 2013) allied to a truly comprehensive taxon sampling may well reclaim the importance of selected morphological characters as diagnostic markers for even higher taxa, such as families and orders, even in groups with a particularly simple morphology such as Calcarea.

Diagnoses

Descriptions of genera

Asterisks designate species that were tested in our molecular phylogeny. Names between brackets are the original genus of the listed species.

Genus *Clathrina* Gray, 1867

Type species: *Grantia clathrus* Schmidt, 1864 currently accepted as *Clathrina clathrus* (Figs. 2A and 3A, B).

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

Species: *C. angraensis* (Azevedo and Klautau 2007); *C. antofagastensis** (Azevedo et al. 2009); *C. (Guancha) arnesenae* (Rapp 2006); *C. aurea** Solé-Cava et al. 1991); *C. (Guancha) blanca** (Miklucho-Maclay 1868); *C. broendstedti* (Rapp, Janussen, and Tendal 2011); *C. (Guancha) camura* (Rapp 2006); *C. ceylonensis* (Dendy 1905); *C. chrysea* (Borojevic

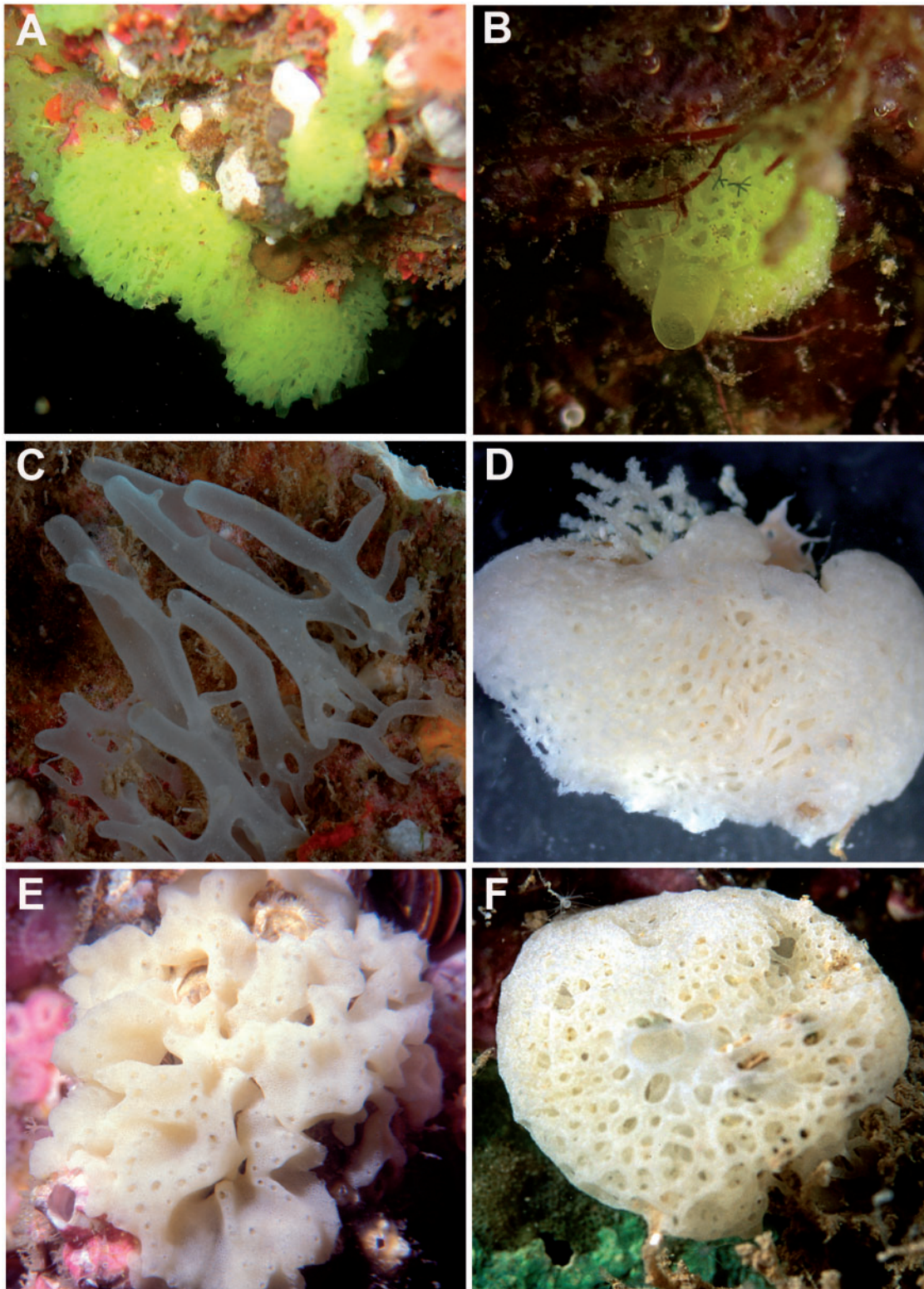


Fig. 2 Photographs of specimens of the new and rediagnosed genera of Clathrinida. (A) *Clathrina aurea* (photo *in situ*: André Padua). (B) *Ernstia* sp. nov. 2 (photo *in situ*: André Padua). (C) *Ascandra* sp. nov. 9 (photo *in situ*: Cristina Diaz and Belinda Alvarez). (D) *Arthuria hirsuta* (photo *in vitro*: Fernanda Azevedo). (E) *Borojevia brasiliensis* (photo *in situ*: Eduardo Hajdu). (F) *Brattegardia nansenii* (photo *in situ*: Bjørn Gulliksen; this photo was previously published in Rapp 2006).

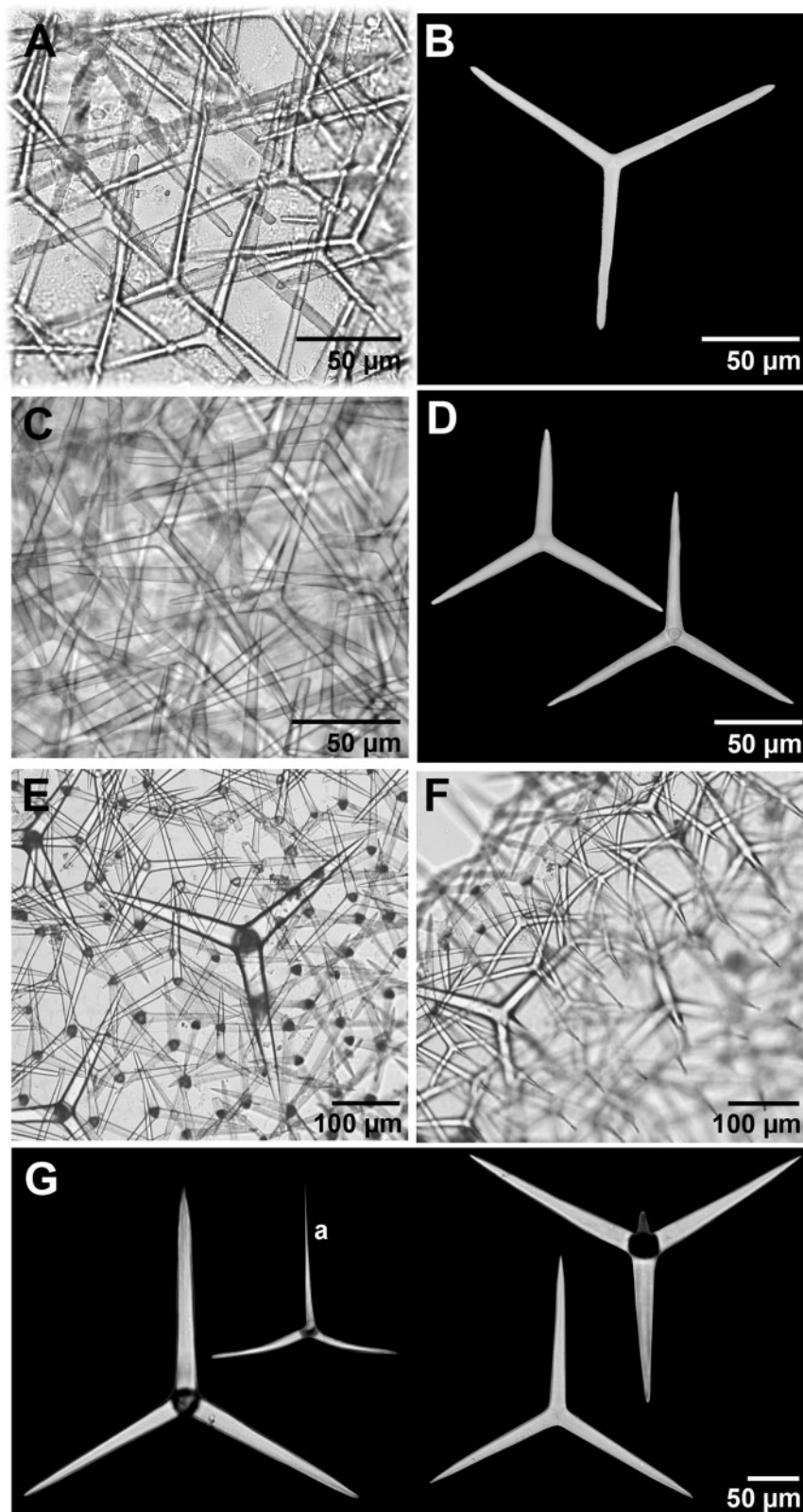


Fig. 3 Photographs of spicules and skeleton of the new and rediagnosed genera of Clathrinida. (A, B) *Clathrina aurea*. (C, D) *Ernstia* sp. nov. 2. (E, F, G) *Ascandra* sp. nov. 9. (a) apical actine.

and Klautau 2000); *C. clara* (Klautau and Valentine 2003); *C. clathrus** (Schmidt 1864); *C. conifera** (Klautau and Borojevic 2001); *C. coriacea** (Montagu 1818); *C. cribrata* (Rapp, Klautau, and Valentine 2001); *C. cylindractina** (Klautau, Solé-Cava, and Borojevic 1994); *C. fjordica** (Azevedo et al. 2009); *C. hispanica** (Klautau and Valentine 2003); *C. hondurensis* (Klautau and Valentine 2003); *C. jorunnae* (Rapp 2006); *C. (Guancha) lacunosa** (Johnston 1842); *C. laminoclathrata* (Carter 1886); *C. luteoculcitella** (Wörheide and Hooper 1999); *C. heronensis* (Wörheide and Hooper 1999); *C. parva* (Wörheide and Hooper 1999); *C. (Guancha) pellucida* (Rapp 2006); *C. primordialis* (Haeckel 1872); *C. procumbens* (Von Lendenfeld 1885); *C. (Guancha) ramosa** (Azevedo et al. 2009); *C. rotunda* (Klautau and Valentine 2003); *C. sinusarabica* (Klautau and Valentine 2003); *C. tendali* (Rapp, submitted for publication); and *C. wistariensis** (Wörheide and Hooper 1999) (= *C. helveola** Wörheide and Hooper 1999).

Genus *Ernstia* gen. nov.

Etymology: For Ernst Haeckel in recognition of his building of a tentative phylogenetic classification for Calcarea (Figs. 2B and 3C, D).

Type species: *Clathrina tetractina* (Klautau and Borojevic 2001).

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

Species: *E. (Clathrina) adusta* (Wörheide and Hooper 1999); *E. (Clathrina) quadriradiata* (Klautau and Borojevic 2001); *E. (Clathrina) sagamiana* (Hôzawa 1929); *E. (Clathrina) septentrionalis* (Rapp et al. 2001); *E. (Clathrina) tetractina** (Klautau and Borojevic 2001).

Genus *Ascandra* Haeckel 1872

Type species: *Ascandra falcata* Haeckel 1872 (Figs. 2C and 3E–G).

Proposed neotype: UFRJPOR 5856 (Universidade Federal do Rio de Janeiro, Instituto de Biologia)

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. 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.

Species: *A. (Clathrina) ascandroides* (Borojevic 1971); *A. (Clathrina) atlantica* (Thacker 1908); *A. (Clathrina) biscayae* (Borojevic and Boury-Esnault 1987); *A. (Clathrina) contorta** (Minchin 1905); *A. (Clathrina) corallicola** (Rapp 2006); *A. (Leucosolenia) depressa* (Dendy 1891); *A. falcata** (Haeckel 1872); *A. (Leucosolenia) loculosa* (Dendy 1891); *A. minchini* (Borojevic 1966); *A. (Clathrina) osculum* (Carter 1886).

Arthuria gen. nov.

Etymology: For Arthur Dendy, in recognition of all his precise and detailed work on the taxonomy of Calcarea (Figs. 2D and 4A, B).

Type species: *Clathrina hirsuta* (Klautau and Valentine 2003).

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

Species: *A. (Clathrina) africana* (Klautau and Valentine 2003); *A. (Clathrina) alcatraziensis* (Lanna et al. 2007); *A. (Clathrina) canariensis* (Miklucho-Maclay 1868); *A. (Clathrina) dubia* (Dendy 1868); *A. (Clathrina) hirsuta** (Klautau and Valentine 2003); *A. (Clathrina) sueziana* (Klautau and Valentine 2003); *A. (Clathrina) tenuipilosa* (Dendy 1905).

Borojevia gen. nov.

Etymology: For Radovan Borojevic, in gratitude for teaching his deep knowledge on calcareous sponges and in recognition for all his scientific works (Figs. 2E and 4C, D).

Type species: *Ascaltis cerebrum* Haeckel 1872 currently accepted as *Clathrina cerebrum*.

Diagnosis: Calcinea in which the cormus comprises tightly anastomosed tubes. The skeleton contains regular (equiangular and equiradiate) triactines, tetractines, and tripods. The apical actine of the tetractines has spines. Aquiferous system asconoid.

Species: *B. (Clathrina) aspina** (Klautau, Solé-Cava, and Borojevic 1994); *B. (Clathrina) brasiliensis** (Solé-Cava et al. 1991); *B. (Clathrina) cerebrum** (Haeckel 1872); *B. (Clathrina) paracerebrum* (Austin 1996); and *B. (Clathrina) tetrapodifera* (Klautau and Valentine 2003).

Brattegardia gen. nov.

Etymology: After the Norwegian marine zoologist Torleiv Brattegard for his tremendous effort on exploring and sampling the deeper parts of the

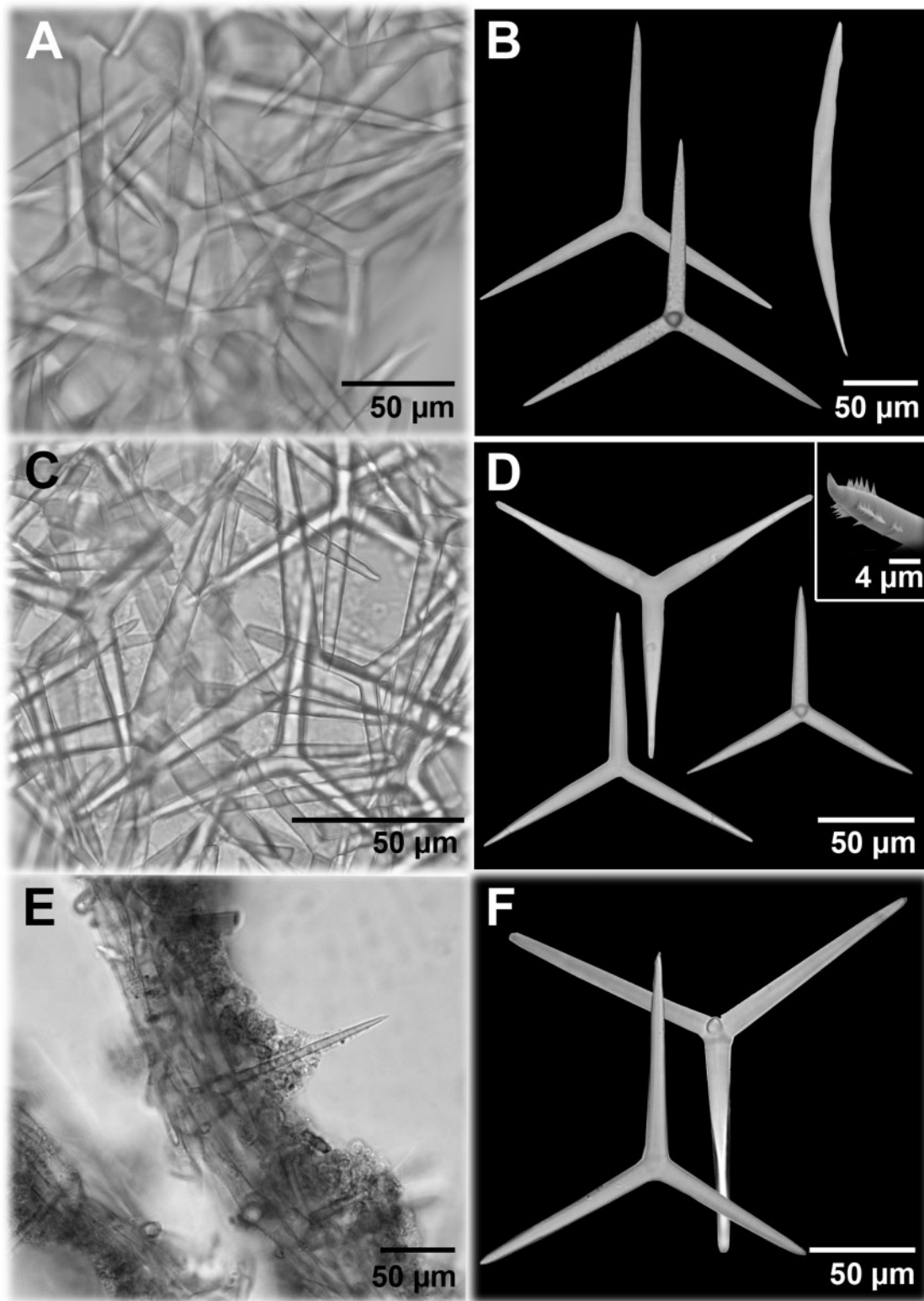


Fig. 4 Photographs of spicules and skeleton of the new and rediagnosed genera of Clathrinida. (A, B) *Arthuria hirsuta*. (C, D) *Borojevia brasiliensis*. In detail, spines on the apical actine of a tetractine. (E, F) *Brattegardia nanseni*.

Norwegian-Greenland-Iceland (GIN) Seas. His collections include numerous new calcareous sponges from abyssal depths, among them a new species of *Brattegardia* (Rapp and Tendal, unpublished results) (Figs. 2F and 4E, F).

Type species: *Leucosolenia nanseni* (Breitfuss 1896) currently accepted as *Clathrina nanseni*.

Diagnosis: Calcinea in which the cormus is formed by anastomosed tubes covered by a thin membranous layer, at least in young specimens. Cormus is massive/globular with or without a stalk. The skeleton contains regular (equiangular and equiradial) triactines and tetractines, but parasagittal triactines may be present. Triactines are the most numerous spicules. Aquiferous system asconoid.

Species: *B. (Clathrina) nanseni** (Breitfuss 1896).

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