Integrating morphological and molecular taxonomy with the revised concept of Stelligeridae (Porifera: Demospongiae)

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ABSTRACT

This study reinforces and extends the findings of previous molecular studies that showed that there is a close relationship between species assigned to *Halicnemia*, *Higginsia*, *Paratimea* and *Stelligera a*nd that the family Heteroxyidae is polyphyletic. In the present study the re-examination of a large number of specimens and species of *Halicnemia, Higginsia* and *Paratimea* has resulted in the description of one new species of *Halicnemia* and six new species of *Paratimea*; *H. caledoniensis* sp. nov., *P. aurantiaca* sp. nov., *P. dentata* sp. nov., *P. hoffmannae* sp. nov., *P. lalori* sp. nov., *P. mosambicensis* sp. nov. and *P. rosacea* sp. nov. respectively; the resurrection of *Halicnemia gallica* and a better understanding of the characters uniting Stelligeridae. A new species of *Heteroxya, H. beauforti* sp. nov. is also described. We demonstrate that many of the taxa assigned to Heteroxyidae (on the basis of the possession of smooth or acanthose microxeas) are more closely related to other families and we propose several changes to the classification of Heteroscleromorpha.

Desmoxyidae is resurrected from synonymy and transferred to Poecilosclerida; *Higginsia anfractuosa* is transferred to Hymedesmiidae and a new genus *Hooperia* gen. nov. is erected for its reception; *Higginsia durissima* is returned to *Bubaris* (Bubaridae); *Higginsia fragilis* is transferred to *Spanioplon* (Hymedesmiidae); *Hemiasterella camelus* is transferred to *Paratimea*; *Raspailia (Parasyringella) australiensis* and *Ceratopsion axiferum* are transferred to *Adreus* (Hemiasterellidae).

ADDITIONAL KEYWORDS: Axinellida-Desmoxyidae-*Halicnemia*-Hemiasterellidae-Heteroxyidae-homoplasy-*Paratimea*-*Plenaster*-polyphyletic-*Stelligera*-*new species*

INTRODUCTION

Sponges are one of the most ancient groups of multicellular organisms with a fossil history dating back to the Cambrian (Botting & Muir, 2017) and with steroid biomarkers in the Neoproterozoic some 635-717 Myr (Love *et al.*, 2009). Recent molecular studies confirm that they are one of the earliest diverging metazoans (Pisani *et al*., 2016; Simion *et al*., 2017; Dohrmann & Wörheide, 2017). Sponges occupy a wide range of aquatic, benthic environments, from temporary freshwater pools to abyssal depths. They are a very

successful group with over 9000 described species recorded in the World Porifera Database [herein referred to as WPD (van Soest *et al*., 2018)], however Hooper & van Soest (2002) state that there is likely to be a similar number of undescribed taxa and Appeltans *et al*. (2012) estimate that there may be as many as 18000 undescribed species. Traditional taxonomy and classification of sponges are based on morphology, cytology and reproductive traits but particularly spicule morphology and skeletal architecture (Hooper & van Soest, 2002). The combination of very ancient lineages, a high proportion of undiscovered taxa and relatively few morphological characters make understanding evolutionary relationships among sponge taxa difficult and has resulted in many orders, families and genera composed of polyphyletic assemblages (Boury-Esnault, 2006). Fortunately, a growing number of molecular phylogenetic studies, many of which integrate observations on morphology, are improving our understanding of the phylogenetic relationships among sponges; these were reviewed by Cárdenas *et al*. (2012).

The current study expands on the preliminary work of Morrow *et al*. (2012) on Stelligeridae using a combination of molecule-based phylogenetic hypotheses derived from 18S and 28S rRNA and CO1 barcoding fragments together with a careful re-examination of skeletal morphology to try and resolve the phylogenetic relationships within this group of sponges. It is not a monographic revision, but rather a contribution towards a better understanding of the systematics of Stelligeridae and, more broadly, Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012 in general.

The class Demospongiae Sollas, 1885 represents almost 80% of all known sponges. Based on molecular results (Morrow & Cárdenas, 2015) Demospongiae is subdivided into three subclasses; Keratosa Grant, 1861; Verongimorpha Erpenbeck, Sutcliffe, de Cook, Dietzel, Maldonado, van Soest, Hooper & Wörheide, 2012 and Heteroscleromorpha. Heteroscleromorpha is by far the largest of all the subclasses with approximately 6773 valid species. Morrow *et al*. (2012) used data derived from partial 28S rRNA and CO1 barcoding fragments to propose a new classification for Heteroscleromorpha. They demonstrated that there was a close relationship between *Halicnemia* Bowerbank, 1864, *Paratimea* Hallmann, 1917 and *Stelligera* Gray, 1867 and resurrected Stelligeridae Lendenfeld, 1898 for this well supported clade. Erpenbeck *et al*. (2012b) using CO1 barcoding sequences confirmed these results and also showed that *Higginsia*, a genus which is morphologically similar to *Halicnemia,* also clustered within Stelligeridae.

The taxonomic interpretation of *Halicnemia* and *Paratimea* using traditional morphological characters has resulted in very different classifications. Historically *Paratimea* and *Stelligera* have always been considered to be closely related. The spicules in both genera are styles or tylostyles, oxeas (often centrotylote) and oxyaster microscleres. However, their relationship with *Halicnemia* is more controversial. In *Halicnemia* the megascleres are also styles/tylostyles and centrotylote oxeas however the microscleres are centrangulate spined microxeas (acanthoxeas) instead of asters. Table 1 summarizes the various relationships espoused by the main authors who have written on this subject. Below we discuss the confused taxonomic history of *Halicnemia* and *Paratimea*.

Topsent (1897) reasoned that *Halicnemia patera* Bowerbank, 1864*, Hymeraphia verticillata* Bowerbank, 1866 and *Bubaris constellata* Topsent, 1893 belonged in a single genus (*Halicnemia*) on the basis of the shared possession of tylostyles and distinctive, centrotylote oxeas forming the ectosomal skeleton. He considered that the microscleres could be either oxyasters or acanthoxeas. He also speculated that the microxeas in *Halicnemia* could have been derived from asters; however, Dendy (1922) regarded it as more likely that the asters found in *Halicnemia constellata* were pseudasters derived from the spined microxeas. Carter (1875) was the first to notice the similarities in spicule composition between *Halicnemia patera* and *Hymeraphia verticillata.*

By contrast, Hallmann (1917, in postscript) disagreed with Topsent's placement of *Hymeraphia verticillata* and *Bubaris constellata* in the genus *Halicnemia* (along with the type

Halicnemia patera). Hallmann considered that the differences between these three species necessitated the allocation of each to a separate genus. *Halicnemia* was maintained for the type species *H. patera*, while he established *Laonaenia* for *Hymeraphia verticillata* and *Paratimea* for *Bubaris constellata.* He tentatively included *Paratimea* which has euaster microscleres in the family Spirastrellidae Ridley & Dendy, 1886. Hallmann (1917) divided Axinellidae Carter, 1875 into four subfamilies: Axinellinae; Trachycladinae Hallmann, 1917; Desmacellinae Ridley & Dendy, 1886 and Desmoxyinae Hallmann, 1917. To Desmoxyinae he allocated his new genera *Desmoxya*, *Laonaenia* and *Allantella* and also *Halicnemia*, *Higginsia* and *Holoxea* Topsent, 1892. The genus *Allantella* was created for *Trachytedania arborea* Keller, 1891. Hooper (2002a) synonymised *Allantella* with *Higginsia* on the basis of the presence of spined microstrongyles. Re-examination of type material of *Holoxea furtiva* Topsent, 1892 (type species of *Holoxea*) by Uriz (2002) led to *Holoxea* being reassigned to Astrophorina Sollas, 1888.

Dendy (1905) established the subfamily Heteroxyinae for *Heteroxya* Topsent, 1898 and his new genus *Acanthoxifer.* Later, Dendy (1922) added *Higginsia* and *Halicnemia* to a section, Heteroxyeae, within the sub-family Axinellinae. Dendy, 1922 was in agreement with Topsent (1897) and treated *Bubaris constellata* as a species of *Halicnemia*, he stated “Nor do I imagine that *H. constellata*, on account of its pseudasters, need be considered as the type of a distinct genus”. Topsent (1928) proposed the family Astraxinellidae Dendy, 1905 for the group *Halicnemia, Higginsia, Vibulinus* Gray, 1867 (= *Stelligera*: synonymy by Hooper, 2002) and *Hemiasterella* and retained Heteroxyinae for *Heteroxya* and *Acanthoxifer*. He assigned four families to Halichondrina Vosmaer, 1887: Axinellidae; Astraxinellidae; Heteroxyidae Dendy, 1905 and Bubaridae Topsent, 1894. Dendy (1905) introduced the name Astraxinellidae briefly and rather flippantly:

> “Some of the old group Axinellidae (e.g. *Vibulinus*, with asterose microscleres) must likewise be included in this sub-order, and it may prove necessary to institute a new family - Astraxinellidae - for their reception.”

As there is no genus *Astraxinella,* the family name Astraxinellidae is invalid. For reasons of priority, Morrow *et al*. (2012) resurrected Stelligeridae for the clade that contained *Halicnemia, Paratimea* and *Stelligera.* Lendenfeld (1898) gave the following diagnosis for Stelligeridae:

> “Euastrosa with a spongin skeleton, which either just glues the megascleres together or is highly abundant like in *Axinella*. This family includes the two genera, *Stelligera* (with monactine megascleres and additional diactines) and *Hemiasterella* (with only diactine megascleres). In the Adriatic, they are represented by genus *Stelligera*.”

Laubenfels (1936) subdivided Axinellidae into two subfamilies, Axinellinae and Higginsinae, for genera that are similar to typical Axinellidae but possess microsclere oxeas that are frequently spiny. Lévi (1955) synonymized Higginsinae with Desmoxyinae as *Higginsia,* the type genus of de Laubenfels’ subfamily, was included in Desmoxyinae and *Desmoxya* the type genus of Desmoxyinae was included in Higginsinae by de Laubenfels. Lévi (1955) also then raised Desmoxyinae to family rank. Lévi (1953) erected a new order Axinellida, containing the family Axinellidae, which had previously been classified within Halichondrida (Topsent, 1928; Laubenfels, 1936) and assigned Desmoxyidae Hallmann, 1917, Heteroxyidae and Astraxinellidae (for axinellids with asterose microscleres) to it. Later, Lévi (1973) used Hemiasterellidae, rather than Astraxinellidae, for axinellids with asters (*Hemiasterella, Adreus*, *Paratimea* and *Stelligera*).

Bergquist (1970) and Wiedenmayer (1977) adopted the classification of Lévi (1955) and used Desmoxyidae for axinellids with microscleres in the form of spined or smooth microxeas. Bergquist (1970) established the genus *Acanthoclada* and assigned it to Desmoxyidae.

Van Soest, Díaz & Pomponi (1990), in a study using morphocladistics, called for the abandonment of Axinellida and the allocation of Desmoxyidae to Halichondrida and Hemiasterellidae to Hadromerida Topsent, 1894 (see Morrow *et al*., 2013). Van Soest *et al*. (1990) assigned *Myrmekioderma* Ehlers, 1870 and *Didiscus* Dendy, 1922 to Halichondriidae Gray, 1867, a classification followed by Díaz *et al*. (1993). Later van Soest & Lehnert (1997) returned *Myrmekioderma* and *Didiscus* to Desmoxyidae.

The monotypic genus, *Desmoxya* Hallmann, 1917 was created for *Higginsia lunata* Carter, 1885. Hooper & Lévi (1993) synonymised *Desmoxya* and *Dendropsis* Ridley & Dendy, 1886 with *Higginsia* Higgin, 1877 based on the shared apomorphy of spined microxeas, however they highlighted that there were major skeletal differences and that *Desmoxya* may need to be resurrected to accommodate *Higginsia*-like species that lack any evidence of axial and extra-axial skeletons e.g. *Higginsia lunata* and their new species *H. anfractuosa.* The discovery of another species, *Desmoxya pelagiae* van Soest & Hooper, 2005, from the cold water coral reefs of the North Atlantic led to the resurrection of *Desmoxya*. Van Soest & Hooper, 2005 considered Desmoxyidae synonymous with Heteroxyidae and changed the family to which *Halicnemia*, *Higginsia*, *Desmoxya* etc. were assigned from Desmoxyidae to Heteroxyidae.

MATERIALS AND METHODS

COLLECTION OF MATERIAL

This study used a combination of voucher specimens from various institutions together with freshly collected material for DNA analysis. Shallow-water specimens were collected either by SCUBA diving or by shore collecting. Deep-water specimens were collected during the cruise CE10004 of RV *Celtic Explorer*, using the deep-water Remotely Operated Vehicle *Holland I*; the ARK-XXII/1a 2007 expedition on board the RV *Polarstern* to Northern Norway, using the manned-submersible *JAGO*; the PAMELA-MOZ01 2014 expedition (IFREMER) and the BIOMAGLO 2017 expedition (MNHN/IFREMER) to the Mozambique Channel, both using a Warrén dredge (DW). The sponges were photographed *in situ*, then tissue samples approximately 1 cm^3 were collected. The specimens were fixed in 96 % ethanol normally within 1 hour of collection. After 24 hours the ethanol was changed to prevent dilution by seawater. We have attempted to analyse as many species and genera that are currently assigned to Heteroxyidae and Hemiasterellidae as we could obtain. The taxa used in the analyses together with their catalogue numbers and, where relevant, their GenBank accession codes are listed in Supporting information, Data S.1.

The following abbreviations are used for the institutions from which we have examined material:

BELUM, Ulster Museum, Belfast, UK; BMNH, The Natural History Museum, London, UK; QM, Queensland Museum, Australia; MNHN, Muséum National d'Histoire Naturelle, Paris, Français (France); MOM, Musée Océanographique de Monaco; NMNH, National Museum of Natural History, Smithsonian Institution, Washington DC, USA; ZMA, former Zoological Museum Amsterdam, collections now housed at Naturalis Biodiversity Center, Darwinweg 2, 2333 CR Leiden, Netherlands; ZMBN, Natural History Museum of Bergen, Bergen, Norway; SMNH, Swedish Museum of Natural History, Stockholm, Sweden; UCMPWC, University of California Museum of Paleontology, USA; WAMZ, Western Australian Museum, Perth, Australia.

PREPARATION AND EXAMINATION OF MATERIAL

Nitric acid spicule preparations and thick section mounts were made following the protocols described by Picton & Goodwin (2007). Photomicrographs of the spicules and sections were made using Nikon Eclipse 80i light microscope equipped with a Jenoptic ProgRes CT3 digital camera and software. Spicules were measured from nitric acid histological preparations using this equipment. Unless otherwise stated (n: number of spicules measured) 20 spicules of each category were measured for each specimen. Spicule

dimensions are either given as a range, or as a minimum–*mean*–maximum throughout the manuscript.

At NUI Galway, nitric acid preparations of spicules were made directly onto cut microscope slides, air dried then mounted on scanning electron microscope (SEM) discs and coated with gold then viewed in the HBB SEM. At the Netherlands Centre for Biodiversity Naturalis, spicules were air dried directly onto the SEM studs, sputter-coated with gold and examined and photographed using a JEOL SEM. At the Queensland Museum, tissue was dissolved in 12.5% sodium hypochlorite, neutralized in distilled water, rinsed twice in 70% ethanol, then in 98% ethanol and then air dried. SEM preparations were sputter coated in gold to improve resolution. The scanning electron micrograph photos were taken using a Hitachi TM-1000 SEM. All plates were assembled in Adobe Photoshop.

DNA EXTRACTION AND AMPLIFICATION

DNA extraction and amplification followed the protocols outlined in Morrow *et al*. (2012). The CO1 Folmer fragment was obtained using primers LCO1490 and HCO2198 (Folmer *et al*., 1994), the 28S D1–D2 marker used Por28S-15F and Por28S-878R, the 28S D3–D5 marker used Por28S-830F and Por28S-1520R and the 28S D6–D8 marker used Por28S-1490F and Por28S-2170R (Morrow *et al*., 2012). New primers were designed in the present study to amplify a shorter fragment (192 nucleotides, from the 28S D3 region) from older museum material in which the DNA was fragmented: Por28S-1010F; GTCTTGAAACACGGACCAAG; Por28S-1277R; GTTCACCATCTTTCGGGTC. The following protocol was used for the D3 primers; 94.0°, 5 min; (94.0°, 30 s; 45.0°, 30 s; 72.0°, 30 s) x 40 cycles; 72.0°, 5 min.

PHYLOGENETIC ANALYSES

The 18S rRNA sequences were obtained from GenBank and were primarily generated by Redmond *et al*. (2013). Many of these sequences were from the same specimens and DNA extracts used by Morrow *et al.* (2012, 2013). The 28S rRNA and CO1 sequences are a combination of previously published sequences available on GenBank (primarily from Morrow *et al*., 2012; 2013), and sequences newly generated for this study.

This study used complete 18S rRNA, 28S rRNA (D1–D2; D3–D5; D6–D8 regions) and CO1 Folmer fragments as a number of studies have shown that they are useful phylogenetic markers in sponges (Borchiellini *et al*., 2004; Erpenbeck *et al.,* 2007; Wörheide & Erpenbeck, 2007; Cárdenas, 2010) and there is a relatively high number of sponge sequences available for these markers on GenBank.

Sequences were managed in Geneious R10 (http://www.geneious.com, Kearse *et al*., 2012). Forward and reverse reads were assembled into contigs using the assembly function of the software and checked for inconsistencies. Where inconsistencies arose Geneious used the better quality of the two reads or introduced IUPAC ambiguity codes into the consensus sequence. Sequences were aligned with MAFTT (Katoh *et al*. 2002) and trimmed in Geneious. Complete or nearly complete sequences of 18S rRNA, 28S rRNA (D1–D8) and CO1 Folmer fragments were concatenated for Figure 1. The best fitting model for each of the three partitions was separately selected using JModelTest (Darriba *et al*., 2012). The GTR+G+I model was identified as the best-fit model of molecular evolution for all datasets.

Several molecular studies have suggested Haplosclerida Topsent, 1928 as the sister group to the remaining Heteroscleromorpha (Borchiellini *et al.,* 2004; Lavrov, Wang & Kelly, 2008; Sperling, Peterson & Pisani, 2009; Morrow & Cárdenas, 2015). However, Erpenbeck *et al.* (2004) demonstrated that ribosomal sequences in Haplosclerida have increased evolutionary substitution rates, making them unsuitable outgroup taxa for Heteroscleromorpha rRNA analyses. The keratose sponges *Dysidea arenaria* Bergquist, 1965, *Dactylospongia elegans* (Thiele, 1899) and *Aplysina cauliformis* (Carter, 1882) were chosen as the outgroup for our concatenated tree (Fig.1) as previous studies have shown these to form a suitable outgroup (Redmond *et al*., 2013; Thacker *et al.,* 2013) for the Heteroscleromorpha used in this study. The keratose sponges *Dysidea arenaria, D. etheria*

Laubenfels, 1936, *Dactylospongia elegans*, *Euryspongia lobata* Bergquist, 1965 and *Lamellodysidea herbacea* (Keller, 1889) were used as outgroups for our other gene trees.

Maximum Likelihood (ML) bootstrap analyses (1000 replicates) were performed under the general GTRGAMMAI nucleotide evolution model using RaxML (Stamatakis, Hoover & Rougemont, 2008). Bayesian inference (BI) used MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with 10^6 generations. The best tree from each RAxML analysis is illustrated showing bootstrap support >70 and posterior probabilities >0.7 from the Bayesian analysis (Fig. 1; Supporting Information Figs.S2–S5).

RESULTS

TREES

In this study we used five different markers, complete 18S rRNA, 28S rRNA (D1–D2, D3–D5 & D6–D8 regions), and the mitochondrial CO1 Folmer fragment to try and resolve the phylogeny of taxa currently assigned to Heteroxyidae and Stelligeridae (Hooper & van Soest, 2002; van Soest & Hooper, 2005; van Soest *et al*., 2018). A phylogenetic hypothesis based on RAxML analysis of the combined 18S, 28S (D1–D8 region) and CO1 barcoding sequences of 39 taxa was constructed (Fig. 1). In order to have representatives of *Higginsia* and *Axinella* Schmidt, 1862 in the combined 18S, 28S, CO1 analysis, the 18S and 28S rRNA sequences of *Higginsia mixta* Hentschel, 1912 and *Axinella pyramidata* Stephens, 1916 were concatenated with the CO1 sequences of *Higginsia* cf. *petrosioides* Dendy, 1922 and *Axinella infundibuliformis* (Linnaeus, 1759) respectively (separate analyses of CO1 sequences (Supporting Information, Fig. S3) show *H.* cf. *petrosioides* grouping within Stelligeridae and *A. infundibuliformis* clustering with Axinellidae).

Phylogenetic trees were also reconstructed for the separate datasets. Supporting Information Figure S2 represents the 18S rRNA tree, Figure S3 the 28S (D3–D5 region) tree and Figure S4 the CO1 Folmer fragment tree. Supporting Information Figure S5 is a 28S rRNA (D1–D2 region) genetree consisting mainly of raspailiid taxa. For each of the trees, taxa assigned to Heteroxyidae are shown in bold.

DESCRIPTION OF TREES

The topology based on the combined analysis of 18S, 28S rRNA and CO1 sequence data (Fig. 1) from 39 taxa shows strong support (100 BS/1 PP) for a clade containing *Halicnemia gallica* (Topsent, 1893)*, Higginsia*, *Stelligera stuposa* (Ellis & Solander, 1786), *S. rigida* (Montagu, 1818) and *Paratimea loennbergi* (Alander, 1942). This supports the findings of Morrow *et al*. (2012) who resurrected Stelligeridae for this assemblage. Heteroxyidae (*sensu* van Soest & Hooper, 2005) appears to be a polyphyletic assemblage as *Didiscus* sp. and *Myrmekioderma granulatum* (Esper, 1794) are clustering within Raspailiidae Hentschel, 1923 and *Desmoxya pelagiae* groups with Poecilosclerida Topsent, 1928.

The 18S tree (Supporting Information Fig. S2) is congruent with our combined genetree (Fig. 1) but includes a larger dataset of 87 taxa. It also shows strong support for Stelligeridae *sensu* Morrow *et al*. (2012) (95 BS/1 PP) and indicates that Heteroxyidae is polyphyletic. A specimen identified as *Higginsia anfractuosa* Hooper & Lévi, 1993 (OCDN 3725J from Tanzania) in Redmond *et al*. (2013) has been re-examined and identified as Hymedesmiidae sp. Topsent, 1928. It does not cluster with other *Higginsia* and *Halicnemia*, but with *Hemimycale columella* (Bowerbank, 1874) in Hymedesmiidae. *Myrmekioderma granulatum* and *Myrmekioderma* sp. (both from the Indo-Pacific region) and *Didiscus* sp. cluster with Raspailiidae, however *M. rea* (de Laubenfels, 1934), from the Caribbean Sea, clusters with Axinellidae indicating that *Myrmekioderma* may be polyphyletic.

The 28S genetree (Supporting Information Fig. S3) includes sequence data from 78 taxa and shows *Halicnemia gallica*, *H. verticillata, H. caledoniensis* sp. nov., *Acanthoclada prostrata*, *Paratimea loennbergi*, *P. aurantiaca* sp., *P. mosambicensis* sp. nov., *P. rosacea* sp. nov., *Stelligera rigida* and *S. stuposa* clustering within Stelligeridae. It supports the

phylogeny obtained using 18S sequences and shows Hymedesmiidae OCDN 3725J clustering with *Hemimycale* (in Hymedesmiidae: Poecilosclerida) and *Desmoxya pelagiae* clustering with Poecilosclerida. Again, *Myrmekioderma granulatum* and *Didiscus* cluster with Raspailiidae.

Our CO1 Folmer tree (Supporting Information Fig. S4) is based on sequence data from 66 taxa. In this phylogenetic tree members of Stelligeridae form a polytomy at the base of Raspailiidae. *Paratimea camelus* comb. nov. (= *Hemiasterella camelus*) clusters closely with *Paratimea oxeata*. *Desmoxya pelagiae* clusters with *Tedania ignis* (Duchassaing & Michelotti, 1864) in Poecilosclerida and not with heteroxyid taxa. Unlike our other genetrees, the CO1 tree shows strong support (99 BS/1 PP) for *Myrmekioderma granulatum* (EF519652) grouping with *Heteroxya corticata* Topsent, 1898 and *H. beauforti* sp. nov. in Heteroxyidae and not with raspailiids. *Hooperia anfractuosa* comb. nov. (= *Higginsia anfractuosa*) clusters with *Hemimycale* Burton, 1934. Supporting Information Data S6. is a pairwise identity matrix for *Paratimea* spp. and *Stelligera* spp. based on these CO1 sequences: the sequences are all very similar to each other with a maximum difference of approximately 2% between *Paratimea* spp. and *Stelligera* spp.

In our 28S D1–D2 genetree (Supporting Information Fig. S5) Stelligeridae is strongly supported (100 BS/1 PP) and is the sister clade to Raspailiidae. Stelligeridae is represented by *Halicnemia gallica*, *H. verticillata*, *H. caledoniensis* sp. nov., *Paratimea loennbergi*, *P. oxeata*, *P. aurantiaca* sp. nov., *P. hoffmannae* sp. nov., *Stelliegra rigida* and *S. stuposa. Raspailia* (*P.*) *australiensis* and *Ceratopsion axiferum* which are currently placed in Raspailiidae, group instead with *Adreus* (Tethyida). The 28S D1–D2 tree has more resolution than the CO1 tree and supports the monophyly of *Halicnemia, Paratimea* however is paraphyletic with respect to *Stelligera. Paratimea loennbergi* and *P. aurantiaca* cluster with *Stelligera* spp. wheras *P. oxeata*, *P. hoffmannae* sp. nov. and *P. rosacea* form a sister clade.

DISCUSSION

Our combined hypothesis (Fig. 1) and single-gene trees (Supporting Information, Figs S2–S5) are congruent and show that there is strong molecular evidence for a close relationship between some former 'hemiasterellid' taxa (*Paratimea* and *Stelligera*) and taxa that were previously assigned to Heteroxyidae (*Halicnemia*, *Higginsia* and *Acanthoclada*). Our results strongly support the resurrection of Stelligeridae *sensu* Morrow *et al*. (2012) for this clade. The recently established *Plenaster* Lim & Wiklund, 2017 was tenatively assigned to Stelligeridae on the basis that "Stelligeridae is the only family in the order Axinellida that has members bearing styles and euasters like *Plenaster.*" However, their molecular trees (28S rRNA D1-D2 region and CO1 Folmer fragment) clearly show that *Plenaster* is not a stelligerid; the taxonomic affinities of this genus will be part of a future manuscript.

Hooper (1986) suggested *Paratimea* might be an encrusting form of *Stelligera* given that their respective type species had similar aster morphology, whereas Voultsiadou-Koukoura & van Soest, 1991 considered *Paratimea* a valid genus. On our 28S D1–D2 tree (Supporting Information, Fig. S5) *Paratimea* appears polyphyletic, *P. loennbergi* and *P. aurantiaca* sp. nov. are paraphyletic with respect to *Stelligera* spp. and *P. oxeata*, *P. hoffmannae* sp. nov. and *P. rosacea* sp. nov. form a sister clade to *Stelligera* spp. + *Paratimea* pars. It may be that in the future encrusting *Paratimea* spp. such as *P. loennbergi, P. aurantiaca* sp. nov., *P. dentata* sp. nov. and *P. constellata* (the type species) are transferred to *Stelligera* which has priority over *Paratimea*, and that a new genus is established for *P. oxeata*, *P. hoffmannae* sp. nov., *P. rosacea* sp. nov. and other massive, mostly deep-water '*Paratimea*' species that have large oxeas as their principal spicules and relatively large, often asymmetric, asters. Until we have more sequence data to test the validitiy of this, we retain *Paratimea*.

There are several morphological characters that unite Stelligeridae, in spicule morphology, surface architecture, cell types, reproduction, and these are discussed below.

Spicules: Megascleres; the principal spicules in *Paratimea* and *Halicnemia* are long, slender tylostyles, styles or oxeas whereas in *Stelligera* and *Higginsia* they are only styles. *Acanthoclada* is unusual in that it has styles and rhabdostyles. Some species of *Paratimea* and *Halicnemia* have distinctive short, club-like tylostyles (Figs 2F, *P. loennbergi*; 11B*, Halicnemia patera*; 15B*, H. caledoniensis* sp. nov.) which may be a synapomorphy for the clade. Alander (1942), in his description of *P. loennbergi* (as *Halicnemia loennbergi*) remarked that the genus *Halicnemia* comprised species of two categories. One group to which *H. constellata T*opsent, 1897 belonged has one type of tylostyle plus oxyasters. The other group with *H. patera* has two types of tylostyles plus spined microxeas. In common with *H. patera* his new species *H. loennbergi* had two types of tylostyles but the microscleres were oxyasters. He considered that his new species united this group. These short, stout club-like tylostyles have also been observed in *Halicnemia caledoniensis* sp. nov.

Secondary spicules are small ectosomal styles, oxeas or anisoxeas: These spicules often form distinctive bouquets around the protruding larger styles, tylostyles or oxeas. In *Paratimea* and *Halicnemia* they are often centrotylote (Fig. 24A, D & G). In *Halicnemia gallica* there are usually two or three equidistant tylote swellings. The centrotylote oxea of *H. verticillata* are very distinctive, the ends of the oxea being fissurate (Fig. 24E). It shares this character with *Higginsia bidentifera* (Ridley & Dendy, 1886) and *Higginsia petrosioides* Dendy, 1922. In *Paratimea dentata* sp. nov., included in our 18S tree (Supporting Information Fig. S2), the centrotylote oxeas are also fissurate (Fig. 24C). This is the first time this distinctive spicule has been found in *Paratimea,* it has not been reported elsewhere within Demospongiae and is further evidence for *Paratimea* and *Halicnemia* being closely related.

Microscleres; in *Stelligera* and *Paratimea* the microscleres are smooth rayed oxyasters (Fig. 5B) whereas in *Halicnemia* and *Higginsia* they are acanthoxeas (Fig. 5F & I). Topsent (1897) speculated that the acanthoxea were derived from asters and that the two structures were homologous, however Dendy (1922) thought that it was more probable that the oxyasters were merely pseudasters derived from the acanthoxea. Topsent (1928) went further and suggested that the centrotylote oxeas could also have their origin in asters. Sollas (1882) considered the microrhabds in *Pachymatisma* Bowerbank in Johnston, 1842 to be homologous with the asters in *Geodia* Lamarck, 1815 as both share a similar position within the ectocortex. By examining the ontogeny of the microscleres he reported that it was possible to trace the development of the microrhabd from the adult form which is cylindrical with rounded ends and a roughened surface, to a smooth fusiform spicule with a central globular enlargement and pointed ends, which he regarded as a biradiate aster. In contrast, the developing asters though progressively smaller, remained multiradiate. Sollas described the number of rays in the asters as very variable with a reduction to four, three or even two rays frequent. The two rayed asters have a central enlargement and closely resemble developing microrhabds. Sollas considered this as evidence that asters descended from microxea. Conversely, Cárdenas *et al*. (2010) noted that microrhabds are frequently centrotylote and that the tylote swelling may represent the ancestral centre of the aster. We consider the asters and acanthoxea in Stelligeridae to be homologous, if we consider the acanthoxea in *Halicnemia caledoniensis* sp. nov., they occupy a similar position within the ectosome as the asters in *Paratimea* and *Stelligera*. Unfortunately, our molecular trees do not give any clues as to whether the aster or acanthoxea is the ancestral state in Stelligeridae.

In terms of morphology, *Acanthoclada* is quite different to other stelligerids, in addition to the unusual megascleres (rhabdostyles), it is the only member that has acanthose cladotoxas and birotules, however the 28S D3-D5 tree (Supporting Information, Fig. S3) strongly supports its status as a stelligerid.

Surface architecture: At least some species of *Halicnemia, Higginsia, Paratimea* and *Stelligera* share a strikingly similar surface architecture to typical raspailiid species, with large robust megascleres 2–3 mm long protruding from the surface surrounded by a bouquet

of thin spicules which are variously described as styles, anisoxea or oxea (Morrow *et al*., 2013 p. 443). We consider the possession of a raspailiid surface architecture to be an apomorphy for the clade containing Raspailiidae and Stelligeridae. Desqueyroux-Faúndez & van Soest (1997) described a new species *Halicnemia diazae* from the Galapagos that they reported as "having characters that are typical of both *Halicnemia* and *Higginsia* and between the halichondrid family Desmoxyidae and the poecilosclerid families Raspailiidae and Rhabderemiidae". They suggested that further revisions of these genera might result in the merging of Desmoxyidae and Raspailiidae. Although this ectosomal surface architecture appears to be confined to Raspailiidae and Stelligeridae and is strong morphological support for a close relationship between these two families it is not ubiquitous for all the taxa. This highlights the difficulties in defining higher taxonomic groups on the basis of one or few morphological characters.

Cells with inclusions: The presence of cells characterised by granules or vesicles appears to be constant in *Stelligera*, *Paratimea* and *Halicnemia* (Fig. 25A–C). Although not ubiquitous, these cells are also widespread in Raspailiidae and Axinellidae. These cells are also abundant in *Heteroxya beauforti* (Fig. 26D) which clusters within Axinellida. Topsent (1891) referred to these cells as 'spherulous cells,' and speculated that they could be responsible for the excretion of slime in *Halicnemia* (*Halicnemia*, *Paratimea*, *Stelligera* and *Acanthoclada* all produce copious amounts of slime on collection). As yet no indisputable function has been ascribed to these cells and the same term has been used to describe cells in different Demospongiae orders although it has not been demonstrated that the structure and function are equivalent. Thompson, Barrow & Faulkner (1983) demonstrated that in *Aplysina fistularis* (Pallas, 1766) the spherulous cells contain secondary metabolites that have an antibacterial function. They speculated that these secondary metabolites may prevent the growth of biofouling organisms, control bacterial communities or deter predators.

Oviparity: Stelligera, *Paratimea*, *Halicnemia* and *Acanthoclada* all appear to be oviparous, Fig. 25A–C shows the oocytes are often surrounded by cells with granular inclusions. The interaction between the oocytes and these cells is unknown. The observation that Stelligeridae is oviparous is further support for Axinellida since Raspailiidae and Axinellidae are oviparous (Alvarez & Hooper, 2002).

Using a combination of molecular phylogenies and careful re-examination of morphological characters we return to a classification system which is very similar to the earlier classifications of Topsent (1928) and Dendy (1922) who recognised the morphological similarities between *Halicnemia*, *Higginsia*, *Paratimea* and *Stelligera*. One important difference between our results and the classification of Topsent (1928) is the position of *Hemiasterella*. Topsent included *Hemiasterella*, *Halicnemia* (including *Paratimea*), *Higginsia* and *Vibulinus* (= *Stelligera*) in Astraxinellidae (see Table1), whereas in our molecular genetrees (Fig. 1; Supporting Information Figs S2–S5), Hemiasterellidae (represented by *Adreus* and *Axos*) groups with Tethyidae and Timeidae and not with Stelligeridae. In *Stelligera* and *Paratimea* the asters are smooth rayed whereas in *Hemiasterella, Adreus* and *Axos* the asters are often microspined and come in a variety of size classes. In *Hemiasterella typus* Carter, 1879 (type taxon) the megascleres are exclusively stylote whereas *Hemiasterella s.l.* contains a diverse group of species in which the megascleres can be stylote, oxeote or a combination of both. In order to resolve the higher taxonomic placing of *Hemiasterella* and hence Hemiasterellidae comparable DNA sequences from *Hemiasterella* species and in particular from *H. typus* are needed.

The surprising discovery that *D. pelagiae* clustered close to Tedaniidae (Poecilosclerida) and not with *Halicnemia* and *Higginsia* in our molecular trees (Fig. 1; Supporting Information Figs S2–S5) caused us to return to the specimens of *D. pelagiae* and indeed to the type material of *D. lunata* and re-examine the morphology more carefully. Using SEM we found morphological characters such as the presence of onychaetes and acanthostyles, that unite *Desmoxya pelagiae* with *Tedania* and gained an insight into the homoplasious nature of the acanthoxea that wrongly led to it being classified with *Halicnemia* and *Higginsia*. Whilst we

are lacking any molecular data from the type species *D. lunata,* on the basis of the structure of the skeleton and the presence of onychaetes, we resurrect Desmoxyidae for *Desmoxya* only, and transfer it to Poecilosclerida.

Hooper & Lévi (1993), in their description of their new species *Higginsia anfractuosa* from a lagoon in New Caledonia, stated that morphologically it was most similar to *Higginsia lunata* Carter, 1885 in growth form, papillose surface features and skeletal architecture and that both species are atypical of other *Higginsia*. Hooper (2002a) speculated that *Desmoxya* may need to be resurrected for *Higginsia*-like species that lack any evidence of axial compression (citing *H. lunata* and *H. anfractuosa*), having instead a halichondroid, meandering reticulation of choanosomal tracts. Our 18S and 28S trees (Supporting Information, Figs S2, S3) contain a specimen from Tanzania, Hymedesmiidae OCDN 3725J, (previously identified as *H. anfractuosa*) in Redmond *et al.* (2013); Morrow *et al*. (2013) and Thacker *et al*. (2013). Our CO1 tree (Supporting Information Fig. S3) has sequence data from the type specimen of *H. anfractuosa* (QM G300723) which clusters with Hymedesmiidae G 304373 (identified on GenBank as *Crella* sp.), at the base of a clade containing species of *Hemimycale* and not with *Higginsia* species or indeed *Desmoxya pelagiae*. We have erected the genus *Hooperia* gen nov. for *Higginsia anfractuosa* comb. nov.; we consider it closely related to *Hemimycale* but easily distinguished from it by the presence of rugose oxea. Although we have no supporting molecular data, we transfer *Higginsia fragilis* Lévi, 1961 to *Spanioplon* Topsent, 1890 (Hymedesmiidae) as morphologically it is much closer to *Spanioplon* than to *Higginsia*.

Erpenbeck, Breeuwer & van Soest (2005) using partial 28S rRNA were the first authors to show *Didiscus* spp. clustering with *Myrmekioderma granulatum* within Raspailiidae. Erpenbeck *et al.* (2012a) using CO1 barcoding sequences again showed *Didiscus* grouping within Raspailiidae, however their *Myrmekioderma granulatum* sequences clustered with Axinellidae and not with *Didiscus*. Morphologically *Myrmekioderma* and *Didiscus* share similar skeletal architectures, and a distinctive surface crust with sculptured grooves and subdermal drainage canals. The results of Redmond *et al.* (2013), using complete 18S rRNA, indicate that *Myrmekioderma* is polyphyletic with *M. granulatum* clustering within Raspailiidae and *M. rea* with Axinellidae. From the molecular data it is clear that *Didiscus* belongs in Raspailiidae however the potential polyphyly of *Myrmekioderma* needs further investigation.

The remaining genera that are classified in Heteroxyidae by van Soest *et al.* (2018) and for which we have no molecular data (*Alloscleria* Topsent, 1927; *Alveospongia* Santos, Pinheiro, Hajdu & Van Soest, 2016; *Julavis* Laubenfels, 1936; *Microxistyla* Topsent, 1928; *Negombo* Dendy, 1905 and *Parahigginsia* Dendy, 1924) are retained in Heteroxyidae.

Summary of Taxonomic Changes: Desmoxyidae is resurrected from synonymy with Heteroxyidae and transferred to Poecilosclerida; a new genus, *Hooperia* Morrow gen. nov. is established for *Higginsia anfractuosa*; *Higginsia durissima* is returned to *Bubaris*; *Higginsia fragilis* is transferred to *Spanioplon*; *Hemiasterella camelus* is transferred to *Paratimea*; *Halicnemia gallica* is resurrected from synonymy with *Halicnemia patera*; *Raspailia* (*Parasyringella*) *australiensis* and *Ceratopsion axiferum* are transferred to *Adreus*.

CONCLUSIONS

Boury-Esnault & Solé-Cava (2004) and Cárdenas *et al*. (2012) pointed out that when morphological and molecular trees are not congruent we need to sample additional genes but above all to reassess very carefully the morphological characters without any preconceived ideas. Then the molecular trees can be reinterpreted in light of reconsidered morphological data. Using this approach has helped us gain a better understanding of the phylogenetic relationships within Stelligeridae and Heteroscleromorpha in general. Our study shows strong morphological and molecular support for the family Stelligeridae *sensu* Morrow *et al*. (2012). In Figure 1, which represents our largest dataset in terms of number of nucleotides but with fewer taxa, *Paratimea* is closest to *Stelligera* and *Halicnemia* and

Higginsia form a sister relationship. The inclusion of more species and more molecular markers, especially from the type species are needed to determine whether *Halicnemia* and *Higginsia* are monophyletic. Our molecular trees do not support the monophyly of *Paratimea*, the 28S tree (Supporting Information Fig. S5) shows several of our new species of *Paratimea* (*P. hoffmannae* sp. nov., *P. lalori* sp. nov. and *P. rosacea* sp. nov.) clustering separately to other *Paratimea* spp. These new species are all from deep-water habitats, share a massive growth form, have oxeas as their principal spicules and have relatively large asters, often with unequal lengthed rays.

It is clear that within Stelligeridae microscleres can be either asters or microxeas (including acanthose cladotoxas and birotules) and the argument for the separation of taxa into separate families and orders based on whether they possess asters or microxeas can no longer be supported. This illustrates well the difficulties for sponge systematics, deciding which characters are diagnostic and which are variable or homoplasious. The molecular trees generated in this study have helped us to distinguish characters that are homologous e.g. a raspaillid surface architecture and those that are homoplasious e.g. the occurrence of acanthoxeas.

The inclusion in this study of species that are new to science e.g. *Paratimea dentata* sp.nov. and *Halicnemia caledoniensis* sp. nov., has enhanced our understanding of evolutionary relationships within Heteroscleromorpha.

SYSTEMATICS

The classification used here follows Morrow & Cárdenas, 2015

CLASS DEMOSPONGIAE SOLLAS, 1885

SUBCLASS HETEROSCLEROMORPHA CÁRDENAS, PÉREZ & BOURY-ESNAULT, 2012

ORDER AXINELLIDA LEVI, 1953

STELLIGERIDAE LENDENFELD, 1898

Emended Diagnosis: Axinellida in which the choanaosomal skeleton can be composed of styles, tylostyles, oxeas or rhabdostyles. Ectosomal region often with protruding megascleres surrounded by bouquets of smaller, slender accessory oxeas or styles. Ectosomal crust heavily reinforced with microscleres. Accessory oxeas often with centrotylote swellings, occasionally with fissurate terminations. Microscleres can be smooth-rayed euasters; spined microxeas often bent or centrangulate or acanthose cladotoxas and birotules. Where known, reproduction is oviparous. Presence of cells with granular inclusions in most species. All produce slime on collection.

Included genera: *Acanthoclada* Bergquist, 1970 (p22, pl.5b, pl.10a,f, pl.16a,b); *Paratimea* Hallmann, 1917 (p675); *Halicnemia* Bowerbank, 1864 (p184); *Higginsia* Higgin, 1877 (p291); *Stelligera* Gray, 1867 (p545); **Plenaster* Lim & Wiklund, 2017 (although see remarks below).

Remarks: A detailed description of the morphological characters that unite Stelligeridae is given in the discussion. The molecular trees of Lim *et al.* (2017) clearly show that *Plenaster* is not a stelligerid, the taxonomic affinities of this genus will be part of a future manuscript.

STELLIGERA GRAY, 1867

Diagnosis (modified from Hooper, 2002b): Stelligeridae with erect branching growth form; choanosome composed of axial region of styles and oxea and extra-axial region of long projecting styles perpendicular to axis and styles/oxea forming an irregular reticulation; ectosomal skeleton composed of bouquets of slender styles surrounding protruding extra-axial styles; smooth-rayed euaster microscleres form an ectosomal crust. Produces copious amounts of slime on collection.

Type species: *Stelligera stuposa* (Ellis & Solander, 1786)

Included species: WPD (van Soest *et al*., 2018) lists five valid species, however only *S. stuposa* and *S. rigida* were examined in this study.

Remarks: Voultsiadou-Koukoura & van Soest (1991) suggested that *Stelligera mutila* (Topsent, 1928) is a probable synonym of *Hemiasterella elongata* Topsent, 1928. It is possible that re-examination of species assigned to *Stelligera* in WPD (van Soest *et al*., 2018) will result in some species being transferred to other families and genera therefore we have not included a list of assigned species.

STELLIGERA STUPOSA (ELLIS & SOLANDER, 1786)

Spongia stuposa Ellis & Solander, 1786

Dictyocylindrus stuposus (Ellis & Solander, 1786)

Raspailia stelligera Schmidt, 1862

Stelligera stelligera (Schmidt, 1862)

Stelligera stuposa f. *stuposa* (Ellis & Solander, 1786)

Vibulinus stuposus (Ellis & Solander, 1786)

Material examined: BELUM Mc814, N. of Chapel Island, Strangford Lough, Northern Ireland, 54°23.60'N, 5°35.94'W, 25 m, 15.06.1982, coll. B. Picton, det. B. Picton.

BELUM Mc4330, Sligneach Mor, Loch Sunart, Scotland, 56°40.272'N, 5°58.731'W, 25 m, 30.06.2008, coll. C. Goodwin, det. C. Goodwin.

DNA SEQUENCES:

DNA sequences: From BELUM Mc4330 we sequenced partial CO1 (Genbank accession no. HQ379421) & 28S (D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379220; HQ379286 & HQ379354 respectively). The GenBank 18S sequence (KC902232) is from this specimen.

REMARKS

Remarks: Voultsiadou-Koukoura & van Soest (1991) suggested that *Stelligera stelligera* (Schmidt, 1862) (type locality Adriatic Sea) might be the same species as *Stelligera stuposa* (Ellis & Solander, 1786) (type locality British Isles). Hooper (2002b) re-examined the type material and synonymised *S. stelligera* with *S. stuposa,* making *S. stuposa* the type species of *Stelligera.*

STELLIGERA RIGIDA (MONTAGU, 1818)

Spongia rigida Montagu, 1818

Stelligera stuposa f. *rigida* (Montagu, 1818)

Vibulinus rigidus (Montagu, 1818)

Material examined: BELUM Mc975, Blockhouse Island, Carlingford Lough, Northern Ireland, 54°01.45'N, 6°04.70'W, 15 m, 21.07.1983, coll. B. Picton, det. B. Picton.

BELUM Mc4357, Wreck of the Rondo, Sound of Mull, Scotland, 56°32.33'N, 5°54.81'W, 32 m, 02.07.2008, coll. B. Picton, det. B. Picton.

DNA sequences: From BELUM Mc4357 we sequenced partial CO1 (Genbank accession no. HQ379420) & 28S (D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379219; HQ379285 & HQ379353 respectively). The GenBank 18S sequence (KC902164) is from this specimen.

Remarks: Hooper (2002b) speculated that *S. rigida* (Montagu, 1818) from the British Isles and *S. nux* Lendenfeld, 1898 from the Mediterranean might be synonyms of *S. stuposa* although the types were not examined. From extensive collecting of *S. stuposa* and *S. rigida*

from areas close or adjacent to the type locality we can verify that differences in external morphology are consistent with small differences in aster morphology. There were also small differences between the two taxa using complete 18S rRNA (Supporting Information, Fig. S2) and partial fragments of 28S rRNA (Supporting Information, Figs S3, S5) however the CO1 Folmer fragment (Supporting Information, Fig. S4) was identical for the two species.

PARATIMEA HALLMANN, 1917

Emended diagnosis: Stelligeridae with encrusting or massive growth form. Choanosomal skeleton lax, encrusting species have hymedesmioid skeletal architecture consisting of erect tylostyles and paratangential tracts of centrotylote or polytylote ectosomal oxea. Massive species have oxeote megascleres, arranged without order. Oxea are also scattered throughout the choanosome in dragmata. Microscleres are smooth rayed euasters, most abundant in surface layer. Produce slime on collection.

Type species: *Paratimea constellata* (Topsent, 1893)

Included species: *P. alijosensis* Austin, 1996, *P. arbuscula* (Topsent, 1928), *P. aurantiaca* sp. nov., *P. azorica (*Topsent, 1904), *P. camelus* (van Soest, 2017) comb. nov., *P. constellata* (Topsent, 1893), *P. dentata* sp. nov., *P. duplex* (Topsent, 1927), *P. galaxa* Laubenfels, 1936, *P. globastrella* van Soest, Kaiser & van Syoc, 2011, *P. hoffmannae* sp. nov., *P. lalori* sp. nov., *P. loennbergi* (Alander, 1942), *P. loricata* (Sarà, 1958), *P. mosambicensis* sp. nov., *P. oxeata* Pulitzer-Finali, 1978, *P. pierantonii* (Sarà, 1958), *P. rosacea* sp. nov.

PARATIMEA CONSTELLATA (TOPSENT, 1893)

(Fig. 2A–D)

Bubaris constellata Topsent, 1893 (p.33–34)

Halicnemia constellata (Topsent, 1893)

Material examined: **Holotype**: MNHN-DT-2361 Roscoff (only a microscope slide of the type specimen remains).

Description: *Megascleres* long, slender tylostyles 2500–3000 x 13–14 µm (these measurements are taken from the original description. In the slide from the holotype that we examined only one tylostyle was intact, it measured 2 mm), sub-trilobate head 17 µm (Fig. 2A).

Accessory oxeas centrotylote oxeas 379–*670*– 900 x 8–10 µm (Fig. 2B).

Microscleres: smooth rayed euasters 14–*30*–46 µm (Fig. 2C).

Remarks: This species is very similar to *P. loennbergi* with the exception that in *P. loennbergi* there are two categories of tylostyles: long, slender tylostyles with a globular to sub-trilobate head (>2 mm x 32 µm) similar to those of *P. constellata* and, in addition, distinctive short tylostyles that are stout and club-like with a pear-shaped, annulated head. These are only found in the base of the sponge where it is in contact with the substratum. It is possible that these two taxa are conspecific but that the microscope preparations made from *P. constellata* did not include the basal layer with the distinctive short tylostyles. Unfortunately, the type material of *P. constellata* is missing and it is not possible to ascertain whether the basal layer, if present, contained the short, club-like tylostyles. Therefore, we retain *P. constellata* and *P. loennbergi* as two separate species until fresh material from the type locality can eventually be examined and sequenced.

We have examined specimens from the Ulster Museum collection identified as *P. constellata*. The spiculation and skeletal architecture is identical to that of *P. loennbergi* and the material has been re-identified accordingly. Records of *P. constellata* from the coasts of Britain & Ireland (Picton, Morrow & van Soest, 2007) should be attributed to *Paratimea loennbergi*. The GenBank sequences for *Paratimea constellata* listed in Morrow *et al*. (2012)

(HQ379218; HQ379397; HQ379284; HQ379352; HQ379419) have been changed to *P. loennbergi*.

PARATIMEA CAMELUS (VAN SOEST, 2017) **COMB. NOV.**

Hemiasterella camelus van Soest, 2017: pp 174–176, figs 109 a–e, original description.

Material examined: **Holotype** RMNH Por. 9924, Suriname, 'Luymes O.C.P.S.II' Guyana Shelf Expedition, station M97, 7°18.498'N, 54°.10.002'W, depth 130 m, bottom coarse sand, 16 April 1969.

DNA sequences: We sequenced the CO1 Folmer fragment from the holotype, GenBank accession XXXX.

Remarks: The CO1 genetree (Supporting Information, Fig. S4) shows *P. camelus* clustering closely with *P. oxeata*, inside Stelligeridae, the pairwise identity matrix (Supporting Information, Fig. S6) shows 99.85% similarity between the two species. A re-examination of the holotype found a ‚raspailiid surface architecture', typical of many stelligerids. The smaller oxyspherasters reported by van Soest (2017; fig. 109e) are considered to be contaminants as we did not find any in our spicule preparations or tissue section. These asters are very different to those found in other Stelligeridae and are likely to be contamination from a tethyid sponge.

PARATIMEA DUPLEX (TOPSENT, 1927)

(Fig. 3 A–D, reproduced from Topsent, 1928 pl. 6, fig. 21)

Material examined: Type material: **Holotype** MNHN-DT-1094, dried specimen, station 1116, 50 miles off the coast of Mogador, Morocco, 31°43.50'N, 10°46.75' W, 2165 m, 11.07.1901, habitat: pink mud made up of Foraminifera (slide only).

Paratypes: MNHN-DT-1093, station 1116 (see above), specimen in alcohol; MNHN-DT-1191, station 1242, Banc de Seine, 240m, 10.09.1901, habitat: broken shell and gravel (slide only).

ZMA POR19447 (slide only), Porcupine Bank, west of Ireland, 55°26.64'N, 16°04.5'W, 06.09.2004, 773 m, Box Core, R.V. *Pelagia*, coll. R.W.M. van Soest.

Description: Paratype *Halicnemia duplex* MNHN-DT-1093, station 1116, 31°43.5'N, 10°46.75'W, 50 miles from Mogador, Morocco, 11.07.1901, 2165 m, Prince Albert of Monaco cruises.

Outer morphology Topsent (1928) describes two specimens from station 1116, as growing on the deep-water coral *Desmophyllum pertusum* (L.), the type is cushion shaped, 3 mm thick with a conulose surface.

Colour Topsent (1928) describes the colour as greyish in alcohol.

Choanosomal skeleton principal spicules have a disordered arrangement, spongin lacking, making the skeleton lax and friable. Asters abundant throughout skeleton.

Ectosomal skeleton tufts of centrotylote oxeas encircle large oxea which occupy the axis of the conule, asters very abundant (Fig. 3D).

Megascleres are centrotylote oxeas 2–2.6 mm x 20–40 mm and styles to subtylostyles 1.6–1.8 mm x 25–35 µm (Fig. 3A–C).

Accessory oxeas weakly centrotylote oxeas, 360–770 µm x 7–9 µm.

Microscleres are oxyasters without centrum, smooth rayed, rays of inequal lengths. Asters 50–100 µm in diameter with 10–15 rays (Fig. 3A & C).

Remarks: Topsent notes that *P. duplex* is unusual in having a mix of oxeas, styles and tylostyles as the principal megascleres. The oxea are much more common and also larger than the styles or tylostyles.

*PARATIMEA LOENNBERGI (*ALANDER, 1942)

(Fig. 2E & F)

Halicnemia loennbergi Alander, 1942 (p. 68)

Material examined: **Holotype**: SMNH 1229 (wet specimen), Väderöfjord, Sweden, 60 m, coll. H. Alander. New spicule preparations were made.

BELUM Mc5290, Aberreidy Quarry, Pembrokshire, Wales, 51°56.273'N, 5°12.512'W, 13 m, 30.07.2009, coll. B. Picton. det. C. Morrow.

BELUM Mc4323, Loch Caolisport, Firth of Lorn, Scotland, 55°51.159'N, 5°43.142'W, 34 m, 24.06.2008, coll. B. Picton, det. C. Morrow.

BELUM Mc7417, Ramsö, Kosterfjord, Sweden, 58°49.710'N, 11°04.992'E, 25 m, 07.09.2010, coll. B. Picton, det. C. Morrow.

ZMA POR20296, Mingulay Reef, Outer Hebrides, Scotland, 56°49.404'N, 7°23.862'W, 139 m, 11.07.2006, coll. R.W.M. van Soest, det. C. Morrow.

Description: Outer morphology forms a thin, hispid crust, usually covered in silt trapped by projecting spicules.

Colour pale yellow.

Choanosomal skeleton comprised of long tylostyles with their heads embedded in a basal layer of spongin, the shafts project through the surface. Smaller, club-like tylostyles also present in the basal layer. Cells with granular inclusions abundant throughout choanosome.

Ectosomal skeleton slender accessory oxea form bouquets around the projecting tylostyles. Asters form a dense layer at surface.

Megascleres Holotype SMNH 1229 - large tylostyles (Fig. 2E), slightly bent, 1350–3000 x 10–*12.6*–15 µm (n = 4); head: 15.5–*20.4*–27 µm (n = 5). Often the tylostyles have a second or third tylote swelling near the base (Fig. 2E & F). Small tylostyles not found in the spicule preparation we made but mentioned and illustrated in Alander (1942). He states that they are stout and club-like with a pear-shaped, annulated head and measure 180–225 x 12–15 µm. The diameter of the head is approximately 25 µm. Figure 6F is a photomicrograph of the large and small small tylostyles from a specimen of *P. loennbergi* in the Ulster Museum Porifera collection (BELUM Mc5290).

Accessory oxeas (SMNH 1229) slightly bent, 530–*712*–930 x 5–*5.3*–6 µm (n = 7).

Microscleres (SMNH 1229) smooth oxyasters 22–*28*–36 µm (Fig. 2F).

Reproduction the presence of oocytes has been observed in several specimens collected at depths between 20–30 m during June to August.

Slime produces copious amounts of slime on collection.

DNA sequences: From (BELUM Mc4323) we sequenced the CO1 folmer fragment (Genbank accession no. HQ379419) & 28S (D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379218; HQ379284 & HQ379352 respectively). The GenBank 18S sequence (KC902409) is also from this specimen.

Remarks: This species may be synonymous with *P. constellata* (see notes on *P. constellata* above). The main difference is the presence of short, stout tylostyles in *P. loennbergi,* however these are relatively scarce and might have been missed by Topsent. In *P. loennbergi* the larger tylostyles frequently have a second or third tylote swelling near the base (Fig. 2E, F). This has not been observed in *P. constellata* but does occur in *Halicnemia patera*.

PARATIMEA OXEATA PULITZER-FINALI, 1978

(Fig. 4A–E)

Material examined: S153, Trémies Cave, Cassis, France, Mediterranean Sea, 43°11.8573'N, 5°30.6022'E, 04.07.1981, (Jean Vacelet's Collection, Marine Station of Endoume).

Description: Outer morphology massive lobose, surface conulose, oscules arranged on top of raised humps (Fig. 4A).

Colour pale yellow-cream.

Choanosomal skeleton composed of irregularly arranged large oxeas and asters.

Ectosomal skeleton typical 'raspailiid surface architecture' whereby bundles of smaller oxeas surround large oxea, these in turn support a thick layer of asters.

Megascleres large oxeas match the description given by Pulitzer-Finali (1978), they are curved, sometimes double bent or flexuous, they measure 1–1.5 mm x 14–24 µm (Fig. 4B–D).

Accessory oxeas are curved or abruptly bent, often with a centrotylote swelling, they measure 250–650 x 3.7 µm.

Microscleres are oxyasters without a centrum, with 4–12 tapering rays, rays have slight annulations. Asters are typically 20–40 µm however where the rays are reduced in number they are generally much larger, up to 60 µm (Fig. 4E).

Reproduction the presence of oocytes were noted in one specimen collected from Cap Morgiou, Mediterranean Sea, 25.05.1992 (J. Vacelet pers. comm.).

Slime produces copious amounts of slime on collection.

Habitat: Submarine caves, 15–20 m.

DNA sequences: We sequenced CO1 Folmer fragment from this specimen, GenBank accession XXXX.

PARATIMEA AURANTIACA MORROW **SP. NOV.**

(Fig. 5A–F)

Material examined: Holotype BELUM Mc5226, Abercastle, North Pembrokeshire, Wales, 52°00.069'N, 5°05.655'W, 27.6 m, 29.07.2009, coll. B. Picton.

Paratype BELUM Mc2937, Duncan's Bo, Rathlin Island, Northern Ireland, UK, 55°18.7183'N, 6°15.1238'W, 32 m, 06.09.2005, coll. B. Picton.

Description: *Outer morphology* thinly encrusting with a hispid surface (Fig. 5E).

Colour: Bright yellow-orange.

Choanosomal skeleton: Hymedesmoid arrangement consisiting of erect, long tylostyles and ascending bundles of centrotylote oxeas scattered throughout the skeleton. Cells with granular inclusions are abundant (Fig. 5F).

Ectosomal skeleton bundles of centrotylote oxeas penetrate the surface giving it its hispid appearance, oxyasters are common in the surface layer (Fig. 5F).

Megascleres are tylostyles-subtylostyles, 1230–*1830*–2400 µm x 8–11 µm, the tylote base is 14–16 µm (Fig. 5A).

Accessory oxeas centrotylote oxeas 560–*700*–850 µm x 1–*5*–8 µm. The ends of the oxea taper to a fine point (Fig. 5B–C).

Microscleres are smooth oxyasters, 25–*30*–36 µm in diameter, the diameter of the centrum is approximately 10 µm. In the SEM of the aster (Fig. 5D), 12 conical rays are visible.

Slime produces copious amounts of slime on collection.

Habitat: Vertical to overhanging sublittoral rocky reefs with strong tidal currents.

Etymology: *Aurantiaca* L. = orange-coloured, refers to the yellow-orange colour of this species.

DNA sequences: From the holotype we sequenced 28S D1–D2, D3–D5, D6–D8 regions, GenBank accession nos. HQ379217; HQ379283 & HQ379351 respectively. The 18S sequence KC902401 on GenBank is from this specimen. From the paratype (BELUM Mc2937) we sequenced 28S D1–D2 GenBank accession no. KF017191.

PARATIMEA DENTATA MORROW **SP. NOV.**

(Fig. 6 A–F)

Material examined: **Holotype** BELUM Mc6884, Les Dents, Channel Isles, 49°25.5270'N, 2°23.7130'W, 26 m, 28.06.2010, coll. B. Picton.

Description: *Outer morphology* thinly encrusting with a hispid surface (Fig. 6E).

Colour bright yellow, Methuen colour code 3A4 (Kornerup & Wanscher, 1978) (Fig. 6E).

Choanosomal skeleton hymedesmoid arrangement consisiting of erect, long tylostyles and ascending bundles of centrotylote oxeas scattered throughout the skeleton (Fig. 6F). Cells with granular inclusions are abundant throughout the choanosome.

Ectosomal skeleton bundles of centrotylote oxeas penetrate the surface giving it its hispid appearance, oxyasters are common in the surface layer.

Megascleres tylostyles-subtylostyles, 1660–*1890*–2100 µm x 8–10 µm (n=4), the base is 13–16 µm (Fig. 6A).

Accessory oxeas centrotylote oxea 370–*412*–460 µm x 2–4–5 µm, centrotylote swelling 3–5.7–7.6 µm (Fig. 6B). Both ends of the oxea are dentate with approximately 6 'teeth' (Fig. 6C).

Microscleres are smooth oxyasters, 15–*17*–19 µm in diameter, the diameter of the centrum is approximately 6 µm. In the SEM of the aster (Fig. 6D), 23 conical rays are visible.

Slime Produces slime on collection.

Habitat: Vertical to overhanging sublittoral rocky reef with strong tidal currents.

Etymology: From the Latin for toothed *dentate*, refers to the 'toothed' ends of the oxea.

DNA sequences: The 18S sequence on GenBank, accession no. KC902076 if from the holotype.

Remarks: *Paratimea dentata* can easily be distinguished from other species of *Paratimea* by the presence of robust, centrotylote oxea in which both ends are flanged, with approximately 6 'teeth'. It shares this character with *Halicnemia verticillata*.

PARATIMEA HOFFMANNAE MORROW & CÁRDENAS **SP. NOV.**

(Fig. 7A–G)

Material examined: **Holotype** ZMBN 125735 and BELUM Mc 2018.2 (small piece of the holotype), Røst reef, Northern Norway, 67°31' N, 9°28' E, *Polarstern* ARK-XXII/1a expedition, 10.06.2007, 328 m, manned-submersible *JAGO*, station 17-1 (dive 994), coll. Paco Cárdenas and Friederike Hoffmann.

Paratypes: ZMBN 125736, Røst reef, Northern Norway, 67°30' N, 9°25' E, *Polarstern* ARK-XXII/1a expedition, 09.06.2007, 282 m, manned-submersible *JAGO*, station 14-4 (dive 991), coll. Friederike Hoffmann; BELUM Mc 2018.3, west of Ireland, 54°03.7806'N, 12°24.987'W,

Celtic Explorer Biodiscovery Cruise, 07.06.2013, 1500 m, ROV *Holland I*, coll. Christine Morrow.

Description: *Outer morphology* massive, subspherical, holotype is approximately 7 cm in diameter. Paratype ZMBN 125736 (Fig. 7A) was approximately 10 x 15 cm, however only a fragment of this specimen was collected by the manned-submersible. The surface is covered in large conules, 1–4 mm in height (Fig. 7F).

Colour creamish-white.

Choanosomal skeleton ascending bundles of large oxeas with scattered oxea and abundant asters throughout choanosome (Fig. 7B).

Ectosomal skeleton tufts of smaller oxeas are present in the surface layer. Surface conules are dense with asters (Fig. 7F & G).

Megascleres large, curved oxeas (occasionally centrotylote), gently tapering at both ends to a fine point 2056–*2187*–2250 x 25–*26*–28 µm (Fig. 7C).

Accessory oxeas are rare, bent (usually off-centre) and occasionally centrotyle (Fig. 7D). They are scattered in loose bundles close to the surface but do not appear to form surface bouquets around the principal oxeas. They measure 353–*446*–520 x 3–*4*–5 µm.

Microscleres large, asymmetic asters 42–*60*–81 µm in diameter, centrum 9–11 µm. There are 7–18 smooth, tapering rays (Fig. 7E).

Slime produces copious amounts of slime on collection.

Etymology: This species is named in honour of the sponge biologist and microbiologist Friederike Hoffmann, for her role in making the investigation of the sponge fauna, a vital part of the *Polarstern* ARK-XXII expedition in 2007.

DNA sequences: We sequenced 28S D1–D2 from the holotype and from BELUM Mc 2018.3, GenBank accessions XXXX and XXXX respectively and CO1 Folmer fragment GenBank accessions KC869429 and XXXX respectively.

Remarks: This species is similar in spicule morphology to *P. duplex* and *P. lalori* (see remarks above regarding *P. lalori* sp. nov.), but can be distinguished from it by the absence of tylostyles, stylote spicules are rare, the presence of large, surface conules and by it's massive growth form. The ectosomal skeleton consists of ascending bundles of large oxea and scattered oxea whereas in *P. duplex* and *P. lalori* sp. nov. the oxea are without order. In *P. hoffmannae* sp. nov. the accessory oxea do not appear to form bouquets around the larger oxea.

PARATIMEA LALORI MORROW **SP. NOV.**

(Fig. 8 A–G)

Material examined: **Holotype** BELUM Mc7732, west of Ireland, 54°03.7806'N, 12°24.987'W, *Celtic Explorer* Biodiscovery Cruise, 25.05.2010, 1500 m, ROV *Holland I*, coll. Christine Morrow.

Description: Outer morphology the type specimen was growing on dead *Desmophyllum pertusum*, it is spherical in shape, approximately 2.5 cm in diameter with oscules arranged in a cluster on the upper surface of the sponge. The surface is slightly uneven due to the presence of minute conules (Fig. 8A).

Choanosomal skeleton very little spongin present giving a lax, friable texture, megascleres distributed without order, asters distributed throughout choanosome.

Ectosomal skeleton ectosomal oxeas form bouquets around central large oxea but do do not protrude much beyong the surface. (Fig. 8B). Asters from a dense ectosomal layer.

Colour pale yellow to cream in life and in alcohol.

Megascleres predominantly large, gently bent oxeas (Fig. 8C), ocassional oxeas have an off-centred tylote swelling (Fig. 8D, E). Stylote spicules are rare (Fig. 8F), occasional styles have a small inflation towards the base. Oxeas measure 1439–*1760*–2020 x 19–*30*–35 µm.

Accessory oxeas are cigar-shaped with a conspicuous centrotylote swelling, they are usually straight or only slightly bent and measure 278–*335*–422 x 9–*10.5*–12 µm (Fig. 8F).

Microscleres are oxyasters without centrum, smooth rayed, rays of inequal lengths. Asters 29–*60*–71 µm in diameter with 12–16 rays (Fig. 8G).

Slime produces copious amounts of slime on collection.

Habitat: On a branch of dead *Desmophyllum pertusum*, 1500 m depth.

DNA sequences: From the holotype we sequenced CO1 Folmer fragment GenBank accession XXXX

Etymology: This species is named in recognition of the kind assistance of Pierce Lalor of the Centre for Microscopy and Imaging, Department of Anatomy, NUIG.

Remarks: *Paratimea lalori* sp. nov. is similar to *P. duplex* and *P. hoffmannae* sp. nov., all three species have large oxeas as their principal spicules and large asters with rays of inequal lengths. Externally the three species are quite different, Topsent (1928) describes *P. duplex* as a 3 mm thick, greyish coloured cushion, *P. hoffmannae* sp. nov. is cream-white in colour, massive-subspherical and covered in large conules whilst *P. lalori* sp. nov. is pale yellow-cream in colour, spherical in shape with only very small conules. In *P. duplex* the accessory oxea are longer and more slender (360–770 µm x 7–9 µm) than those in *P. lalori* sp. nov. (278–*335*–422 x 9–*10.5*–12 µm), and form obvious bouquets surrounding large oxea. In *P. lalori* sp. nov. the accessory oxea are also arranged in bouquets around the principal oxea however this is not so obvious as in *P. duplex*, since they only project a very short distance beyond the ectosomal surface (Fig. 8B). The accessory oxea in *P. hoffmannae* sp. nov. are long and much more slender than those of *P. lalori* sp. nov., they are usually bent in the middle region and sometimes there is a centrotylote swelling. They measure 353–*446*–520 x 3–*4*–5 µm. *P. lalori* sp. nov. and *P. hoffmannae* sp. nov. also differ from *P. duplex* in the make up of their principal spicules, in addition to large oxea, *P. duplex* also has tylostyles and styles whereas *P. lalori* sp. nov. and *P. hoffmannae* sp. nov. have only occasional stylote spicules.

PARATIMEA MOSAMBICENSIS MORROW & CÁRDENAS **SP. NOV.**

(Fig. 9A–F)

Material examined: **Holotype** MNHN-IP-20XX-XXXX, PAMELA-MOZ-01 expedition, Iles Glorieuses, Mozambique Channel, 11°22.75604’ S, 47°16.40977’ E, 28.09.2014, station DW01, Warén Dredge, field# sponge10, 753 m (start of dredge), coll. Karine Olu.

Paratypes MNHN, five other specimens were collected at the same DW01 station.

Description: *Outer morphology* thick cushion, approximately 12 x 10 cm across by 5 cm thick. The oscules are arranged on the upper surface. The surface is slightly hispid (Fig. 9C).

Colour greyish-beige.

Choanosomal skeleton a disordered arrangement of large oxea and large asters (Fig. 9D).

Ectosomal skeleton densely packed with large asters, bouquets of accessory oxea surrounding principal oxea although present (Fig. 9E), are not ovious.

Megascleres principal spicules are large, gently curved oxeas, 1583–*2000*–2322 x 31–*36*–44 µm (Fig. 9A).

Accessory oxeas are rare and are very variable in length, sometimes they have a centrotylote swelling although this is also variable. They are relatively long and thin measuring 666–*900*–1200 x 10–*12*–15 µm (Fig. 9E).

Microscleres are huge asters with markedly inequal lengthed rays. The rays are very long relative the centrum, the asters are 73–*84*–100 µm across and the centrums measure 10–*14*–18 µm. The number of rays is very variable, in the smaller asters the rays are more numerous (Fig. 9B).

Etymology: *Mosambicensis* L. = from Mozambique, this specimen was collected from the Mozambique Channel.

DNA sequences: From the holotype we sequenced 28S D3–D5 GenBank accession XXXX and CO1 Folmer fragment GenBank accession XXXX.

Remarks: This species can be distinguished from other cushion shaped to massive *Paratimea* species by the absence of surface conules; its distinctive asters which at 73–100 µm, are amongst the largest found so far in *Paratimea* (see Supporting Information, Figure S7 for comparison of asters in *Paratimea*). The texture is much firmer relative to other *Paratimea* species which tend to have a very lax skeleton.

There is only 1 base pair difference of the CO1 Folmer fragment between the holotype of *P. mosambicensis* sp. nov. and that of *P. hoffmannae* sp. nov., however the external morphology, colour and aster size and morphology are all different.

PARATIMEA ROSACEA MORROW & CÁRDENAS **SP. NOV.**

(Fig. 10 A–F)

Material examined: **Holotype** MNHN-IP-2015-1236, Iles Glorieuses, Mozambique Channel, 11°29'S, 47°29'E, BIOMAGLO expedition, 25.01.2017, station DW4812, 390–417 m, field# PMG158, coll. Cécile Debitus.

DESCRIPTION

Description: *Outer morphology* globular, approximately 4 cm in diameter with oscules and radiating oscular channels on the upper surface (Fig. 10A).

Colour rose-pink.

Choanosomal skeleton large oxeas scattered without order, brown pigment bodies are common throughout choanosome. Asters are abundant. The skeleton is lax, with only small quantities of spongin.

Ectosomal skeleton composed of a dense layer of asters, accessory oxeas surround principal oxea however which penetrate the surface however this is not very obvious (Fig. 10B).

Megascleres are large, robust, gently curving oxea, lacking a centrotylote swelling (Fig. 10C). They measure 2500–*2670*–3035 x 46–*55*–60 µm at their widest section. Tylostyle spicules are rare, they are much smaller than the oxeas (600–800 µm) (Fig. 10D, E).

Accessory oxeas this category of spicule is rare. They are thin, parallel sided oxea, they can be straight or gently curving and lack a centrotylote swelling. They measure 503–*600*–790 x 5–*8*–10 µm (Fig. 10B).

Microscleres unlike other deep-water, massive *Paratimea* spp., the asters in *P. rosacea* sp. nov. have relatively short rays of more or less equal lengths, more like those in encrusting *Paratimea* spp. (e.g. *P. constellata*, *P. loennbergi* etc.) and *Stelligera* spp. The asters are 30–*38*–47 µm in diameter with a centrum 13–*15*–17 µm (Fig. 10F).

Slime produces copious amounts of slime on collection.

Etymology: *Rosacea* L. = rose-coloured, refers to the pink colour of this species.

DNA sequences: From the holotype we sequenced 28S D1–D2 GenBank accession XXXX; 28S D3–D5 GenBank accession XXXX and CO1 Folmer fragment GenBank accession XXXX.

Remarks: This species is distinguished from other massive *Paratimea* species by its pink colouration and asters with more or less equal lengthed rays. Although the asters are similar to those found in encrusting *Paratimea* species (e.g. *P. constellata*, *P. loennbergi*, *P. aurantiaca* sp. nov.) it can be distinguished from them by its globular growth form; having oxeas as the principal spicules as opposed to tylostyles; choanosomal spicules with a disordered arrangement rather than hymedesmoid and relatively large asters.

HALICNEMIA BOWERBANK, 1864

Diagnosis: see Hooper (2002)

Type species: *Halicnemia patera* Bowerbank, 1864

Included species: WPD (van Soest *et al*., 2018) lists eight species however only three of these species (*H. patera*; *H. salomonensis* Dendy, 1922 and *H. verticillata*) were examined in this study.

HALICNEMIA PATERA BOWERBANK, 1864

(Fig. 11A–F)

Hymedesmia inflata Bowerbank, 1866

Crella inflata (Bowerbank, 1866)

Quindesmia inflata (Bowerbank, 1866)

MATERIAL EXAMINED

Material examined: Syntypes: BMNH 10.1.1.2459/2460 Shetland, 1863 (Norman Collection) (Fig. 7A) (dried specimen and slide).

BMNH 1925.11.1.326 North Sea, near Shetland Is. 330 m 60°34'N, 2°04'E ("Goldseeker" Collection).

BMNH Bk471 from Shetland (collected by Mr Peach) (identified as the holotype for *Halicnemia inflata*).

Description: (BMNH 10.1.1.2459/2460) *Outer morphology* discoid, free-living sponge with a fringe of spicules bordering the outer margin approximately 2cm in diameter.

Megascleres Long tylostyles (Fig. 11B) 2450–2670 x 21–29 µm (at neck) (n = 3), slightly trilobate head 40 µm across, occasionally with a second tylote swelling near the base (Fig. 11C). In addition to the large tylostyles there are also short bulbous tylostyles (Fig. 11E) 294–*488*–833 x 12–*19*–26 µm (at neck), with a globular-shaped head, 25–*33*–40 µm. Frequently there is a second tylote swelling on the shaft of the short tylostyles (Fig. 11E).

Accessory oxeas centrotylote (Fig. 11D), usually with one central swelling although two equidistant of centre swellings is also common. Occasional oxeas have 3 swellings and some have no swellings, only a V-shaped bend in the middle, reminiscent of a hairpin (Fig. 11E). They measure 1350–*1620*–1930 x 6–*10.4*–12 µm.

Microscleres centrangulate acanthoxeas (Fig. 11F) 127–*154*–175 x 7–*9*–12 µm.

Remarks: *Halicnemia patera* BMNH 1996.10.639 off Jenny's Cove, Lundy, Bristol Channel, 22 m, coll. J. D. George 1985, det. S. M. Stone coll. no. L5639 non. *patera*. This specimen matches Topsent's description of *Halicnemia gallica* (Topsent, 1893) which was later synonymised with *H. patera* by Topsent (1897), but is here recognised as a valid species.

HALICNEMIA GALLICA (TOPSENT, 1893)

(Fig. 12A–D)

Bubaris gallica Topsent, 1893

Naenia gallica (Topsent, 1893)

Material examined: Neotype (here designated) BELUM Mc5427, Huw's Reef, Pembrokeshire, Wales, 51°57.845'N, 5°07.546'W, 17 m, 04.08.2009, coll. B. Picton, det. C. Morrow [*in situ* photo (Fig. 8A)].

BELUM Mc6677, Channel Isles, 49°26.73'N, 2°22.15'W, 23 m, 23.06.2010, coll. Jen Jones, det. C. Morrow.

ZMA POR4835, Baie de la Tortue, Roscoff, France, 48°41.28'N, 3°53.04'W, 15 m, 08.06.1982, coll. W.H. de Weerdt, det. C. Morrow.

Description: (BELUM Mc5427) *Outer morphology* the neotype is a small encrusting sponge, approximately 3 x 4 cm in area by 2–3 mm in thickness. The surface is covered in conules (Fig. 12A).

Colour yellowish-orange in colour.

Megascleres very long tylostyles (Fig. 12B) 1700–2930 x 18–20 µm, head ovoid to trilobate, measuring approximately 26 µm across (n = 2).

Accessory oxeas with 1–4 tylote swellings but most frequently with 2 (Fig. 12B, C). Oxeas measure 823–*1172*–1444 x 6–*10*–13 µm. The diameter of the tylote swellings varies from 10 to 18 µm.

Microscleres centrangulate acanthoxeas (Fig. 12D) 115–*118*–120 x 4–*5*–6.8 µm.

Reproduction the presence of oocytes has been observed in several specimens collected between 20–30 m during June to August.

Slime produces copious amounts of slime on collection.

DNA sequences: From the neotype (BELUM Mc5427) we sequenced the CO1 Folmer fragment (GenBank accession no. HQ379422) & 28S D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379221; HQ379287 & HQ379355 respectively). The 18S GenBank sequence KC902045 is also from the neotype.

Remarks: Topsent (1897) synonymised *H. gallica* with *H. patera,* however our re-examination of the type material of *H. patera* showed significant differences to the description given by Topsent (1893) for *H. gallica.* The type specimen of *H. patera* is disc-shaped and free-living (Fig. 11A) and was dredged from deep-water (330 m) at Shetland. By contrast Topsent's description of *H. gallica* was based on thickly encrusting specimens collected from shallow-water from the Roscoff area of the Celtic Sea and the Banyuls area of the Mediterranean Sea. In addition to the differences in habitat and growth form, Topsent (1897) also noted that the spiculation of the specimens from the French coast was less robust than that of the Shetland specimen and that there were also some differences in spicule morphology and skeletal architecture between the samples. Topsent (1897) considered the disc-shaped specimens from Shetland as a local form which was perhaps linked to the nature of the sea-bed. It is possible that Topsent's description of *H. gallica* was based on two different species. Topsent (1897) mentions how the colour of the specimens varies from yellow to orange-red. He linked the colour to the spherulous cells, specimens with large, uncoloured spherulous cells were yellow, specimens with smaller dark red-coloured spherulous cells were orange-red. Topsent also mentions that among the tylostyles there are short tylostyles similar to those of the Shetland specimens but that their presence is not constant. We have collected two species of *Halicnemia* from around the southwest coasts of Britain and Ireland, one is orange coloured and the second, *Halicnemia* Mc4307 sp. nov. is bright yellow and has short tylostyles in the base that are similar to those in *H. patera* from Shetland. Our orange *Halicnemia* matches the original description of *H. gallica.* Having compared numerous specimens of this orange encrusting *Halicnemia* with the type material of *H. patera* we consider the two sufficiently different and resurrect *H. gallica* for this species. All type material of *H. gallica* is believed to have been discarded from the Biological Oceanography Laboratory of Banyuls and no material remains in the porifera collection at

the MNHN (Paris). In the absence of type material for *H. gallica* and the confusion in Topsent's description mentioned above, to define the nominal taxon objectively

we designate a neotype, BELUM Mc5427: Huw's Reef, North Pembrokeshire: 51°57.8449'N 5°07.5460'W, depth: 17.4 m, 04.08.2009 coll. B. E. Picton (Fig. 12A) from the Porifera collection at the Ulster Museum, Belfast. Records of *H. patera by* Descatoire (1966), van Soest (1987), Ackers, Moss & Picton (1992), Picton *et al*. (2007), Morrow *et al*. (2012) should be reattributed to *H. gallica.*

Higginsia coralloides var. *arcuata* Higgins, 1877 from Ireland may be synonymous with *Halicnemia gallica* however the type material, which had been deposited at the Museum of Liverpool, was destroyed in the 1941 Blitz. The description and illustrations are not sufficient for confirmation.

HALICNEMIA SALOMONENSIS DENDY, 1922

(Fig. 13A, B)

Material examined: **Holotype**, *Halicnemia salomonensis* Dendy, 1922 (p. 128, pl. 17 fig. 9A–C) BMNH 21.11.7.109 RN CXXIV.4, Salomon Islands, Indian Ocean, 75 fathoms (137 m), (spicule slide only).

Description: *Megascleres* are stout styles, often irregularly bent (Fig. 13A).

Microscleres the acanthoxea are different to the acanthoxea in other *Halicnemia* species. Dendy (1922) described them as 'trichites' they measure 49–80–119 µm (Fig. 13B). It is not possible to say with any certainty whether this species belongs in *Halicnemia* or elsewhere in the classification, for now we retain it in *Halicnemia*.

HALICNEMIA VERTICILLATA (BOWERBANK, 1866)

(Fig. 14A–F)

Bubaris verticillata (Bowerbank, 1866)

Hymeraphia verticillata Bowerbank, 1866 (p. 145–146)

Laonaenia verticillata (Bowerbank, 1866)

Naenia verticillata (Bowerbank, 1866)

MATERIAL EXAMINED

Material examined: Syntypes BMNH 10.1.1.2389–2392, Porcupine Expedition 1868, 345 fathoms (631 m), 40 miles NNW of Shetland, Norman Collection (slide only).

BMNH 10.1.1.2388, Trondheim, Norway, Norman Collection (slide only).

BMNH 1406.70.5.3.21, Marquesas, Florida (Schmidt, 1870).

BELUM Mc6786, Boue Tirlipois, Channel Isles, 49°24.427'N, 2°23.183'W, 36 m, 25.06.2010, coll. B. Picton, det. C. Morrow.

BELUM Mc5018, Galway Bay, Ireland, 53°15.080'N, 9°59.420'W, 38 m, 17.08.1993, coll. C. Morrow, det. C. Morrow.

BELUM Mc2018.4, Whittard Canyon, Ireland, 54°03.36'N, 12°33.28'W, Event 15, 1400 m, 04.06.2013, coll. C. Morrow, det. C. Morrow.

Field# 40-10(6), off Tromsø, Sotbakken, Northern Norway, 70°45.46'N, 18°40.48'E, *Polarstern* ARK-XXII/1a expedition, van veen grab, station 40-10, 18.06.07, 273 m, coll. P. Cárdenas., det. P. Cárdenas.

Description: (BMNH 10.1.1.2389) *Megascleres* large tylostyles 2–3 mm x 4–7 µm (n = 4), tylostyle head 10–16 µm (Fig. 8A);

Accessory oxesa centrotylote oxea 600–800 x 5–10 µm, centrotylote swelling 10–17 µm. The centrotylote oxeas have distinctive fissurate ends (Fig. 14C).

Microscleres acanthoxeas in a wide range of sizes (50–400 x 5–10 µm) with spines arranged in verticils (Fig. 14D).

Reproduction the presence of oocytes were observed in BELUM Mc6786, collected in june from the channel Islands, 36 m.

Slime produces copious amounts of slime on collection.

DNA sequences: From BELUM Mc5018 we sequenced CO1 Folmer fragment, GenBank accession no. HQ379414) & 28S D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379211; HQ379276 & HQ379344 respectively. We sequenced 28S D1–D2 from BELUM Mc2018.4 (GenBank accession no. XXXX).

Remarks: On *Hymeraphia verticillata*, Bowerbank (1866, p.146) states; “this species differs from other British *Hymeraphia* species in having the primary skeleton spicules surrounded by fascicules of secondary skeleton spicules.” He also states that it differs in the spicules of the dermal and interstitial membranes which have verticillate spines which is not known to occur in any other British sponge. Bowerbank does not justify his allocation of *verticillata* to *Hymeraphia*.

Topsent (1897) attempts to explain the confused taxonomic affinities between *Hymeraphia verticillata* and *Halicnemia patera* and comments on how Bowerbank (1866) overlooked the similarities between the two species described by him.

Morrow *et al.* (2012) reassigned *H. verticillata* to *Halicnemia*. Using 28S rRNA they showed that *verticillata* was more closely related to the genus *Halicnemia* than to *Hymeraphia* which was also in their trees.

BMNH 1406.70.5.3.21 (slide) was examined, it was identified as *Hymeraphia verticillata* from Marquesas, Florida (Schmidt, 1870). The megascleres are oxeas with a double bend with fine microspining at the tips, they measure 515 x 5 µm (Fig. 14E & F). Centrotylote oxeas were observed but unmeasurable. Acanthoxeas (Fig. 14E & F) were very large and robust 558 x 40 µm, very acanthose, spines not arranged in verticils as in *H. verticillata*. The oxeas in this specimen (with microspined tips and a double bend) are similar to those of *Heteroxya corticata* Topsent, 1898. The spicules of this specimen are very different to those of *Halicnemia verticillata* and this specimen should be considered as an undescribed species, perhaps of *Heteroxya.*

In addition to tylostyles, verticillate acanthoxea and centrotylote oxea with fissurate ends, Topsent (1928; pl VI fig. 16, station 3144, Azores, 919 m), also illustrates oxyaster microscleres. Topsent considered the presence of asters in *verticillata* as further evidence of a close relationship with *Paratimea*. In the specimens collected by SCUBA diving from relatively shallow-water (38 m) the asters were never found, however they were present in the deep-water specimen (BELUM Mc2018.4) from the Whittard Canyon area. On the 28S D1–D2 tree (Supporting Information, Fig. S5) we can see that the shallow-water specimen which lacks asters is identical to the deep-water specimen with asters. This marker contains a region that usually shows variation between closely related species (see Morrow *et al*. 2012), therefore it seems likely that we are dealing with a single species. Uriz & Maldonado (1995) and Maldonado *et al.* (1999) demonstrated experimentally that there was a link between the spicule content of the sponge *Crambe crambe* and the silica concentration in seawater, while Cárdenas & Rapp (2013) observed this with Geodiidae in the environment. It is possible that the asterose microscleres in *H. verticillata* are only expressed in deeper water where the concentration of silica is relatively high.

HALICNEMIA CALEDONIENSIS MORROW **SP. NOV.**

(Fig. 15 A–G)

Material examined: **Holotype**: BELUM Mc4307, NW of Cath Sgeir, Gigha, Firth of Lorn, Scotland, 55°39.87'N, 5°47.68998'W, 29 m, 24.06.2008, coll. B. Picton.

Paratypes: BELUM Mc3493 E of Black Rock, Skerries, Northern Ireland, 55°13.51272'N, 6°36.54282'W, 29 m, 25.08.2006. coll. B. Picton; BELUM Mc3736 Labhra Cliff, Lough Hyne, Co Cork, Ireland, 51°30.0546'N, 9°18.1338'W, 12 m, 10.04.2007, coll. B. Picton; BELUM Mc5406 Ynys Deullyn, North Pembrokeshire, Wales, 51°57.91998'N, 5°08.45802'W, 16 m, 03.08.2009, coll. B. Picton.

Description: *Outer morphology* thinly encrusting with a hispid surface covered in silt. The holotype is approximately 15 x 10 mm (Fig. 15G).

Colour pale yellow, Methuen colour code 3A4 (Kornerup & Wanscher, 1978) (Fig. 15G).

Choanosomal skeleton hymedesmoid arrangement consisiting of erect, long tylostyles and bundles of long, slender, centrotylote oxeas scattered throughout the skeleton. Smaller, club-like tylostyles present in basal layer. Cells with granular content are abundant throughout the choanaosomal tissue (Fig. 15).

Ectosomal skeleton large tylostyles penetrate the surface, surrounded by supporting bundles of centrotylote oxeas. Acanthoxeas form a dense paratangential layer beneath the surface (Fig. 15F).

Megascleres are very long, thin tylostyles 1000–2800 µm x 5–7 µm (n=6) with only a slightly swollen base (9–12 µm) (Fig. 15A). In addition to long tylostyles, there are small club-like tylostyles 160–*215*–340 x 7–*13*–20 at the widest part of the shaft and 13–*20*–29 µm at the base (Fig. 15B).

Accessory oxesa centrotylote oxeas, centrotylote swelling not always obvious, the oxeas are 430–*560*–660 x 2.5–*3.5*–5 µm (Fig. 15C–D).

Microscleres are angular acanthoxeas 90–*100*–110 µm x 4–*5*–9 µm (Fig. 15E) . The spines are relatively large, approximatley 3–4 µm in length. The term *microsclere* is widely used for the acanthoxeas in *Halicnemia* (e.g. Hooper, 2002), although they can be quite large (up to 110 µm in *H. caledoniensis* and up to 400 µm in *H. verticillata*).

Reproduction the presence of oocytes has been observed in several specimens collected between June and August.

Slime produces copious amounts of slime on collection.

Habitat: Vertical to overhanging sublittoral rocky reef with strong tidal currents.

Etymology: *Caledonia* L. = Scotland, refers to the type locality.

DNA sequences: From the holotype we sequenced partial CO1 (Genbank accession no. HQ379423) & 28S (D1–D2, D3–D5, D6–D8, GenBank accession nos. HQ379222; HQ379288 & HQ379356 respectively).

Remarks: *H. caledoniensis* sp. nov. differs from other species of *Halicnemia* from the Atlantic area by the distinctive acanthoxea which are sharply bent in the mid section and have much larger spines than other *Halicnemia* spp. It shares the presence of short, stout, club-like tylostyles with *H. patera* and *Paratimea loennbergi*.

HIGGINSIA HIGGIN, 1877

Diagnosis: See van Soest (2002)

Type species: *Higginsia coralloides* Higgin, 1877

Included species: Van Soest *et al*. (2018) in WPD lists 26 species. We have only examined the type material of six of these species. *Higginsia durissima* (Burton, 1928, p. 131, fig. 2) is returned to *Bubaris* Gray, 1867 as there is no justification for its transferral to *Higginsia*, *H.*

anfractuosa Hooper & Lévi, 1993, and *H. fragilis* Lévi, 1961 are reassigned to Hymedesmiidae.

Remarks: Van Soest (2002) states that the spined microscleres can be curved and that raphides or trichodragmata may occur. He was referring to *Higginsia lunata* Carter, 1885. Van Soest & Hooper (2005) subsequently resurrected the genus *Desmoxya* for this species (see remarks on *Desmoxya*).

HIGGINSIA PETROSIOIDES DENDY, 1922

(Fig. 13C)

Higginsia petrosioides Dendy, 1922 (p.126, pl. 7 fig. 9)

Material examined: **Holotype** BMNH RN CXXXII.2, Seychelles, Indian Ocean 20.10.05, 44 fathoms (80 m), 'Sealark' (slide only).

DNA sequences: CO1 Folmer fragment GenBank accession EU146439 from QM G300611.

Remarks: In this species the centrotylote swelling on the oxea is only slightly noticeable. The oxea are often bifurcate at one or both ends (Fig. 13C). Flanged ends to the oxea are also found in *Higginsia bidentifera, Halicnemia verticillata* and *Paratimea dentata* sp. nov. (BELUM Mc 6884).

HIGGINSIA ROBUSTA BURTON, 1959

(Fig. 13D)

Higginsia robusta Burton, 1959 (p. 255, Fig. 32)

Material examined: **Holotype** BMNH 1936.3.4.342, Gulf of Aden, 11°57.2'N, 50°35'E, 12.10.1933, 38 m (slide only).

Remarks: This species is characterised by the possession of relatively short and thick styles and acanthoxea (Fig. 13D). The styles are approximately 740 x 36 µm, occasionally short thick oxea of similar proportions are present. The acanthoxea are 42–58 µm.

HIGGINSIA THIELEI TOPSENT, 1898

(Fig. 13E)

Axinella thielei (Topsent, 1898) (p. 245).

Material examined: Lectotype (here designated) MNHN DT885, *Princess Alice*, Prainha de Pico, Azores, 523 m, 1895.

MNHN DT886, MNHN DT884 (slides only), Princess Alice Bank, Azores, 200 m, 1897.

Description: *Megascleres* robust styles to tylostyles 630–*735*–845 x 25–40 µm.

Microscleres acanthoxea 50–*73*–98 x 2–4.5 µm (Fig. 13^{E}).

Remarks: MNHN DT884 was not mentioned in the original description. The acanthoxea in this specimen are shorter and more robust, with sparser spination than the type material.

ACANTHOCLADA BERGQUIST, 1970

Diagnosis: see Bergquist (1970).

Type species: *Acanthoclada prostrata* Bergquist, 1970 (p22, pl.5b, pl.10a,f, pl.16a,b)

ACANTHOCLADA PROSTRATA BERGQUIST, 1970

Material examined: NMNZ Por 145 Takatu Point, New Zealand, 36°23'S, 174°50'E, 10 m.

Description: Bergquist (1970) describes this species as "thickly encrusting and slimy" and notes the presence of oocytes in one specimen.

DNA sequences: From NMNZ Por 145, We sequenced 28S D3 region, GenBank accession XXXX.

Remarks: Bergquist (1970) proposed *Acanthoclada* for sponges with a *Higginsia*-like skeleton but with the addition of echinating rhabdostyles. In addition to centrotrangulate oxea, *Acanthoclada* also has cladotoxa and curved birotule microscleres. Bergquist had misgivings regarding the allocation of *Acanthoclada* to Desmoxyidae due to the absence of oxeote microscleres. Hooper (2002a) retained *Acanthoclada* in Desmoxyidae with reservation, stating, "being most similar to *Higginsia* based largely on their affinities in skeletal structure, whereas this assignment is still not certain." Our 28S genetree (Supporting Information, Fig. S3) strongly supports a close relationship between *Acanthoclada*, *Halicnemia* and *Higginsia*.

HETEROXYIDAE DENDY, 1905

Diagnosis: Encrusting to massive growth forms. Surface hispid, sinuous or straight canals or grooves may be present. Choanosome consisting of (acanth)oxea either loosely scattered or forming a confused reticulation. Ectosomal skeleton consisting of dense brushes of (acanth)oxea perpendicular to surface. Megascleres, two size classes of smooth or spined oxea, some of the oxea have a characteristic double flex, occasionally styles present. Microscleres when present consist of raphides in trichodragmata in one or more size categories, larger raphides sinuous or curved.

Included genera: *Heteroxya** Topsent, 1898 (pp231–234, fig. 2a); *Myrmekioderma** Ehlers, 1870 (p32); *Alloscleria* Topsent, 1927 (p6);*; Julavis* Laubenfels, 1936 (p79); *Microxistyla* Topsent, 1928 (p179); *Negombo* Dendy, 1905 (p127) and *Parahigginsia* Dendy, 1924 (p375). (* = genera for which molecular data is available, the remaining genera for which we have no molecular data are retained in Heteroxyidae).

Remarks: The family Heteroxyidae was originally proposed for *Heteroxya* and *Acanthoxifer* Dendy, 1905 [= *Myrmekioderma* Bergquist (1965) compared the type species of *Myrmekioderma* with the type species of *Acanthoxifer* and concluded that they were conspecific]. Erpenbeck, Breeuwer & van Soest (2005) using partial 28S rRNA sequences showed *Myrmekioderma granulatum* (Esper, 1794) (type species of *Myrmekioderma*) clustering with *Didiscus* spp. in Raspailiidae. Redmond *et al.* (2013) using 18S rRNA showed *M. granulatum* clustering with Raspailiidae but *M. rea* clustered with Axinellidae suggesting that the genus is polyphyletic. Erpenbeck *et al.* (2012) using CO1 barcoding sequences showed *M. granulatum* and *M. gyroderma* clustering with Axinellidae and not with *Didiscus* in Raspailiidae. In our CO1 tree (Supporting Information, Fig. S4) we have used the *Myrmekioderma* sequences from Erpenbeck *et al*. (2012) and they cluster with *Heteroxya corticata* Topsent, 1898 and *H. beauforti* sp. nov., close to Axinellidae. Pending further investigation of the possible polyphyly of *Myrmekioderma* we retain the genus in Heteroxyidae.

Figure 1 provides strong molecular evidence for the exclusion of *Didiscus* and *Desmoxya* from Heteroxyidae. Whilst previous molecular studies have consistently shown *Didiscus* clustering within Raspailiidae this is the first study that provides molecular and morphological support for the allocation of *Desmoxya* (and Desmoxyidae) to Poecilosclerida.

In our CO1 gene tree (Supporting Information, Fig. S4), *Heteroxya* spp. cluster with *Myrmekioderma* spp. close to Axinellidae. Some Axinellidae e.g. *Axinella parva* Picton & Goodwin, 2007 and *A. pyramidata* Stephens, 1916 have oxea with a double flex which are similar to the oxea in *Heteroxya corticata* (type taxon of Heteroxyidae) (Fig. 14A–D) and *H. beauforti* (Fig. 14D–F). Cells with granular inclusions are very abundant in *A. pyramidata, A. parva, H. corticata* and *H. beauforti.* These characters appear to unite *Heteroxya* and Axinellidae and support the recent allocation of Heteroxyidae to Axinellida (van Soest *et al*., 2018 in WPD).

HETEROXYA TOPSENT, 1898 (P. 231)

Diagnosis emended: Encrusting growth form; surface highly hispid; choanosome with a condensed basal layer of spongin lying on the substrate, containing (acanth)oxeas distributed without appreciable order on basal spongin and strewn throughout the mesohyl; subectosomal skeleton consists of oxeas arranged perpendicular to the ectosome, protruding through the surface, but not embedded in basal spongin; ectosomal skeleton with a perpendicular palisade of smaller (acanth)oxeas, through which the larger subectosomal oxeas protrude; very long styles present in one species. Two categories of oxea, smooth or spined; larger oxea sometimes with a double flex; microscleres absent.

Type species: Heteroxya corticata Topsent, 1898 (pp231–234, fig. 2A)

Included species: *H. corticata*; *H. beauforti* Morrow sp. nov.

HETEROXYA CORTICATA TOPSENT, 1898

(Fig. 16B–D)

Material examined: Syntypes MOMINV-INV-22285, Topsent 1888, Azores, stn 578, 869, depth 1165 m, 1240 m Collections du Prince Albert Ier de Monaco.

DNA sequences: The CO1 Folmer fragment on GenBank, accession KP939318 is from the holotype.

Remarks: Fig. 14A is a photograph of an ethanol preserved specimen from the type lot. It is dirty-beige in colour with a hispid surface and is growing on coral. Fig. 16B is a photomicrograph showing the large oxea that often have a characteristic double flex. The large oxea measure 1600–*1700*–2000 x 26–*32*–37 µm. The ends of the oxea occasionally have some microspination (Fig. 16C). The small oxea (Fig. 16D) measure 235–*310*–420 x 12–23 µm. The spination is more pronounced in the smaller oxea, particularly towards the ends of the oxea but some spination may also be present over the entire surface of the spicule.

HETEROXYA BEAUFORTI MORROW **SP. NOV.**

(Fig. 17A–F)

Material examined: **Holotype** BELUM Mc7794, 54°03.67998'N, 12°33.105'W, CE10004 of RV Celtic Explorer, 31.5.2010, 1300 m, coll. C. Morrow, det. C. Morrow.

Paratypes BELUM Mc7750, 54°03.79758'N, 12°24.78846'W, CE10004 of RV Celtic Explorer, 26.5.2010, 1469 m, coll. C. Morrow, det. C. Morrow.

ZMA POR20081, 55°26.676'N, 16°04.308'W, Expedition Biosys/Hermes 2005, Field Bx173/rest, 629 m, 12.7.2005, coll. RWM van Soest, det. C. Morrow.

Description: *External morphology* Thin encrustation on dead scleractinian cup coral *Desmophyllum* sp. (Fig. 17A). The holotype is almost completely overgrowing the underside of the cup coral that measures approximately 35 mm in diameter, thickness approximately 1.5 mm. Surface is hispid to hirsute. Oscules not apparent in preserved material.

Colour dirty white in alcohol.

Ectosomal skeleton composed of a layer of large and small oxea arranged in tufts, perpendicular to the surface, the larger oxea penetrate the surface for most of their length giving surface its hispid appearance (Fig. 17B).

Choanosomal skeleton relatively aspicular, consists of scattered oxea and very long styles up to 6 mm, arranged perpendicular to the base of the sponge. The styles penetrate the surface for most of their length and are responsible for the hirsute appearance of the surface (Fig. 17B–C). Cells with granular content (Fig. 17B) are particularly abundant in the basal region of the choanosome.

Megascleres consist of two size classes of oxea (Fig. 17D) and very long styles (Fig. 17E). The larger oxea (Fig. 17D) are slightly bent in the middle region and taper to a smooth point

at each end. They measure 622–*1030*–1385 x 10–*16*–21 µm. The smaller oxea (Fig. 17F) frequently have a double flex and taper to a fine point at each end, they measure 207–*280*–370 x 11–*14*–16 µm. Both categories of oxea are entirely smooth. The styles (Fig. 17E) are very long and slender and are parallel sided for most of their length. They measure 5000–*5650*–6300 x 23–*25*–27 µm.

Reproduction: Oocytes were observed in the holotype which was collected in May.

Habitat: Encrusting dead coral in the deep cold water coral reefs off the west coast of Ireland between depths of approximately 600–1500 m.

Etymology: Named after the Beaufort Marine Biodiscovery Research Award which provided funding for part of this research and in honour of the Irish hydrographer Francis Beaufort.

DNA sequences: We sequenced the CO1 Folmer fragment from the holotype, GenBank accession no. KF017197.

Remarks: Topsent (1898) established the genus *Heteroxya* for his new species *H. corticata* from deepwater (approximately 1200 m) off the Azores. Van Soest *et al*. (2007) recorded *H. corticata* from SE Rockall Bank (off the west coast of Ireland) at a depth of 629 m. We have re-examined the specimen from Rockall (ZMA POR20081) and it appears to agree with our new species *H. beauforti*. The new species differs from *H. corticata* in the following characteristics; both the large and small oxeas in *H. beauforti* are smooth whereas in *H. corticata* the smaller oxeas (Fig. 16D) are covered in spines and some of the large oxeas have scattered spines near the apices (Fig. 16C). The large oxeas in *H. corticata* measuring 1600–*1700*–2000 x 26–*32*–37 µm are longer and more robust than the large oxeas in *H. beauforti* (622–*1030*–1385 x 10–*16*–21 µm). In addition to oxeas the new species also has very long slender styles that measure up to 6.3 mm. The CO1 Folmer fragment from the holotype of *H. corticata* (GenBank KP939318.1) is identical to the sequence from *H. beauforti.* Whilst this marker is frequently used in species barcoding (Herbert *et al.,* 2003), it has been demonstrated that it is highly conserved in sponges and cnidarians and not always suitable for species delimitation in these groups. Our CO1 tree (Supporting Information, Fig. S4) shows *Heteroxya* clustering close to Axinellidae.

ALLOSCLERIA TOPSENT, 1927

Type species: Alloscleria tenuispinosa Topsent, 1927

Included species: *A. tenuispinosa* Topsent, 1927 (p. 6)

Remarks: Topsent (1927) erected the genus *Alloscleria* for his new species *A. tenuispinosa* and *Topsentia glabra* (Topsent, 1898) and allocated the genus to Spongosoritidae (=Halichondriidae). He considered *A. tenuispinosa* most closely allied with *Topsentia glabra*. Hooper (2002b), synonymised *Alloscleria* with *Halicnemia*, primarily on the basis of the presence of acanthoxea. However, Erpenbeck & van Soest (2002) on a discussion of *Topsentia* Berg, 1899, subsequently resurrected *Alloscleria* although they retained the genus in Desmoxyidae.

ALLOSCLERIA TENUISPINOSA TOPSENT, 1927

(Fig. 13F)

Halicnemia tenuispinosa (Topsent, 1927) (Hooper, 2002)

Material examined: **Holotype** MNHN DT1990, stn 1242, Banc de Seine, Azores, 10.09.1901, 240 m (slide only).

Description: *Megascleres* this species has fusiform styles 310–*400*–444 S.D. = 40 x 7–*8*–10 µm.

Microscleres very fine acanthose oxea 60–*88*–106 x 1.5–*1.8*–2.5 µm that are only slightly curved in the middle (Fig. 13F).

Remarks: The spicule and skeletal morphology of *A. tenuispinosa* differs substantially from typical *Halicnemia* and *Higginsia* species. Without molecular evidence it is difficult to identify the correct family and order for this genus.

BUBARIDA MORROW & CÁRDENAS, 2015

BUBARIDAE TOSENT, 1894

BUBARIS GRAY, 1867

Type species: *Bubaris vermiculata* (Bowerbank, 1866) (p.141)

Included species: *B. ammosclera* Hechtel, 1969; *B. carcisis* Vacelet, 1969; *B. conulosa* Vacelet & Vasseur, 1971; *B. durissima* Burton, 1928; *B. murrayi* Topsent, 1913; *B. salomonensis* Dendy, 1922; *B. sarayi* Ilan, Ben-Eliahu & Galil, 1994; *B. sosia* Topsent, 1904; *B. subtyla* Pulitzer-Finali, 1983; *B. vermiculata* (Bowerbank, 1866).

BUBARIS DURISSIMA BURTON, 1928

(Fig. 18)

Higginsia durissima (Burton, 1928) WPD (van Soest *et al*., 2018)

Material examined: **Holotype** BMNH R.N.XXXI.i, stn 532, Andaman Sea, Merqui Archipelago, 113 m.

Description: *Megascleres* thick, short rhabdostyles and oxea 450 x 34 µm, axial canal often visible in rhabdostyles (Fig. 18).

Remarks: Although this species was transferred to *Higginsia* (van Soest *et al*., 2018 in WPD), we do not know of good morphological evidence to support placing it here. This species has shared affinities with Axinellidae, Hymerhabdiidae and Dictyonellidae. On spicule and skeletal morphology these three families are apparently indistinguishable. There is some evidence that there are chemical characters that characterise Hymerhabdiidae such as the presence of isocyanids (Braekman *et al*. 1992) and using DNA sequences they are easily distinguishable. Given that it is not possible to say with confidence whether *durissima* should be assigned to Axinellidae, Dictyonellidae or Hymerhabdiidae, and in the absence of molecular data, we return it to its original genus (*Bubaris*) the most conservative option.

POECILOSCLERIDA TOPSENT, 1928

DESMOXYIDAE HALLMANN, 1917 (HERE RESSURECTED)

Diagnosis (emended): Thinly encrusting or massive sponges; megascleres smooth diactinal strongyles and onychaetes, acanthostyles present in one species; microscleres are crescent-shaped acanthoxea. Choanosomal skeleton comprised of ascending, plumose columns of strongyles interspersed with bundles of onychaetes. Basal layer of erect acanthostyles present in one species. Acanthoxea distributed throughout the choanosome but concentrated in surface layer.

Type genus: *Desmoxya* Hallmann, 1917

Included genera: *Desmoxya* Hallmann, 1917

Remarks: We resurrect Desmoxyidae for *Desmoxya* and transfer it to Poecilosclerida. The presence of acanthose raphides (onychaetes) indicate an affinity with Tedaniidae (see remarks on *Desmoxya*).

DESMOXYA HALLMANN, 1917

Desmoxya Hallmann, 1917 (p. 650–654)

Diagnosis (emended): Desmoxyidae with thinly encrusting or massive growth form. Choanosomal skeleton hymedesmioid to plumose, composed of tracts of strongylote megascleres and crescent-shaped, acanthose microscleres especially abundant in surface

membrane. Bundles of onychaetes scattered throughout choanosome. Basal layer of erect acanthostyles present in one species.

Type species: Desmoxya lunata (Carter, 1885)

Included species: *D. lunata* (Carter, 1885); *D. pelagiae* van Soest & Hooper, 2005

Hallmann, 1917 speculated that the microscleres in *Desmoxya lunata*, which are terminally spined, crescent shaped or sigmoidal microxea, may have been derived from sigmata. He questioned whether genera with spined microxea such as *Higginsia*, *Halicnemia* and *Desmoxya* should be included in Axinellidae. He further speculated that the acanthoxea in *Desmoxya* and *Halicnemia* were similar to those of *Acanthoxa* Hentschel, 1914 and could be homologous with the acanthoscleres of myxillids. Our 18S tree (Supporting Information, Fig. S2) shows that there is molecular support for Hallmann's hypothesis (in part) as *Desmoxya pelagiae* clusters in Poecilosclerida; however *Halicnemia* and *Higginsia* group with *Stelligera* and *Paratimea* in Stelligeridae: Axinellida.

DESMOXYA LUNATA (CARTER, 1885)

(Fig. 19A–G)

Higginsia lunata Carter, 1885

Material examined: **Holotype** BMNH P.P.H.'86:12:15:138 Brit.Mus.Sp. 68, (Carter, 1885, p. 358), 35 m, South Australia.

Remarks: A re-examination of the type specimen of *D. lunata* using SEM revealed that the raphides are acanthose (Fig. 19D–G), suggesting that it may have affinities with Tedaniidae. The raphides are unusual, they appear to occur in at least three length categories and are 'compound'. They consist of several slightly overlapping raphides glued together, whereas in *D. pelagiae* the raphides are spined mostly at the ends and are in trichodragmas with individual raphides single, not compound. The acanthoxea in *D. lunata* are diactinal (Fig. 19C) whereas in *D. pelagiae* they are monactinal. In other respects the spicules and skeletal arrangement are closest to those of *D. pelagiae.* We therefore take *D. pelagiae* as representative of *Desmoxya* and propose the transfer of *Desmoxya* from Heteroxyidae: Axinellida to Desmoxyidae: Poecilosclerida.

DESMOXYA PELAGIAE VAN SOEST & HOOPER, 2005

(Fig. 20ª–H)

Desmoxya pelagiae van Soest & Hooper, 2005 (pp. 1368–1370, fig. 2ª–H).

Material examined: Holotype ZMA POR18145, M2004 BX33A, Rockall Bank, S part, 55°30.288'N, 15°47.148'W, 02.09.2004, 673 m.

BELUM Mc7764, 54°4.435'N, 12°31.507'W, CE10004 of RV *Celtic Explorer*, 27.05.2010, coll. C. Morrow, det. C. Morrow.

DNA sequences: From BELUM Mc7764 we sequenced partial CO1 (Genbank accession no. KC876696) & 28S (D3–D5, GenBank accession no. KF018109). The 18S GenBank sequence (KC902064) is from the same specimen.

Remarks: A re-examination of the type specimen of *D. pelagiae* found acanthostyle spicules and unidirectional spines on the raphides (similar to onychaetes) that were overlooked in the original description (Fig. 20 is an SEM of the spicules from the type specimen). The acanthostyles are relatively scarce and confined to the basal region of the sponge. They measure 288–*314*–353 x 9–10 µm n = 6 (measurements taken at middle portion of shaft), the head of the acanthostyles are approximately 12 µm across.

The presence of acanthostyles and onychaetes suggest an affinity with Tedaniidae (Poecilosclerida). Figure 20 G & H illustrates the spicule composition in *D. pelagiae* and *Tedania* (*Trachytedania*) cf. *ferrolensis* (Cristobo & Urgorri, 2001), respectively. Both species

share strongyles, raphides and acanthostyles. In addition *D. pelagiae* has bow-shaped acanthose microscleres with uni-directional spines.

HYMEDESMIIDAE TOPSENT, 1928

Diagnosis: See Van Soest (2002)

Included genera: *Acanthancora* Topsent, 1927; *Hamigera* Gray, 1867; *Hemimycale* Burton, 1934; *Hymedesmia* Bowerbank, 1864; *Kirkpatrickia* Topsent, 1912; *Phorbas* Duchassaing & Michelotti, 1864; *Plocamionida* Topsent, 1927; *Pseudohalichondria* Carter, 1886; *Spanioplon* Topsent, 1890.

Remarks: Morrow *et al*. (2013) and Redmond *et al*. (2013) indicated that this family is polyphyletic and in need of revision.

HOOPERIA MORROW **GEN. NOV.**

(Figs 21C–F, 22A–C)

Diagnosis: Erect, globular, cylindrical digitate growth form. Surface with non-detatchable dermis with distinctive, evenly distributed areolated porefields up to 2.5 mm in diameter. Choanosome consisting of ascending, meandering, anastomosing bundles of strongyles. Around papillae/areolae spicule bundles diverge. Ectosome heavy layer of collagen containing rugose oxea arranged paratangentially to surface. Megascleres are straight, slender strongyles with symmetrical rounded ends. Microscleres are slender, straight or only slightly curved rugose oxea with tapering ends.

Type species: *Higginsia anfractuosa* Hooper & Lévi, 1993 (p. 1455, Figs 39–40), herein designated.

Remarks: This genus is erected for *Higginsia anfractuosa*. In assigning this species to *Higginsia* Hooper & Lévi (1993) emphasized the presence of acanthoxea and raphides of two size classes. They stated that morphologically it was most similar to *Higginsia lunata* Carter, 1885 in growth form, papillose surface features and skeletal architecture and that both species are atypical of other *Higginsia*. Re-examination of the holotypes of *D. lunata* and *D. pelagiae* using SEM (Figs. 19 & 20 respectively) showed that the 'raphides' were spined, similar to the onychaetes in Tedaniidae. Re-examination of the holotype of *H. anfractuosa* showed that the 'acanthoxea' described by Hooper & Lévi (1993) were slender rugose oxea unlike the crescent-shaped acanthoxea in *Desmoxya*. Thin spicules resembling raphides were present but they were approximately the same sizes as the strongyles and oxea and we would interpret them as developing spicules rather than raphides.

Morphologically we consider *Hooperia anfractuosa* comb. nov. most similar to *Hemimycale*. Both *Hemimycale* and *Hooperia* gen. nov. share conspicuous, raised areolated porefields supported by parallel columns of erect strongyles (Fig. 21C & F respectively). The choanosomal skeleton in *Hemimycale* and *Hooperia* consists of bundles of strongyles that branch and anastomose infrequently (Fig. 21A & D respectively). *Hooperia* gen. nov. can be distinguised from other hymedesmiid genera by the presence of a surface layer of rugose oxea (Fig. 22C) that are morphologically distinct from the acanthoxea in Crellidae.

According to the family diagnoses given in Hooper & van Soest (2002), *Hooperia* would be placed in Crellidae Dendy, 1922 as it has a tangential layer of rugose/acanthose oxea. However, the CO1 genetree (Supporting Information, Fig. S4) shows *H. anfractuosa* clustering with *Hemimycale*: Hymedesmiidae. Morphologically the skeletal architecture and spicule morphology in *Hooperia anfractuosa* comb. nov. (Fig. 21D & E) are more similar to *Hemimycale columella* (Fig. 21A & B) than to *Crella elegans*. Therefore, we have assigned *Hooperia* to Hymedesmiidae.

Etymology: This genus is named in honour of Dr John Hooper, Head of Biodiversity & Geosciences Program, Queensland Museum, for his major contribution to sponge taxonomy.

HOOPERIA ANFRACTUOSA (HOOPER & LÉVI, 1993) **COMB. NOV.**

(Figs 21C–F, 22A–D)

Higginsia anfractuosa Hooper & Lévi, 1993 (p. 1455, figs 39–40)

MATERIAL EXAMINED

Material examined: **Holotype** QM G300723, stn. 181, E. reef flat, Ilôt Maitre, New Caledonia lagoon, 22°20.1'S, 166°25.0'E, 1.5 m, 02.06.1977, coll. G. Bargibant.

NMNH OCDN3725-J, coll. Coral Reef Research Foundation, Tanzania, 12 m, det. Michelle Kelly (non. *H. anfractuosa*).

DNA sequences: We sequenced the CO1 folmer fragment from the holotype, GenBank accession no. XXXX). The sequence is identical to GenBank accession no. HE611614 (*Crella* sp. QM G304373).

Remarks: We examined the Tanzanian specimen (OCDN3725-J) that is included in our molecular trees (Supporting Information Figs S2, S3). The spicule morphology, particularly that of the acanthoxeas is substantially different to *H. anfractuosa* comb nov. and we do not consider it to be the same species.

SPANIOPLON TOPSENT, 1890

Diagnosis: See van Soest (2002).

Type species: *Spanioplon armaturum* (Bowerbank, 1866)

Included species: van Soest *et al*. (2018) in WPD list three valid species. We have only examined material of the type species *S. armaturum* (Bowerbank, 1866).

SPANIOPLON FRAGILIS, LÉVI, 1961 **COMB. NOV.**

(Fig. 23C–F)

Higginsia fragilis Lévi, 1961 (p. 14, Fig. 16)

Material examined: **Holotype** MNHN DCL373 sv 99, Aldabra C. Lévi (microscope slide only).

Description: *Choanosomal skeleton* ascending sinuous columns composed of styles (rhabdostyles) occasionally tylostyles, with interconnecting spicules (Fig. 23F). Heads of principal spicules embedded in spongin. Secondary reticulation with 1–3 spicules interconnecting.

Ectosomal skeleton surface layer of straight acanthoxeas arranged tangentially.

Megascleres robust styles to tylostyles 308–*358*–422 S.D. = 28 x 10–11 µm (Fig. 23C); thin strongyles (tornotes) with basal ends rounded and slightly dilated 178–*270*–317 S.D. = 38 µm (Fig. 23D).

Microscleres Acanthoxeas 91–*102*–110 x 1–2 µm (Fig. 23^{E}).

Remarks: This species appears to be a poecilosclerid, morphologically it is similar to *Spanioplon armaturum* (Fig. 23A & B), both share style/tylostyle megascleres and tornotes with basal ends rounded and slightly dilated. The 'acanthoxeas' in *Higginsia fragilis* are also similar to those of *S. armaturum* (which vary from acanthoxeas to acanthostyles) and are unlike the acanthoxeas in other *Higginsia* and *Halicnemia* species. On the basis of the morphology we propose the transfer of *Higginsia fragilis* to *Spanioplon* (Hymedesmiidae: Poecilosclerida).

TETHYIDA MORROW & CÁRDENAS, 2015

HEMIASTERELLIDAE LENDENFELD, 1889

Diagnosis [emended from Hooper, (2002b)]: Encrusting, cup-shaped, arborescent or branching growth forms; megascleres styles, oxea or both enclosed within axially compressed spongin fibres or basally compressed in encrusting taxa, and plumose to plumo-reticulate extra-axial branches or sometimes with a definite axis. Microscleres are euasters, usually with microspination.

Included genera: Van Soest *et al*. (2018) in WPD list four genera: *Adreus* Gray, 1867; *Axos* Gray, 1867*; Hemiasterella* Carter, 1879 and *Leptosastra* Topsent, 1904.

ADREUS GRAY, 1867

Emended diagnosis: Arborescent growth form; choanosomal skeleton with strongly compressed axis composed of long smooth tylostyles in bundles running longitudinally through branches, poorly developed extra-axial skeleton composed of sparse plumose brushes of smaller smooth styles ascending to periphery; euasters with curved or sinuous, smooth or spined, strongylote or tylote rays often branched, mainly confined to the ectosomal region, absent in some species.

Type species: *Adreus fascicularis* (Bowerbank, 1866).

Included species: Van Soest *et al*. (2018) list three valid species: *A. fascicularis* (Bowerbank, 1866); *A. micraster* (Burton, 1956) and *A. stylifer* (Arndt, 1927). On the basis of the molecular evidence presented in Supporting Information Figure S5, we formally propose the transfer of *Raspailia (Parasyringella) australiensis* Ridley, 1884 and *Ceratopsion axiferum* (Hentschel, 1912) to *Adreus* (see below).

Remarks: In addition to arborescent growth forms, we have also collected an undescribed encrusting *Adreus* (BELUM Mc4982), this species is represented in our 18S rRNA genetree (Supporting Information, Fig. S2).

Erpenbeck *et al*. (2007) in an analysis of the systematics of Raspailiidae using partial 28S rRNA sequences, showed that *R. (P.) australiensis* and *C. axiferum* did not cluster with the main raspailiid group. In an attempt to try and resolve the taxonomic affinities of some of their 'raspailiid' taxa that failed to group with the main raspailiid clade we combined many of their sequences with our own (Supporting Information, Fig. S5). Our analysis indicates that rather than being raspailiids that have lost their acanthostyles, *R. (P.) australiensis* and *C. axiferum* are better interpreted as *Adreus* that have secondarily lost their asters. For these species we propose the new combinations *Adreus australiensis* and *Adreus axiferum*.

ADREUS AUSTRALIENSIS (RIDLEY, 1884) **COMB. NOV.**

Raspailia (Parasyringella) australiensis Ridley, 1884 (p. 460, Pl. 42)

Raspailia australiensis Ridley, 1884

Material examined: QM G320811 & G320826, Gulf of Carpentaria, 15°20.02'S, 140°19.50'E, 28 m, 24.05.2003, coll. C. Bartlett & S. Cook, det. M. Schlacher.

DNA sequences: 28S D1, Genbank accession nos. EU146438 & EU146439 from QM G320811 & G320826 respectively.

ADREUS AXIFERUM (HENTSCHEL, 1912) **COMB. NOV.**

Axinella axifera Hentschel, 1912 (p. 418, Pl. 14 Fig. 2 & Pl. 21 Fig. 56)

Ceratopsion axiferum (Hentschel, 1912)

Material examined: QM G304729, N. of Petherbridge Islets, Turtle Is., Great Barrier Reef, 14°41.04'S, 145°01.60'E, 9.3 m, 02.09.1994, coll. J.A. Kennedy & M. Gwendolyn, det. J. Hooper.

DNA sequences: 28S D1, Genbank accession no. EU146406 from QM G304729.

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CAPTIONS

Table 1. A summary of the different classifications espoused by the main authors who wrote on *Halicnemia* and *Paratimea.* With the exception of Hooper & van Soest (2002), these studies were not complete taxonomic reviews but rather faunas of a particular region and therefore they only include the genera that were represented in their chosen area. * indicates supporting molecular data, **molecular data does not support the allocation of *Plenaster* to Stelligeridae (taxonomic affinities will be discussed in a separate manuscript).

Figure 1. Best tree output from RaxML combined analysis of full length 18S rRNA, 28S rRNA (D1–D8 region) & mitochondrial CO1 barcoding fragment from 41 demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

Figure 2. A–D, photomicrographs illustrating the spicules in *Paratimea constellata* holotype MNHN DT 2361. E – F Photomicrographs illustrating spicules in *P. loennbergi* (E from holotype SMNH 1229; F from BELUM Mc5290). (ty = tylostyle; ast = aster; ox = oxea; cts = centrotylote swelling; ast = aster; tys = tylote swelling; sty = small tylostyle).

Figure 3. A, drawing of the spicules in *Paratimea duplex*, reproduced from Topsent, 1928 Pl. 6 Fig. 21; B, photomicrograph of spicules from holotype showing combination of oxea and styles; C, photomicrograph showing large oxea with centrotylote swelling and large asters with inequal length rays; D, photomicrograph of transverse section of sponge showing accessory oxeas arranged in bouquets around large oxea and dense surface layer of asters.

Figure 4. A, *in situ* photograph of *Paratimea oxeata* S153 Trémies Cave, Cassis (photo by Jean Vacelet); B, photomicrograph showing long flexuous oxeas, styles and asters; C–E, SEM of large oxeas and asters.

Figure 5. *Paratimea aurantiaca* sp. nov. holotype, A–D, SEM's of spicules: A, long tylostyle; B, accessory oxea; C, centrotlyote swelling on accessory oxea; D, aster; E, *in situ* photograph; F, photomicrograph of transverse section of sponge showing hymedesmoid skeleton consisting of erect, long tylostyles and ascending bundles of centrotylote oxea scattered throughout the skeleton. Bundles of centrotylote oxea penetrate the surface, oxyasters are common in the surface layer. Cells with a granular content are abundant.

Figure 6. *Paratimea dentata* sp.nov. holotype, A–D, SEM of spicules: A, large tylostle; B, centrotylote oxea; C, close-up of ends of oxea; D, oxyaster; E, *in situ* photograph (photo Bernard Picton); F, photomicrograph of transverse section of skeleton.

Figure 7. *Paratimea hoffmannae* sp. nov. A, Paratype ZMBN 125736 *in situ*, screenshot of footage taken with the manned-submersible *JAGO*; B, photomicrograph of transverse section of holotype showing ascending bundles of large oxeas and scattered oxeas and asters. Surface conules are dense with asters; C–E, SEM's of spicules: C, principal oxea; D, accessory oxeas; E, asters with inequal length rays; F, elongate conule dense with asters; F & G, tufts of smaller oxea penetrate the surface layer of the conules.

Figure 8. *Paratimea lalori* sp. nov. holotype, A, photograph of ethanol preserved specimen; B, photomicrograph of transverse section showing bouquet of accessory oxeas surrounding principal oxea and dense asters; C–G, SEM's of spicules: C, principal oxea; D, principal oxea with centrotylote swelling; E, enlarged view of centrotylote swelling; F, stylote megasclere; G, asters with inequal length rays; H, accessory oxea with centrotylote swelling.

Figure 9. *Paratimea mosambicensis* sp. nov. holotype, A–B, SEM's of the spicules: A, principal oxea; B, large, inequal length asters; C, photograph of ethanol preserved specimen; D, photomicrograph of transverse section through sponge showing disordered arrangement of oxeas and scattered asters; E, photomicrograph showing long slender accessory oxeas surrounding principal oxea; F, stylote megasclere.

Figure 10. *Paratimea rosacea* sp. nov. holotype, A, photograph of ethanol preserved specimen; B, photomicrograph of transverse section showing slender accessory oxeas surrounding principal oxea and abundant asters; C–F, SEM's of spicules: C, principal oxeas, D, tylostyles; E, enlarged tylostyle base; F, asters.

Figure 11. *Halicnemia patera* syntype BMNH 10.1.1.2459. A, dried specimen (approx. 30 mm in diameter); B–F photomicrographs illustrating spicules (acx = acanthoxea; ty = tylostyle; sty = small tylostyle; dacx = developing acanthoxea; tys = tylote swelling; ox = oxea; k = kink).

Figure 12. *Halicnemia gallica* neotype BELUM Mc5427 A, *in situ* photograph (specimen approx. 36 mm in diameter, photo Bernard Picton); B–D photomicrographs of spicules (ty = tylostyle; acx = acanthoxea; tys = tylote swelling; acx = acanthoxea; ox = oxea; dacx = developing acanthoxea).

Figure 13. A & B, photomicrographs of spicules in *Halicnemia salomonensis* holotype BMNH 21.11.7.109 RN CXXIV:4, C, *Higginsia petrosioides* holotype BMNH RN CXXXII.2; D, *Higginsia robusta* holotype BMNH 1936.3.4.342; E, *Higginsia thielei* lectotype MNHN D.T.885; F, *Alloscleria tenuispinosa* holotype MNHN D.T.1990 (st = style; acx = acanthoxea; ox = oxea; fs = fissurate end).

Figure 14. A–D *Halicnemia verticillata* holotype BMNH 10.1.1.2389, photomicrographs of spicules. E–F BMNH 1407.70.5.3-21 non *H. verticillata*, photomicrographs of spicules (ty = tylostyle; acx = acanthoxea; ox = oxea; cts = centrotylote swelling; fs = fissurate end; dacx = developing acanthoxea; ms = microspining).

Figure 15. *Halicnemia caledoniensis* sp. nov. holotype BELUM Mc4307: A–E, SEM's of spicules: A, large tylostyle; B, small, club-like tylostyle; C, centrotylote oxea; D, close-up of end of oxea; E, centrangulate acanthoxea; F, photomicrograph of transverse section of skeleton; G, *in situ* photograph (photo Bernard Picton).

Figure 16. *Heteroxya corticata*, A, photograph of an ethanol preserved specimen growing on coral from type lot INV-22538; B–D, photomicrographs of spicules (st = style; ox = oxea; acx = acanthoxea; ms = microspining).

Figure 17. *Heteroxya beauforti* sp. nov. holotype BELUM Mc7794. A, photograph of the ethanol preserved specimen encrusting the cup-coral *Desmophyllum* sp.; B & C, photomicrographs of transverse section through sponge showing the skeletal architecture; D–F photomicrographs of the spicules (lox = large oxea; sox = small oxea; cgi = cells with granualr inclusions; as = aspicular region; ty = tylostyle).

Figure 18. Photomicrograph of spicules in *Bubaris durissima* holotype BMNH R.N.XXXI;i (st = style; ox = oxea).

Figure 19. SEM of spicules in *Desmoxya lunata* holotype BMNH P.P.H.'86:12:15:138; A, oxea; B, close up of apices of oxea showing telescoped ends; C, crescent-shaped acanthose spicule; D, raphides/onychaetes; E & F, close up of raphides showing spines; G, raphides glued together (compound).

Figure 20. SEM of the spicules in *Desmoxya pelagiae* holotype ZMA POR18145; A, slightly polytylote strongyle; B, rounded ends on strongyle; C, acanthostyle; D, crescent-shaped spicule with unidirectional spines; E, bundle of onychaetes; F, unidirectional spines on onychaetes. G, photomicrograph of spicules in *Tedania* (*Trachytedania*) cf. *ferrolensis* BELUM Mc5348; H, photomicrograph of spicules in *Desmoxya pelagiae* BELUM Mc 7764. (acs = acanthostyle; on = onychaete; acx = acanthoxea; sg = strongyle).

Figure 21. A–C, *Hemimycale columella* BELUM Mc1258; A, transverse section through sponge showing skeletal architecture; B, photomicrograph of strongyle spicule; C, photomicrograph showing structure of areola. D–F, *Hooperia anfractuosa* comb. nov. holotype QM G300723; D, transverse section through sponge illustrating ascending, meandering, columns of strongyles; E, photomicrograph of spicules; F, structure of areola.

Figure 22. A–D, SEM of spicules in *Hooperia anfractuosa* comb. nov. holotype QM G300723; A, stronglyes (90–318 x 2.5–4.5µm); B, close-up of strongyle end; C, acanthostrongyles (106–173 x 2.5–3.5µm); D, close-up of acanthostrongyle showing detail of spination.

Figure 23. A & B, photomicrographs of spicules in *Spanioplon armaturum* BELUM Mc211; C–F, *Spanioplon fragilis* comb. nov. holotype MNHN D-CL-373sv99, C–E photomicrographs of spicules; F, transverse section showing skeletal architecture. (tor = tornote; sg = strongyle; acs = aanthostyle; dacs = developing acanthostyle; st = style; acx = acanthoxea; ty = tylostyle).

Figure 24. SEM showing spicule morphology in *Paratimea* and *Halicnemia.* A–C, *Paratimea dentata* sp. nov. (BELUM Mc6884) A, centrotylote oxea; B, aster; C, fissurate end on oxea. D–F, *Halicnemia verticillata* (BELUM Mc6786) D, centrotylote oxea; E, fissurate end on oxea; F, verticillate acanthoxea. G–I, *Halicnemia caledoniensis* sp. nov. (BELUM Mc4307) G, centrotylote oxea; H, centrangulate acanthoxea.

Figure 25. A, photomicrograph of transverse section of *Stelligera stuposa* BELUM Mc4330 showing oocytes surrounded by cells with granular inclusions (collected 30.06.2008, 25 m, Loch Sunart). B, photomicrograph of transverse section of *Paratimea loennbergi* BELUM Mc4323 showing mature oocytes (eggs) surrounded by cells with granular inclusions (collected 20.06.2008, 25 m, Firth of Lorn, Scotland). C, photomicrograph of transverse section of *Halicnemia gallica* BELUM Mc6677 showing an oocyte surrounded by cells with granular inclusions (collected 23.06.2010, 23 m, Guilleaumesse, Channel Isles). D, photomicrograph of transverse section of *Heteroxya beauforti* sp. nov. BELUM Mc7794 showing an oocyte and cells with granular inclusions (cgi) (collected 31.05.2010, 1300 m, S. off Ireland).

Supporting Information

Data S1. The taxa used in the analyses together with their catalogue numbers, collecting locality and corresponding GenBank accession codes.

Figure S2. Best tree output from RaxML analysis of full length 18S rRNA. Figures at nodes correspond to bootstrap support >70 followed by posterior probabilities >0.7 from the Bayesian analysis.

Figure S3. Best tree output from RaxML analysis of 28S rRNA (D3–D5 region). Figures at nodes correspond to bootstrap support >70 followed by posterior probabilities >0.7 from the Bayesian analysis.

Figure S4. Best tree output from RaxML analysis of mitochondrial CO1 barcoding fragment. Figures at nodes correspond to bootstrap support >70 followed by posterior probabilities >0.7 from the Bayesian analysis.

Figure S5. Best tree output from RaxML analysis of 28S rRNA (D1–D2 region). Figures at nodes correspond to bootstrap support >70 followed by posterior probabilities >0.7 from the Bayesian analysis.

Data S6. Pairwise percentage identitiy matrix for *Paratimea* and *Stelligera* spp. based on CO1 Folmer fragment (658 b.p.).

Figure S7. Comparison of aster morphologies and dimensions in *Paratimea* species.