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Integrative taxonomy justifies a new genus, *Nodastrella* gen. nov., for North Atlantic "*Rossella*" species (Porifera: Hexactinellida: Rossellidae)

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Abstract

Molecular systematic studies have indicated that the hexactinellid sponge species *Rossella nodastrella* Topsent (Lyssacinosida, Rossellidae), previously only known from the NE Atlantic, is only distantly related to its congeners, which are restricted to the Southern Ocean, representing the only case thus far reported of a diphyletic genus in the class Hexactinellida. Here we describe new material of "*Rossella*" *nodastrella* from cold-water coral reefs in the NW Atlantic (Florida). Morphological comparison with the holotype from the Azores and specimens recently reported from off Ireland reveal at least two distinct species, which we corroborate with molecular data. Because the diphyletic nature of "*Rossella*" is further supported with inclusion of the new specimens in the molecular phylogeny, we erect a new genus, *Nodastrella* **gen. nov.**, for these two species. The Irish specimens are synonymized with our new species *Nodastrella asconemaoida* **sp. nov.** Subtle morphological and molecular differences between the E and W Atlantic specimens are for the time being ascribed to intraspecific geographic variation, but indicate that *Nodastrella* might contain more (sub)species, pending investigation of additional specimens, especially from intermediate locations.

Key words: *Asconema*, cold-water coral reefs, glass sponges, molecular systematics, new genus, North Atlantic Ocean, *Rossella nodastrella*

Introduction

Integrative taxonomy aims at combining morphological characters with additional, especially molecular, data to delimit species and higher taxa and is a promising approach to construct more natural classification systems (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010), especially for organisms with depauperate or highly plastic morphology such as sponges (Porifera) (Cárdenas *et al.* 2012). Unlike Demospongiae and Calcarea, for which molecular phylogenies largely contradict morphology-based classification systems (Wörheide *et al.* 2012), sponges of the class Hexactinellida have a higher number of informative morphological characters, especially spicule types and their combinations, and consequently there is a better correspondence between molecular-based phylogenetic hypotheses and traditional taxonomy of this group (Dohrmann *et al.* 2008). However, even in Hexactinellida there are incongruencies, mostly concerning weakly supported taxonomic hypotheses such as subfamilial divisions (Dohrmann *et al.* 2012) or family assignment of certain genera (Dohrmann *et al.* 2011). In this paper, we focus on perhaps the most striking example of conflict between molecular phylogeny and Linnean classification in Hexactinellida, the placement of *Rossella nodastrella* Topsent (Hexasterophora, Lyssacinosida, Rossellidae).

Rossella nodastrella was initially described by Topsent (1915) based on a single small specimen collected in bathyal depths off the Azores, and has thus far been considered the sole North Atlantic species of the genus *Rossella* Carter, which is otherwise restricted to the Southern Ocean (SO) (Tabachnick 2002a). Observations of a mass occurrence ascribed to this species, which was originally attributed to the genus *Asconema* Kent, in a deep-water

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coral reef West of Ireland (van Soest and Lavaleye 2005) prompted a re-description of *Rossella nodastrella* based on new documentation and re-evaluation of its skeletal characters (van Soest *et al.* 2007; see also Tabachnick and Collins 2008 for a later finding of specimens from the N Atlantic Ridge assigned to this species). However, molecular analyses including one of the Irish specimens strongly suggested that *R. nodastrella*, despite having calycocomes—the main diagnostic character of *Rossella*—is not closely related to *Rossella* spp. of the SO, but is in fact more closely related to the genera *Aulosaccus* Ijima and *Acanthascus* Schulze (Dohrmann *et al.* 2008). Here, we report the finding of new specimens from cold-water coral reefs off the Atlantic coast of Florida, and demonstrate by molecular and spicule analysis that there are at least two different species of "*Rossella*" in the N Atlantic. Because it is clear that these are not closely related to SO *Rossella* spp. (i.e., *Rossella* s.s.) we erect a new genus, *Nodastrella* **gen. nov.**, for them, thereby further reconciling the classical taxonomy and molecular phylogeny of Hexactinellida.

Methods

Two specimens of lyssacine hexactinellids were collected in August 2009 in the Straits of Florida using manned submersible *Johnson-Sea-Link II* of Harbor Branch Oceanographic Institute (HBOI) during the "Life on the Edge 2009" cruise (http://deepcoral.wordpress.com/; see below for collection details) and preliminarily identified as "*Asconema* sp.". Pieces of these sponges preserved in ethanol were later sent to the National Museum of Natural History where sections and spicule preparations from different body regions were prepared and mounted for light microscopy (LM) using standard protocols (Boury-Esnault and Rützler 1997). After inspection of these slides by the first author the sponges were identified as "*Rossella*" *nodastrella* and "*Rossella*" aff. *nodastrella*. Additional preparations were made by digesting small pieces of tissue with commercial bleach, washing several times with water and ethanol, drying on LM slides and mounting with Eukitt (Electron Microscopy Sciences, Hatfield, PA, USA); to observe surface spicules in situ, pieces of the dermal and atrial membranes were peeled off, dried on LM slides, and mounted with Eukitt. Further investigations of these specimens, including scanning electron microscopy (SEM) and comparison with the holotype of *R. nodastrella*, were then conducted at the Senckenberg Museum by DJ and CG using previously described methods (e.g., Janussen and Reiswig 2009). A small amount of tissue from a third specimen, which had been collected by HBOI in August 2005 from the Straits of Florida (see below for details), was later sent to MD by Karri Haen (Iowa State University) for identification; since temporary spicule preparations revealed the presence of calycocomes (see Introduction), the specimen was included in molecular analyses.

DNA extraction, PCR amplification, Sanger sequencing, alignment with previously published hexactinellid sequences, and concatenation into a supermatrix of 18S ribosomal DNA (rDNA), partial 28S rDNA, partial mitochondrial (mt) 16S rDNA, and partial mt cytochrome oxidase subunit I (COI) were carried out as previously described (Dohrmann *et al.* 2008, 2009, 2012); new sequences have been deposited in GenBank (accession-numbers HE858263-HE858272). To allow the inclusion of additional nucleotide positions whose alignment was ambiguous within the full set of available hexactinellid sequences, we restricted the ingroup to the Rossellidae and used published sequences of two Leucopsacidae specimens as the outgroup for phylogenetic analysis (see also Dohrmann *et al.* 2011). To avoid excessive missing data, we excluded the 3'-half of the COI gene (I3-M11 partition; cf. Erpenbeck *et al.* 2006) as this region is missing from many rossellid species (see Dohrmann *et al.* 2012: Table S2), and also could not be amplified from the new specimens investigated here (see Results). Phylogenetic analysis of the supermatrix (4110 bp; TreeBase study-nr. 12795) was performed using the Maximum Likelihood (ML) approach as implemented in RAxML (Stamatakis 2006a) v7.2.8 (http://sco.h-its.org/exelixis/software.html) under mixed models with S16 (Savill *et al.* 2001) for 18S + 28S rDNA helices and independent GTR models (Lanave *et al.* 1984) for nuclear rDNA loops, 16S rDNA, and COI. Among-site rate variation was modelled with a 25-category CAT approximation (Stamatakis 2006b) followed by final optimization under a 4-category gamma distribution (Yang 1994). Bipartition robustness, used as a proxy for clade accuracy, was calculated using rapid bootstrapping (Felsenstein 1985; Stamatakis *et al.* 2008), employing the autoMRE bootstopping criterion to determine the sufficient number of pseudoreplicates (Pattengale *et al.* 2010). Bootstrap values ≥ 70% were considered significant, following Hillis and Bull (1993).

Results

Taxonomic descriptions and systematic account

Hexactinellida Schmidt
Hexasterophora Schulze
Lyssacinosida Zittel
Rossellidae Schulze
Rossellinae Schulze[1]

Nodastrella gen. nov.

Synonymy: *Asconema* in part (van Soest and Lavaleye 2005: Fig. 2A–B); *Rossella* in part (Topsent 1915: 1, Figs. 1–5; Topsent 1928: 76, Pl. III Fig. 22, Pl. IV Fig. 3; Tabachnick and Collins 2008: Fig. 15).
Type species: *Nodastrella nodastrella* (Topsent).

Definition: Saccular Rossellinae with both calycocomes and discasters among microscleres, microdiscohexasters concentrated near the dermal surface, and dermalia chiefly stauractins.

Diagnosis: Basiphytous saccular Rossellidae barrel- to amphora-shaped, with a large atrial cavity ending in a wide, unfringed, central osculum, with everted marginal parts in older specimens. Colour in life is white to greyish. Choanosomal main skeleton chiefly of diactins combined with intermediate hexactins and pentactins, dermalia are microspined stauractins and few pentactins, tauactins, and diactins, hypodermalia are large orthotropal pentactins, gastralia mainly hexactins, but also include pentactins, stauractins, and tauactins. Prostalia lateralia, if present, are diactins. Microscleres are calycocomes with reduced primary rays, spherical discasters, microdiscohexasters, (hemi)oxyhexasters, oxyhexactins, and rare onychohexasters. Discasters are concentrated near the atrial surface, microdiscohexasters are concentrated near the dermal surface.

Remarks: The skeleton of *Nodastrella* is similar to that of *Rossella* in the presence of calycocomes, although they are different in morphology by showing reduced primary rays and a central swelling. It differs from *Rossella* mainly by its dermalia, which are chiefly stauractins (whereas the dermalia of *Rossella* are chiefly pentactins), the presence of discasters (never found in *Rossella*), and the absence of tyloidal and rhopaloidal microscleres, discohexactins, and anisodiscohexasters, the latter a typical spicule of most *Rossella* species (although missing in *R. antarctica* Carter and *R. levis* (Kirkpatrick)). Furthermore, the concentration of macrodiscohexasters near the atrial surface and microdiscohexasters near the dermal surface is unique within Rossellidae; in *Rossella* the distribution of microscleres is opposite to that of *Nodastrella* (see also van Soest *et al.* 2007). A further difference is the presence of some stauractins and tauactins and the absence of diactins among the atriala (in *Rossella*, some diactins can be present whereas stauractins and tauactins are absent).

To accomodate removal of *nodastrella* from *Rossella*, we here provide emended definition and diagnosis for the latter genus (from Tabachnick 2002a; additions highlighted in boldface):

Rossella Carter

Definition: Saccular Rossellinae with calycocomes among microscleres**, microdiscohexasters concentrated near the atrial surface, and dermalia chiefly pentactins.**

Diagnosis: Body is saccular, thick-walled, **barrel-shaped with apical narrowing towards the osculum, mode of attachment is** basiphytose or lophophytose. Choanosomal skeleton is composed of diactins and rarely accompanied by hexactins. Hypodermal spicules are pentactins, which can be differentiated into anchorate (which serve as basalia) and commonly with paratropal and orthotropal tangential rays. Prostalia lateralia if present are monaxons and sometimes outward protruding hypodermal pentactines. Dermalia are **chiefly** pentactins or combinations of them with **some** stauractins and hexactins. Atrialia are mainly hexactins, rarely together with pentactins

1. Included here only for completeness; molecular data strongly suggest that the subfamily-division of Rossellidae is artificial (Dohrmann *et al.* 2008, 2012)

or diactins. Microscleres have discoidal, tyloidal, rhopaloidal, oxyoidal rarely onychoidal terminations. Calycocomes always present**, they have well-developed primary rays** and are often accompanied by spherical 'mesodiscohexasters', discohexactins and microdiscohexasters. **Mesodiscohexasters are concentrated near the dermal surface, microdiscohexasters are concentrated near the atrial surface. In most species, microdiscohexasters have secondary rays of unequal length (anisodiscohexasters).** Oxyoidal spicules are combinations of hexasters, hemihexasters, hexactins and rarely other holactinoidal spicules.

The body shape of *Nodastrella* is very variable, urn- to amphora- or trumpet-shaped depending on the size and ontogentic age (as illustrated by van Soest *et al.* 2007: Fig. 1). Young and smaller specimens are commonly tubular or urn-shaped, whereas larger specimens tend to become amphora- or trumpet-shaped. The adult body form is very similar to the typical shape of *Asconema*, another N Atlantic rossellid genus with which *Nodastrella* can easily be confused if no spicule analysis is carried out. Identification of *Nodastrella* is straightforward by the presence of calycocomes and discasters. However, these spicules can be very rare and might be overlooked; in that case the two genera can be distinguished by their dominant dermal megascleres, which are stauractins in *Nodastrella* and pentactins with distally directed unpaired ray in *Asconema* (Tabachnick and Menshenina 2007).

Etymology: The genus name is derived from the species name of the holotype, first described as *Rossella nodastrella* (Topsent 1915).

TABLE 1. Spicule measurements of *Nodastrella nodastrella* holotype (MOM-INV-21666 (04 1353)) and USNM 1150046. Values in µm are given as minimum - mean - maximum (number). D = diameter, L = length. * no complete spicules were found.

Spicule	Holotype	USNM 1150046
Atrial hexactin (D)	140–242.1–520 (7)	200–247.3–310 (30)
Dermal stauractin (D)	210–289.5–360 (14)	190–242–280 (30)
Pappocome-like oxyhexaster		
D	0 *	92.5–120.6–150 (30)
secondary ray (L)	32–56.4–86 (30)	40–54.3–67.5 (30)
primary ray (L)	3–4.6–6 (30)	3.75–5.4–7.5 (30)
Calycocome		
D	80–145–180 (20)	190–202.7–225 (30)
secondary ray (L)	25–55–70 (20)	62.5–75.2–87.5 (30)
primary ray (L)	15–17.5–25 (20)	15–18.1–22.5 (29)
Discaster (D)	90–133.6–160 (14)	130–149–170 (30)

TABLE 2. Spicule measurements of *Nodastrella asconemaoida* sp. nov. holotype (USNM 1150045) and Irish specimen (SMF 10363). Values in µm are given as minimum - mean - maximum (number). Calycocomes were not found in SEM preparations.

Spicule	Holotype	SMF 10363
Atrial hexactin (D)	195–229–262.5 (30)	125–199.5–287.5 (30)
Dermal stauractin (D)	175–213.9–257.5 (30)	100–189.2–250 (30)
Holoxyhexaster		
D	80–103.1–120 (30)	
secondary ray (L)	40–48.6–60 (30)	
primary ray (L)	2–3–5 (30)	
Hemioxyhexaster		
D		42–59–72 (30)
secondary ray (L)		17–26.6–36 (30)
primary ray (L)		2.5–4.1–6 (30)
Discaster (D)		79–101.8–117.5 (11)

Nodastrella nodastrella (Topsent)

Synonymy: *Rossella nodastrella* (Topsent 1915: 1, Figs. 1–5; Topsent 1928: 76, Pl. III Fig. 22, Pl. IV Fig. 3).

Material examined: The holotype (MOM-INV-21666 (04 1353)) described by Topsent (1915) from the Azores, collected by S.A.S. le Prince de Monaco, off San Miguel, St. 3140, on August 18, 1911, depth 1378 m. One specimen (HBOI 7-VIII-09-1-002, USNM 1150046, SMF 11754) from deep-water *Lophelia* coral reefs off Cape Canaveral, Florida, lat. 28°47.621 N, long. 79°37.430 W, depth 759 m, collected August 07, 2009 using manned submersible *Johnson-Sea-Link II*.

Description: The holotype is a small (probably juvenile) specimen, 3.5 cm high and 2 cm max. width with a deep central osculum. The specimen from Florida, USNM 1150046, is 24.5 cm high, the body is vase-shaped with the osculum (ca. 15 cm wide) having outward-flaring margins (Fig. 1). Whereas the dermal surface of the holotype shows a few diactine prostalia, the USNM specimen is smooth. The USNM specimen is white to greyish and is attached to a scleractinian coral (*Lophelia pertusa* L.); the holotype is also basiphytous and attached to the skeleton of another hexactinellid sponge, *Hertwigia falcifera* Schmidt (Topsent 1915).

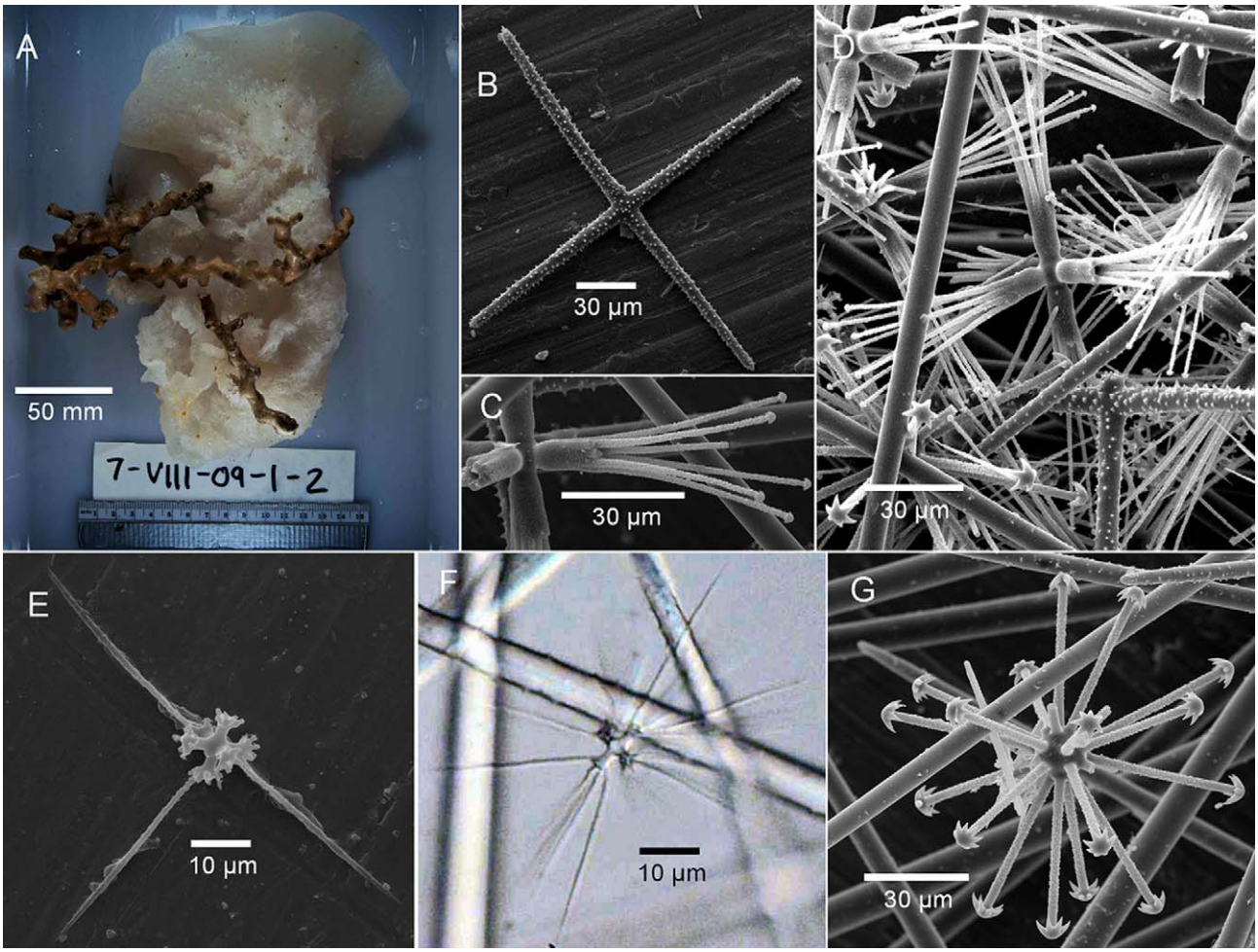

FIGURE 1. *Nodastrella nodastrella* USNM 1150046, specimen (A) and skeleton (B–G). (B–E, G) scanning electron micrographs; (F) light micrograph. (A) Deck photograph of specimen attached to *Lophelia* coral. (B) Dermal stauractin. (C) Detail of calycocome. (D) Cluster of calycocomes and a discaster (lower left). (E) Pappocome-like oxyhexaster with most secondary rays broken off. (F) Complete pappocome-like oxyhexaster. (G) Discaster.

Skeleton (Figs. 1–2, Table 1): Dermal skeleton is a web of microspined stauractins (occasionally with rudimentary tubercle of fifth ray), some tauactins with or without rudimentary fourth ray, some diactins with or without rudimentary third and fourth rays, and very rarely isolated pentactins; the web covers the paratangential rays of large, smooth (except for slightly rugose ray tips), orthotropal hypodermal pentactins. Atrial skeleton is a web of

hexactins and pentactins, combined with some stauractins and rare tauactins. Choanosomalia are chiefly diactins varying in size from few mm to several cm, with rounded to pointed microspined or smooth tips, sometimes with one end swollen. The smaller diactins are commonly centrotylote; in the holotype some smaller diactins protrude as prostalia beyond the dermal surface. Further choanosomal megascleres are microspined hexactins and pentactins of variable sizes. Microscleres are calycocomes most abundant near the dermal surface, spherical discasters most abundant near the atrial surface, and oxyhexasters. We did not observe any microdiscohexasters, but they are documented in the original description, as rare stellate microdiscohexasters with 11–13 secondary rays (Topsent 1915: Fig. 5m; the spicule shown in his Fig. 5a is more likely a small calycocome). Calycocomes have very short primary rays bearing calyces with 7–8 microspiny secondary rays, discasters have ~10–20 microspiny secondary rays and inflated centre hiding the axial cross and entire primary rays. Oxyhexasters are of two types: 1) pappocome-like oxyhexasters, which are always holoxyhexasters, with distinct primary rays ending in conspicuous discs bearing 5–7 straight, microspiny secondary rays, and 2) (only found in the holotype; see Remarks) normal oxy- and few hemioxyhexasters with 1–3 secondary rays and very short primary rays.

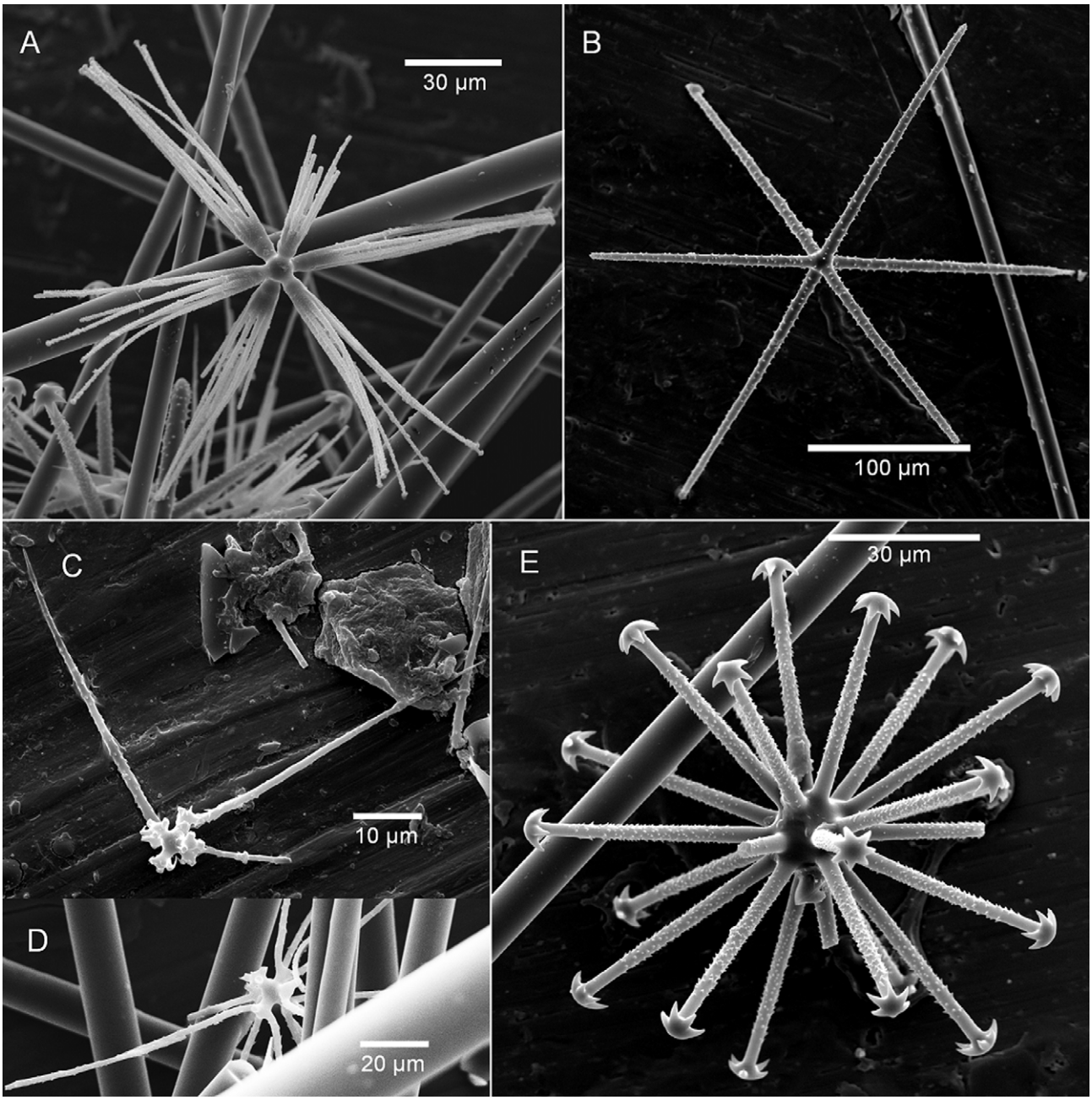

FIGURE 2. *Nodastrella nodastrella* holotype, skeleton. Scanning electron micrographs. (A) Calycocome. (B) Atrial hexactin. (C) Pappocome-like oxyhexaster with most secondary rays broken off. (D) Regular oxyhexaster. (E) Discaster.

Remarks: Despite the fact that Topsent (1915) did not notice, or at least did not mention, the presence of two distinct types of oxyhexasters, the pappocome-like oxyhexasters are the most prominent diagnostic character of *N. nodastrella*. They are holoxyhexasters with a convex, plate-like terminal of each primary ray, on which the secondary rays are attached. The 5–7 straight secondary rays radiate outwards and give the hexaster a spherical appearance, best seen in LM, as the secondary rays tend to break off during preparation and were hardly ever found in situ in SEM. We refrain here from calling these spicules pappocomes because the number of secondary rays is much smaller than in "true" pappocomes (see Tabachnick 2002a; see also Tabachnick and Reiswig 2002 for a discussion of the term). In the holotype, these oxyhexasters are combined with normal (hemi)oxyhexasters, and there seem to be transitional forms between both spicule types, whereas in the Florida specimen only the pappocome-like oxyhexasters were observed. However, because the morphological gap between the two specimens from each side of the Atlantic may be closed or confirmed by further findings we refrain from the erection of subspecies based on the scarce material presently available.

Nodastrella asconemaoida sp. nov.

Synonymy: *Asconema* cf. *foliata* (van Soest and Lavaleye 2005: Fig. 2A–B); *Rossella nodastrella* (van Soest *et al.* 2007: Figs. 1–3); ? *Rossella* aff. *nodastrella* (Tabachnick and Collins 2008).

Material examined: One specimen, the holotype (HBOI 10-VIII-09-1-001, USNM 1150045, SMF 11755) from deep-water *Lophelia* coral reefs off Cape Canaveral, Florida, lat. 28°19.426 N, long. 79°36.925 W, depth 723 m, collected August 10, 2009, and a second specimen (HBOI 5-VIII-05-1-004) from Miami Terrace, Straits of Florida, lat. 25°42.0159 N, long. 79°52.0254 W, depth 337 m, collected August 05, 2005 using manned submersible *Johnson-Sea-Link II*. One specimen (ZMAPOR 19715, SMF 10363) from Rockall Bank, Ireland, lat. 55°29.619 N, long. 15°48.053 E.

Description: The holotype consists of the upper part of a large basiphytous specimen ca. 23 cm high and ca. 13 cm wide; size of the whole specimen is ca. 30 cm high and ca. 23 cm in diameter. The body is vase-shaped with the osculum having outward-flaring margins; colour white to grayish (Fig. 3). The second specimen, from which we had only a small piece of tissue, also had a similar bodyshape and was white when alive; it grew conjoined with another individual (Fig. 4).

Skeleton (Figs. 3 and 5, Table 2): the dermal surface shows a web of microspiny stauractins (occasionally with rudimentary tubercle of fifth ray), some tauactins with or without rudimentary fourth ray, and very rarely isolated pentactins and diactins; the web covers the paratangential rays of large, smooth (except for slightly rugose ray tips), orthotropal hypodermal pentactins. Atrialia are mainly hexactins with often somewhat shortened tangential rays, and pentactins combined with few stauractins. Choanosomalia are diactins of very variable size, up to several mm length, with rounded to pointed microspined or smooth tips, sometimes with one end swollen, with or without central swelling. Shorter diactins can protrude beyond the dermal surface as prostalia (Fig. 4). Further choanosomalia are rough hexactins, numerous regular oxy- and predominantly hemioxyhexasters with very short primary rays and 1–2, rarely 3 secondary rays, less common microhexactins, and very rare onychohexasters. Discasters with inflated centre and ~10–20 secondary rays, and calycocomes are situated mainly near the atrial surface, they are generally rare in the investigated specimens and were not found in SEM preparations. Calycocomes appear as two types, one with 7–8 secondary rays and well-developed calyces, and one with more numerous (up to 13) secondary rays and hardly developed calyces (therefore perhaps better termed "calycocome-like stellate discohexasters", as suggested by one reviewer). Spherical microdiscohexasters are rather common and situated mainly near the dermal surface, their primary rays are roughly of equal length as the ca. 35–40 secondary rays.

Remarks: *N. asconemaoida* differs from *N. nodastrella* by the lack of pappocome-like oxyhexasters, the abundance of hemioxyhexasters, the shape of microdiscohexasters (spherical in contrast to stellate in *N. nodastrella* [according to Topsent 1915]), and the presence of calycocome-like stellate discohexasters; also, the calycocomes/calycocome-like stellate discohexasters are more abundant on the atrial side whereas calycocomes are more abundant on the dermal side in *N. nodastrella*. Furthermore, onychohexasters were not found in *N. nodastrella*, although they might have been overlooked as they appear to be very rare. Spicule composition and morphology of the Florida *N. asconemaoida* correspond well with the descriptions of the Irish specimens (van Soest *et al.* 2007: Fig. 3), an exception being the lack of "proper" calycocomes in the latter (only the calycocome-like stellate discohexasters

were figured in van Soest *et al.* 2007). However, those were very rare in the Florida specimen, and might have been overlooked in the Irish material. Further differences concern the presence of some pentactins and stauractins among atrialia and a greater variation among the choanosomal diactins, which also include non-centrotylote spicules and more than two size classes, in the Florida material. However, we do not consider these differences sufficient evidence to distinguish separate species. After all, the Florida and the Ireland population seem to be genetically very close (see next section). For these reasons we synonymize the Irish species initially identified as *Asconema* cf. *foliata* (van Soest and Lavaleye 2005) and later assigned to *Rossella nodastrella* (Tabachnick and Menshenina 2007; van Soest *et al.* 2007) with our new species *Nodastrella asconemaoida* **sp. nov.** The specimens reported by Tabachnick and Collins (2008) from the N Atlantic Ridge probably also belong in this species, but due to their insufficient documentation this cannot currently be established with certainty.

Etymology: The species name refers to the adult body shape with outward-flaring oscular margin, which is typical for the genus *Asconema*, and was first reported for *Nodastrella* from the Irish population of this species (van Soest *et al.* 2007).

Molecular phylogenetics

For *N. nodastrella* (USNM 1150046) and *N. asconemaoida* (USNM 1150045), coverage of successfully generated sequences was sufficient for inclusion in the supermatrix of genetic markers. We were only unable to amplify the 3'-half of the COI gene from both sponges (which was therefore excluded from analysis; see Methods), and the 5'-half of the 28S rDNA fragment for USNM 1150045. For HBOI 5-VIII-05-1-004, we first sequenced the 16S rDNA and the 3'-half of the 28S rDNA since these are usually the easiest markers to amplify from hexactinellids (Dohrmann, pers. obs.). Those sequences were almost identical to the corresponding sequences of USNM 1150045 (a single substitution in the 28S rDNA and a single 1-bp insertion/deletion [indel] in the 16S rDNA), so we assigned the specimen to *N. asconemaoida* **sp. nov.** and excluded it from further analyses. Genetic differences, as judged by the markers we used, appear very small between the Florida *N. asconemaoida* and the Irish specimen investigated in Dohrmann *et al.* (2008, 2012): 2 bp differences in the COI "barcoding" region, 2 bp in the 16S rDNA[2], and 1 bp in the 18S rDNA (the 28S rDNA sequences were identical). In contrast, the two differ markedly from the Florida *N. nodastrella*, as illustrated by the branch lengths in the ML tree (Fig. 6). Monophyly of *Nodastrella* received 85% bootstrap support; successive sister taxa to this clade are representatives of *Aulosaccus* and *Acanthascus*, whereas the SO *Rossella* spp. are only distantly related, being sister to Rossellinae n. gen. n. sp.[3]

Discussion

Our molecular phylogenetic analysis confirms previous reports (Dohrmann *et al.* 2008, 2009, 2012) that N Atlantic hexactinellid sponges originally assigned to *Rossella* are more closely related to *Aulosaccus* and *Acanthascus* than to Southern Ocean *Rossella* spp. (*Rossella* s.s.). Furthermore, we present morphological and molecular evidence that "*Rossella*" *nodastrella* is actually composed of at least two distinct species, which might in the future be split into subspecies or even more species, pending morphological and molecular evidence from additional populations. Thus, erection of a new genus, *Nodastrella* **gen. nov.**, is justified and necessary.

While there are clear skeletal differences between *Nodastrella* and *Rossella* (see *Remarks* section above), the presence of calycocomes with similar morphology in both taxa is striking, but is best interpreted as convergence (or loss in other genera) in light of the molecular phylogeny. However, calycocomes – with albeit somewhat different morphology – are also found in other genera of Rossellidae (*Caulophacus* Schulze) and Euplectellidae (*Symplectella* Dendy, *Holascus* Schulze, *Neocaledoniella* Tabachnick & Lévi) (Tabachnick 2002a,b), suggesting that their expression is highly homoplastic across Lyssacinosida. Therefore, their diagnostic value lies in the combination with other microscleres, such as discasters in *Nodastrella* and anisodiscohexasters in *Rossella* (see *Remarks* section above).

2. Plus a 4-bp indel within a hypervariable, unalignable region
3. To be described elsewhere by Henry M. Reiswig

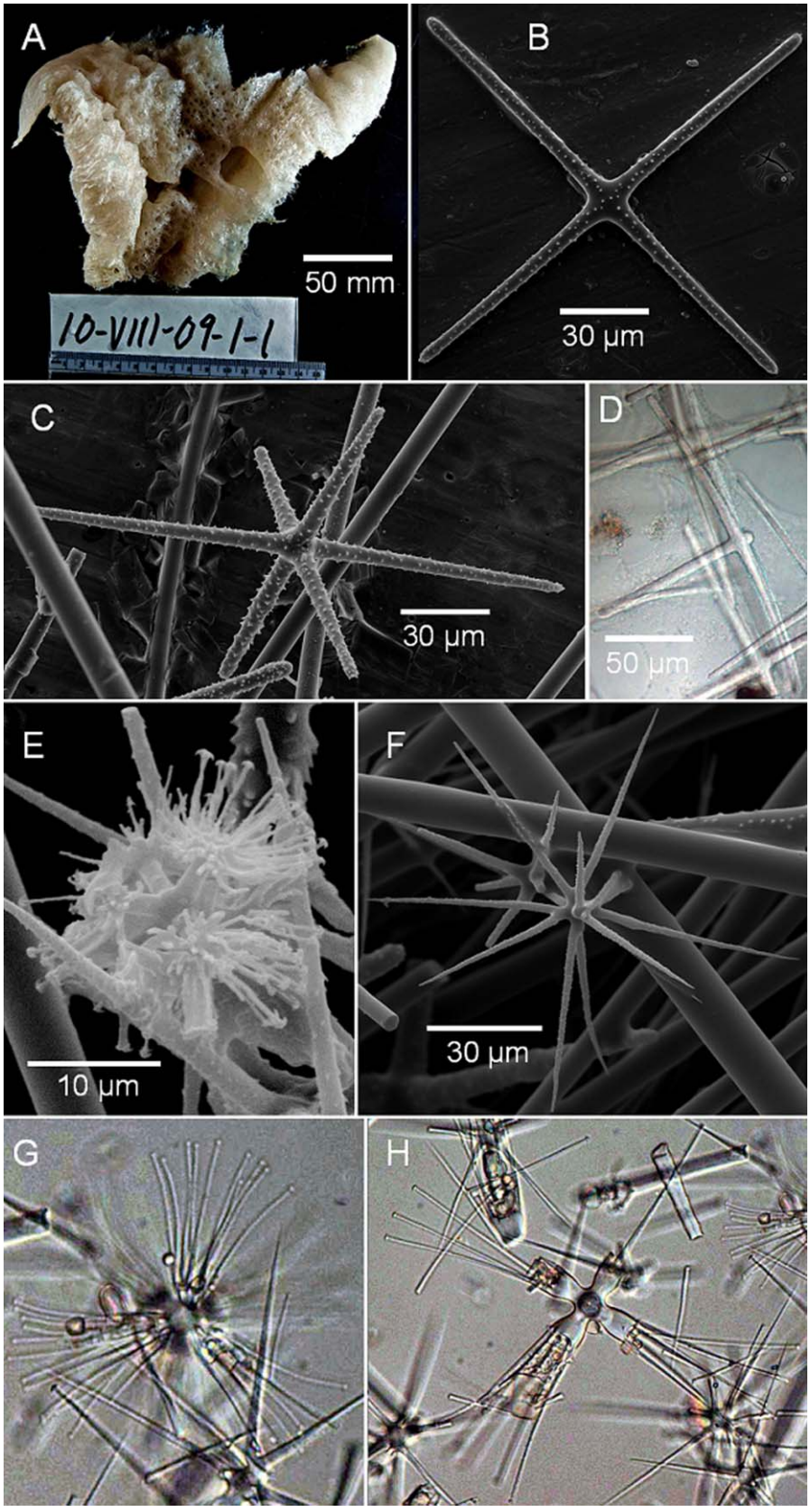

FIGURE 3. *Nodastrella asconemaoida* **sp. nov.** USNM 1150045, specimen (A) and skeleton (B–H). (B, C, E, F) scanning electron micrographs; (D, G, H) light micrographs. (A) Deck photograph of specimen. (B) Dermal stauractin. (C) Atrial hexactin. (D) Dermal tauactin. (E) Microdiscohexaster. (F) Oxyhexaster. (G) "Calycocome" with numerous secondary rays and hardly developed calyces (= calycocome-like stellate discohexaster) (40X). (H) "Proper" calycocome with fewer secondary rays and well-developed calyces (40X).

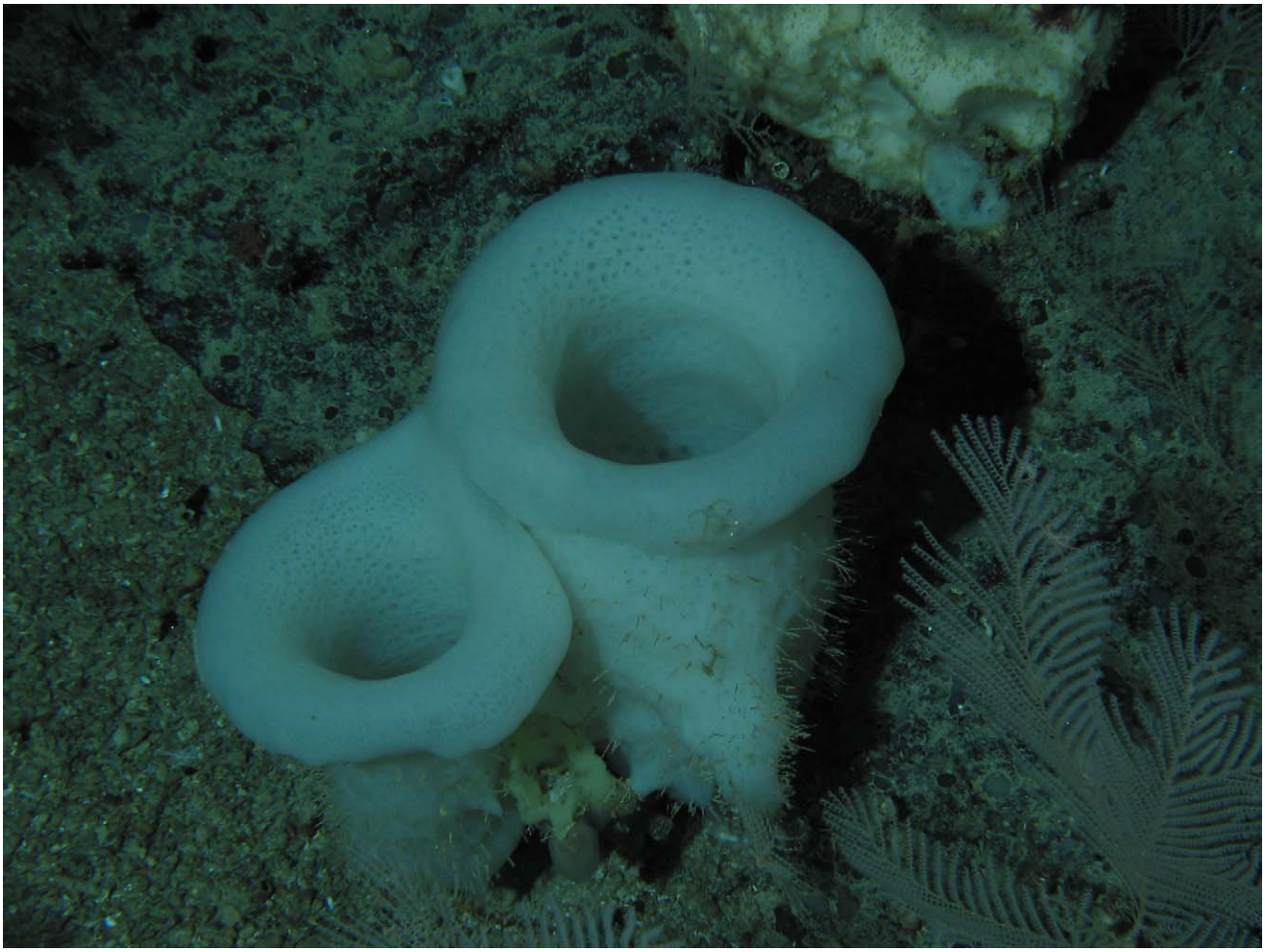

FIGURE 4. Underwater picture of *Nodastrella asconemaoida* **sp. nov.** HBOI 5-VIII-05-1-004, taken by *Johnson-Sea-Link II* on Miami Terrace, Straits of Florida, Reed Site BU2, August 05, 2005. Courtesy of HBOI, Ft. Pierce, Florida. Right specimen is ca. 22 cm tall.

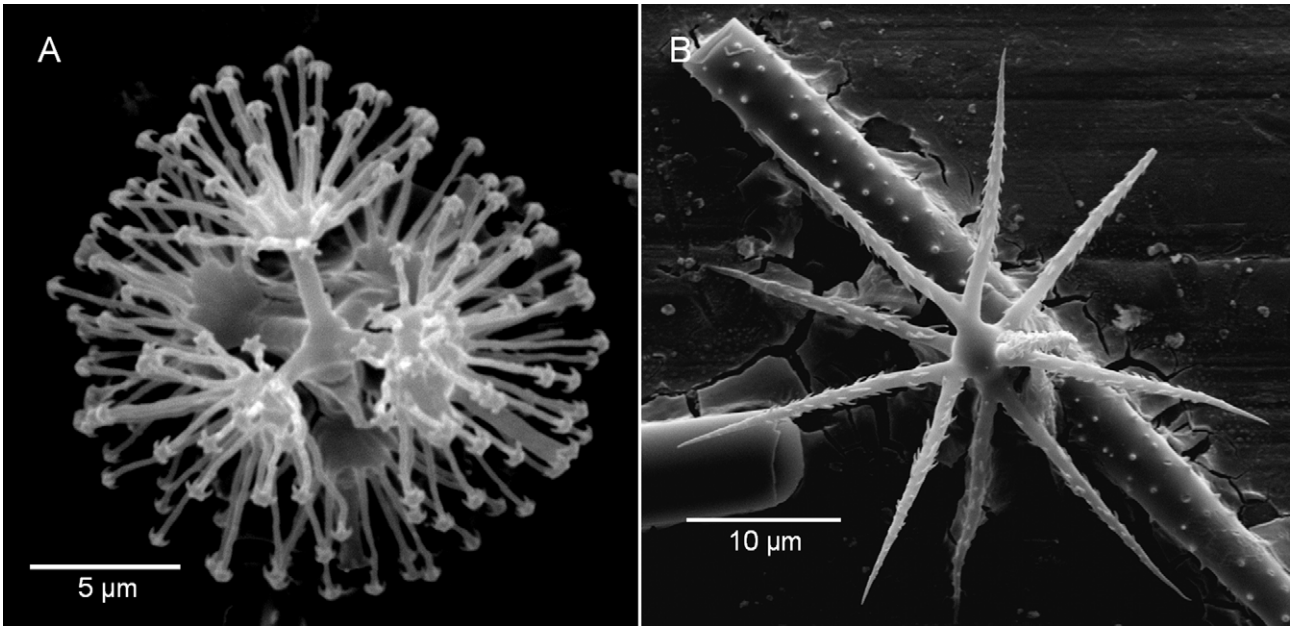

FIGURE 5. *Nodastrella asconemaoida* **sp. nov.** ZMAPOR 19715, skeleton. Scanning electron micrographs. (A) Microdiscohexaster. (B) Oxyhexaster.

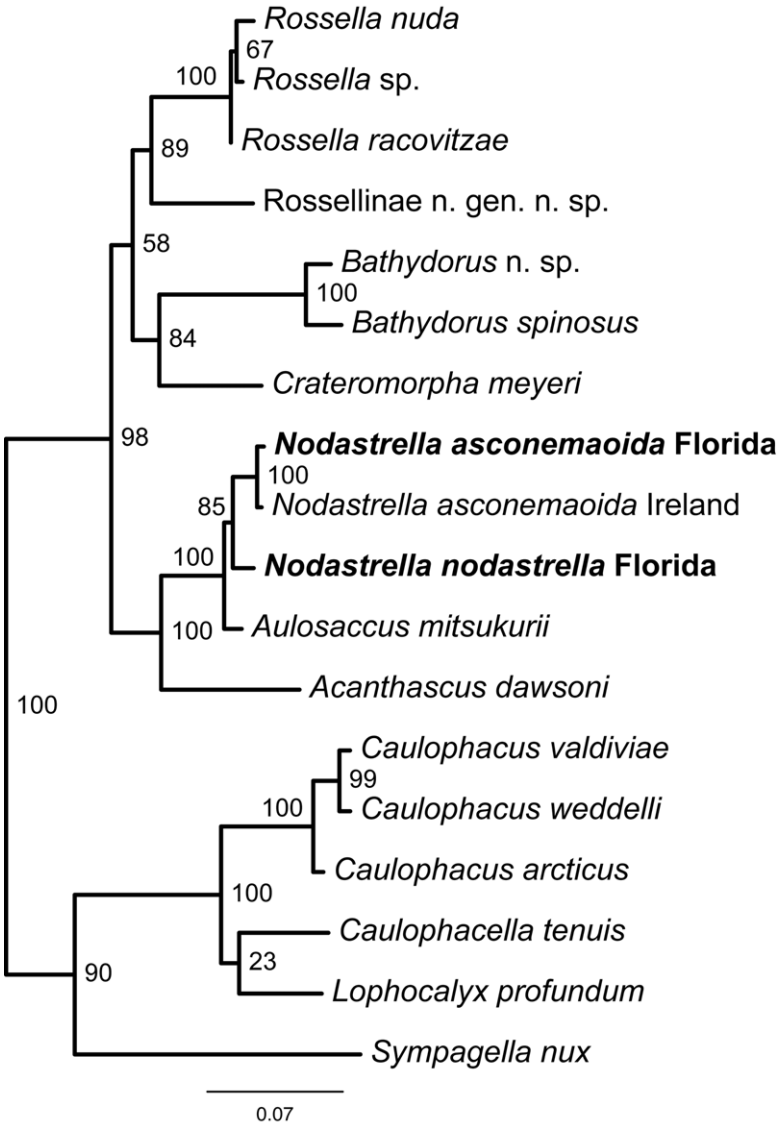

FIGURE 6. Maximum likelihood phylogram of Rossellidae inferred from concatenated 18S, 28S, 16S, and COI sequences using mixed models (see Methods for details). Outgroups (*Oopsacas minuta* + *Leucopsacus* sp.) omitted for clarity. The alignment has 4110 bp, 686 distinct alignment patterns, and 7.84% gaps. Bootstrap proportions are given at nodes; the values are based on 100 pseudoreplicates as determined by the autoMRE bootstopping criterion (Pattengale *et al.* 2010). Scale bar indicates expected number of substitutions per site. Previously unpublished specimens highlighted in boldface. The alignment and the tree have been deposited in TreeBASE under Study-Nr. 12795.

Within the set of rossellid genera sampled so far for molecular phylogenetics, *Nodastrella* forms a highly supported clade with *Acanthascus (Rhabdocalyptus) dawsoni* (Lambe) and *Aulosaccus mitsukurii* Ijima[4]. Among *Acanthascus* and *Aulosaccus* spp. discasters also occur (see Tabachnick 2002a), and *A. mitsukurii*, the sister taxon of *Nodastrella* within the current dataset, also has predominantly spiny stauractins as dermalia, and different types of oxyhexasters, one of which is similar to the pappocome-like oxyhexasters in *Nodastrella nodastrella* (Ijima 1904: Pl. X, Fig. 4). However, as such, these characters are not restricted to those three genera. Clearly, reconstructing the evolution of different spicule types in Rossellidae and teasing apart synapomorphies from homoplasies will require inclusion of many more of the 24 described genera in molecular systematic studies. With respect to finding the sister group of *Nodastrella* it would be especially interesting to sample representatives of *Asconema*, as this

4. Although Tabachnick (2002a) stated that *A. mitsukurii* should be transferred to *Hyalascus* Ijima, we chose to keep the present attribution of this species because it shows the large discohexasters typical of *Aulosaccus* (according to Ijima 1904: Pl. X, Fig. 2, and our own observations on the actual specimen used for molecular analysis)

genus is also restricted to the N Atlantic Ocean and is very similar in adult body shape (hence the initial assignment of the Ireland and Florida specimens; see above). Although body shape can be highly misleading for phylogenetic purposes in hexactinellids (see Dohrmann *et al.* 2009, 2012), discasters and pappocome-like oxyhexasters very similar to those here described for *N. nodastrella*, can be found in some *Asconema* spp. (see Tabachnick and Menshenina 2007).

Conclusions

By integrating molecular and morphological evidence we have shown that N Atlantic hexactinellids originally assigned to the genus *Rossella* are more diverse than initially thought and are unrelated to this taxon, justifying erection of a new genus for them, *Nodastrella* **gen. nov.** While we currently recognize two species, *N. nodastrella* (Topsent) and *N. asconemaoida* **sp. nov.**, future investigation of additional material, especially from other locations, might provide evidence to justify distinction of subspecies or even reveal additional species of *Nodastrella*. This study illustrates the usefulness of reciprocal illumination between inferences made from molecular and morphological data for constructing more natural (i.e., phylogeny-based) classification systems of morphologically difficult groups such as sponges.

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