Molecular and morphological congruence of three new cryptic *Neopetrosia* spp. in the Caribbean (#32201)

First submission

Editor guidance

Please submit by **10 Nov 2018** for the benefit of the authors (and your $200 publishing discount).

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data. Download from the location described by the author.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

5 Figure file(s)
3 Table file(s)
5 Raw data file(s)

Custom checks

DNA data checks

- Have you checked the authors data deposition statement?
- Can you access the deposited data?
- Has the data been deposited correctly?
- Is the deposition information noted in the manuscript?

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

New species checks

- Have you checked our new species policies?
- Do you agree that it is a new species?

Is it correctly described e.g. meets ICZN standard?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Molecular and morphological congruence of three new cryptic *Neopetrosia* spp. in the Caribbean

Jan Vicente Corresp., [1] , **Jaime Ríos Andrés** [2] , **Sven Zea** [3] , **Robert J. Toonen** [1]

[1] School of Ocean and Earth Science and Technology/University of Hawai'i at Mānoa, Hawai'i Institute of Marine Biology, Kāne'ohe, Hawaii, United States of America

[2] Universidad Nacional de Colombia – Sede Bogotá – Departamento de Biología, Ciudad Universitaria, Bogotá, Colombia

[3] Universidad Nacional de Colombia – Sede Caribe – Instituto de Estudios en Ciencias del Mar–CECIMAR, c/o INVEMAR, Calle 25 2–55, Rodadero Sur, Playa Salguero, Santa Marta, Colombia

Corresponding Author: Jan Vicente
Email address: janv@hawaii.edu

Neopetrosia proxima (Porifera: Demospongiae: Haplosclerida) is described as a morphologically variable sponge common on shallow reefs of the Caribbean. However, the range of morphological and reproductive variation within putative *N. proxima* led us to hypothesize that such variability may be indicative of cryptic species rather than plasticity. Using morphological characters and DNA sequences we confirmed the presence of three previously undescribed species of *Neopetrosia*. Morphological differences of each new congener were best resolved by partial gene sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) over nuclear ones (18S rRNA and 28S rRNA). Several new characters for *Neopetrosia* were revealed by each new species. For example, *Neopetrosia dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** showed the presence of grooves on the surface of the sponge body that converge at the oscula, and a more disorganized skeleton than previously defined for the genus. *Neopetrosia sigmafera* **sp. nov.** adds the 1) presence of sigma microscleres, 2) significantly wider/longer oxeas (>200 µm), and 3) the presence of parenchymella larvae. Sampling of conspecifics throughout several locations in the Caribbean revealed larger spicules in habitats closer to the continental shelf than those in remote island locations. Our study highlights the importance of integrating molecular and morphological systematics for the discrimination of new sponge species despite their belonging to one of several polyphyletic groups (families, genera) within the current definition of the order Haplosclerida.

Molecular and morphological congruence of three new cryptic *Neopetrosia* spp. in the Caribbean

Jan Vicente[1], Jaime Ríos Andrés[2], Sven Zea[3], Robert J. Toonen[1]

[1] Hawai‘i Institute of Marine Biology, University of Hawai‘i at Mānoa, Kāne‘ohe, HI, USA
[2] Universidad Nacional de Colombia – Sede Bogotá – Departamento de Biología, Ciudad Universitaria, Bogotá – Colombia
[3] Universidad Nacional de Colombia – Sede Caribe – Instituto de Estudios en Ciencias del Mar–CECIMAR, c/o INVEMAR, Calle 25 2–55, Rodadero Sur, Playa Salguero, Santa Marta – Colombia

Corresponding Author:
Jan Vicente[1]
Hawai‘i Institute of Marine Biology, University of Hawai‘i at Mānoa, Kāne‘ohe, HI, 96744, USA
Email address: janv@hawaii.edu

Abstract

Neopetrosia proxima (Porifera: Demospongiae: Haplosclerida) is described as a morphologically variable sponge common on shallow reefs of the Caribbean. However, the range of morphological and reproductive variation within putative *N. proxima* led us to hypothesize that such variability may be indicative of cryptic species rather than plasticity. Using morphological characters and DNA sequences we confirmed the presence of three previously undescribed species of *Neopetrosia*. Morphological differences of each new congener were best resolved by partial gene sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) over nuclear ones (18S rRNA and 28S rRNA). Several new characters for *Neopetrosia* were revealed by each new species. For example, *Neopetrosia dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** showed the presence of grooves on the surface of the sponge body that converge at the oscula, and a more disorganized skeleton than previously defined for the genus. *Neopetrosia sigmafera* **sp. nov.** adds the 1) presence of sigma microscleres, 2) significantly wider/longer oxeas (>200 μm), and 3) the presence of parenchymella larvae. Sampling of conspecifics throughout several locations in the Caribbean revealed larger spicules in habitats closer to the continental shelf than those in remote island locations. Our study highlights the importance of integrating molecular and morphological systematics for the discrimination of new sponge species despite their belonging to one of several polyphyletic groups (families, genera) within the current definition of the order Haplosclerida.

Introduction

Cryptic species have posed a challenge to taxonomy and biodiversity studies for over 300 years, but access to DNA sequencing has provided relatively simple tools to resolve species boundaries among morphologically similar species (*Bickford et al. 2007*; *Stat et al. 2012*). Particularly for taxa belonging to highly diverse orders with variable growth forms and limited morphological characters, such as corals and sponges, the integration of molecular and morphological approaches can be invaluable (Wörheide & Erpenbeck 2007; Concepcion et al. 2008; Forsman et al. 2009). In sponges, congruence of molecular and morphological datasets have been successful at the subclass level and have reclassified Demospongiae into subclasses Verongimorpha, Keratosa, and the Heteroscleromorpha (Borchiellini *et al.* 2004; Cárdenas *et al.* 2012; Morrow & Cárdenas 2015; Sperling *et al.* 2009). The presence of siliceous megascleres (monaxons and/or tetraxons) and highly diversified microscleres were confirmed as synapomorphies in Heteroscleromorpha were confirmed by partial nuclear gene sequences (28S rRNA and 18S rRNA) and mitochondrial gene sequence (Holmes & Blanch 2007; Lavrov *et al.* 2008). However, congruence of morphological and molecular datasets for lower taxonomic classifications within Heteroscleromorpha (> 5,000 species) have been unsuccessful. Most of these species within Heteroscleromorpha belong to the order Haplosclerida (2,209 species) (Morrow & Cardenas 2015). Although mitochondrial and nuclear genes show Haplosclerida to form a well-supported divergent clade from Heteroscleromorpha (Thacker *et al.* 2013; Lavrov *et al.* 2008), almost every family within Haploslerida is polyphyletic (Redmond *et al.* 2011; Redmond *et al.* 2013).

Among these polyphyletic families is the Petrosiidae which currently consist of 212 species with most of these belonging to *Petrosia* (Vosmaer 1885) (120 species), followed by *Xestospongia* (De Laubenfels 1932) (57 species), *Neopetrosia* (De Laubenfels, 1949) (33 species) and *Acanthostrongylophora* (Hooper 1984) (2 species) (http://www.marinespecies.org/porifera, see Van Soest *et al.* 2018). *Xestospongia* and *Neopetrosia* are mainly distinguished on the basis of spicule size, the former usually having spicules larger than 200 µm and the latter shorter. *Neopetrosia* congeners are distributed worldwide and nine are found in the Tropical Western Atlantic. These include *Neopetrosia carbonaria* (Lamarck, 1814), *Neopetrosia subtriangularis* (Duchassaing 1850), *Neopetrosia proxima* (Duchassaing & Michelotti, 1864), *Neopetrosia rosariensis* (Zea & Rutzler, 1983), *Neopetrosia dominicana* (Pulitzer-Finali 1986), and *Neopetrosia sulcata* (Santos *et al.* 2016), which are found in shallow to deep reefs; and *Neopetrosia dutchi* (Van Soest *et al.* 2014), *Neopetrosia eurystomata* (Van Soest *et al.* 2014), and *Neopetrosia ovata* (Van Soest *et al.* 2014), which are recently discovered mesophotic reef species. Mitochondrial and nuclear sequence data ~~has~~ been published for eight congeners which deeply diverge from one another and show no support for monophyly (Redmond *et al.* 2011; Thacker *et al.* 2013~~, Redmond *et al.* 2013~~). Mindful of the polyphyletic nature of *Neopetrosia*, our purpose for this study was not to find markers that resolve the monophyly for this genus but rather use a pairwise comparison of

mitochondrial and nuclear DNA sequences of our material with those from GenBank to confirm molecular and morphological separation for new congeners in the Caribbean.

Among tropical W. Atlantic *Neopetrosia*, *N. proxima* is a rather widespread species that shows considerable habitat and geographical variability (Zea 1987, Zea *et al*. 2014). In fact, detailed morphological revision of material previously considered to belong to this species has yielded new species (Van Soest *et al*. 2014, Santos *et al*. 2016). While reviewing material of what was believed to be *N. proxima* or close relatives from Colombia, Panama and other Caribbean localities, we found several morphologically distinct morphotypes. After detailed morphological comparisons and molecular barcoding with partial sequence of the cytochrome oxidase subunit 1 (CO1), 28S rRNA, and 18S rRNA, we were able to distinguish three new species from morphologically similar *N. proxima*, which we describe and compare here.

Materials & Methods

Specimen collection. Sponges were photographed in-situ and collected in Bocas del Toro-Panama, Colombia and Martinique by SCUBA at depths ranging between 4 and 36 m. Specimens from Colombia were collected at Golfo de Urabá, Cartagena and Santa Marta in the South American coast, and the San Andrés/Old Providence Archipelago in the SW Caribbean. Field observations (*in vivo*) of each specimen's morphology, color, consistency, surface, oscules, exudates, and odors were recorded. Samples were preserved in 95 % ethanol, and 4 % paraformaldehyde (PFA) for histological examination. Samples preserved in PFA for 2–3 days were later transferred to 70 % ethanol.

Type and other specimens were deposited in the Florida Museum of Natural History (catalogue number beginning with acronym UF) in Florida, USA, the Makuriwa Museum of Marine Natural History of Colombia at the Institute of Marine and Coastal Research (acronym INV POR) and the Natural Science Institute at the National University of Colombia in Bogota – INVEMAR [acronym ICN-MHN(Po)]. Fragments were also deposited in the Zoological Museum of Amsterdam at the Naturalis Biodiversity Center in Leiden, Netherlands (acronym ZMA.POR). Fragments of specimens collected in Panama were deposited in the Museum of Marine Biology and Limnology at the University of Panama as required by the collection permit of fauna Nr. 5 issued by the "Autoridad Nacional del Ambiente (ANAM)". Collecting in Colombia was carried out under Decree 309–2003 of the Ministry of the Environment and Sustainable Development as part of the ongoing project "Sponges of the Colombian Caribbean" of INVEMAR's Makuriwa Museum. Some uncatalogued samples were studied during the "Porifera Tree of Life Project Workshop" in Bocas del Toro, Panama, August 2012. Uncatalogued samples from Martinique were studied during the "2013 Training Course on the sponge biodiversity of the Caribbean Sea, workshop of La Martinique" and the "Kick-off meeting of the Associated International Laboratory MARRIO" in December 2013 (see also Pérez *et al*. 2017).

Sectioning and spicule preparation

Permanent slides with clean spicules and thick (~1 mm) histological sections (tangential and perpendicular) were prepared for each specimen following the methods in Zea (1987). Spicules were digested from small (20 mg) sponge pieces soaked in commercial sodium hipochlorite and shaken for 12 hours. Spicules were subsequently washed and centrifugued three times with DI and resuspended in ethanol; a few drops of spicule suspensions were added to microscope slides, dried on a warm plate and mounted on Permount®. Tissue sections were either dried on a warm plate or dehydrated and stained in successively stronger ethanol solutions (96 %, 100 %) and then cleared in xylene; then sections were mounted on Permount®. Individual spicule types and skeletal framework were photographed with a Zeiss AxioCam ERc5s mounted on a Zeiss AxioLabA.1 light microscope (LM). Photographs were processed in Photoshop and measurements carried out from photos with AxioVision SE64 Rel.4.9.1 and ImageJ® (Abràmofff *et al.* 2005) (http://imagej.nih.gov/ij/). The lengths and widths of 50 spicule measurements per specimen and spicule types are presented as [minimum–*mean* [±1 standard deviation (SD)]–maximum length / width in µm]. A few drops of the spicule suspension from Panamanian specimens were added to a stub, air dried, and imaged under high vacuum with a JEOL 5600 SEM Scanning Electron Microscope (SEM) at the Nano Imaging Facility, University of Maryland Baltimore County. Spicule suspension from Colombian and Martinique specimens were carbon coated with a Quorum Q150R and photographed under a QUANTA 200 FEI SEM. Measurements of spicule tracts, skeletal arrangement of fibers, and meshes were compared across species and specimens from different collection sites.

DNA extraction, sequencing and phylogenetic analysis. Sponge pieces (30 mg) were removed from type material (preserved in 95% ethanol) collected in Panama (UF 3854, UF 3856–3860) and were used for DNA extractions. DNA was extracted using the Promega E.Z.N.A. Tissue DNA Kit, following the manufacturer's instructions. DNA concentrations were checked by absorbance ratios using a UV-visible spectrophotometer (Thermo Scientific NanoDrop). DNA from the first elution was diluted to a working stock concentration of 35 ng μL^{-1}.

A list of primers for PCR amplification targeting the D1-D2 region of the 28S rRNA gene sequence, the 18S rRNA gene sequence and fragments of the cytochrome oxidase subunit 1 (COI) are provided in Table S1. Partial sequences of the different *Neopetrosia* spp. were retrieved from previously reported primer combinations (~~Chombard *et al.* 1998;~~ Folmer *et al.* 1994; Kelly-Borges & Pomponi 1994). Specific primers were then designed when sequence data was missing to complete the gene sequence region of interest. Primers were designed using NetPrimer (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). Polymerase chain reactions were carried out in 25 µl total volume including the following: 9 µL of H_2O, 12.5 µL of BioMix™ Red (Bioline, Taunton, MA) PCR Mastermix, 0.5 µL of each primer (10 mM), 2 µL of BSA (100 µg/ml), and 0.5 µL of template DNA. The PCR program consisted of an initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s; annealing temperatures ranged between 45 and 60 °C for 30 sec to 1 min 30 sec depending on

the primer combination and gene product of interest; and 1 minute extension at 72 °C. A final extension at 72 °C for 8 min finished the reaction. PCR products were all ran on a 1 % agarose gel stained with GelRed and purified using EXOFAP (EXO1 and FastAP). Sequencing reactions were performed using the BigDye TM terminator v. 3.1 and sequencing was done with an ABI Prism 3730XL automated sequencer.

Double sequence coverage was provided for each species and each targeted gene fragment. Sequence chromatograms in forward and reverse directions were assembled and edited by eye using Geneious 10 (Kearse *et al.* 2012). Assembled chromatograms were saved and exported as a fasta file. Each fasta file from targeted gene sequences and each *Neopetrosia* spp. were checked for contamination using the BLAST (Altschul *et al.* 1990) function from GenBank. BLAST results that showed >85 % sequence identity to those belonging to Porifera were exported to Geneious 10 and aligned using the ClustalW function with default parameters. Alignments were generated using 439 bp of the COI gene sequence, 821 bp of the D1-D2 region of the 28S rRNA and 638 bp for the 18S rRNA gene sequence. Phylogenetic trees were rooted on outgroups *Baikalospongia intermedia* DAQ167168.1, *Axinella corrugata* KC869523.1 and EF092264.1 for COI, 28S rRNA and 18S rRNA, respectively. In Geneious 10 plugin for MrBayes version 3.2.1 (Huelsenbeck & Ronquist 2001) for Bayesian inference (BI) and RaxML (Stamatakis 2006) was added for phylogenetic analyses using a maximum likelihood (ML) framework. Both analyses were implemented using the GTRGAMMA model with 1,000 bootstrap replicates. The Bayesian inference was run using 5 million generations sampled every 200 generations. The analysis was stopped when the standard deviation of split frequencies fell below 0.01. At this point convergence was assumed and the burnin value was determined. Phylogenetic trees were generated in Mega7 (Kumar *et al.* 2016). Resulting bootstrap values of >50 from the ML and Bayesian posterior probabilities >0.50 from the BI analysis were incorporated to the tree. Sequences of holotypes and other specimens for each species collected in Panama were deposited in GenBank under accession numbers XXXX–XXXX.

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: [urn:lsid:zoobank.org:pub:B56217F8-FA57-4D93-8A69-BEC98F4B2AE7]. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results

Systematics

Class Demospongiae Sollas, 1885

Subclass Heteroscleromorpha Cárdenas *et al.* 2012

Order Haplosclerida Topsent, 1928

Family Petrosiidae Van Soest, 1980

Definition. Haplosclerida with an ectosomal skeleton consisting of an isotropic reticulation of single spicules or spicule tracts and choanosomal skeleton verging towards an isotropic reticulation of spicule tracts, in which primary and secondary tracts are indistinct (Van Soest, 1980).

Genus *Neopetrosia* De Laubenfels, 1949

Definition. Petrosiidae with finely hispid surface produced by fine brushes of oxeas issued from subectosomal tracts, and a compact choanosomal network combining rounded meshes with a superimposed anisotropic reticulation. Megascleres oxeas less than 200 μm long (De Laubenfels, 1949).

Neopetrosia proxima (Duchassaing & Michelotti 1864)

(Fig. 1; Table 1)

Thalysias proxima Duchassaing & Michelotti, 1864: 84, Pl. VIII, Figs. 2–3.
Densa araminta De Laubenfels, 1934: 14.
Neofibularia proxima; Wiedenmayer, 1977: 147, 255.
Xestospongia proxima; Van Soest *et al.* 1983: 198; Van Soest *et al*. 1984: 143; Zea, 1987: 116, Fig. 34, pl. IX, Figs. 3–4; Van Soest & Stentoft 1988: 132, pl. XII Fig. 4, text, fig. 64; Lehnert & Van Soest 1996: 77, Fig. 29; Díaz, 2005: 470; Collin *et al.* 2005: 648; Rützler *et al.* 2000: 278.
Neopetrosia proxima; Campos *et al.* 2005: 13, Figs. 8A–D; Muricy *et al.* 2011: 106 (with further synonyms from Brazil); Zea *et al.* 2014 (field guide); Santos *et al.* 2016: 336, Fig. 4; Van Soest, 2017: 35, Fig. 21a–d; Pérez *et al.* 2017: 10.

Material examined. Bocas del Toro, Panama: UF 3856, Punta Caracol (9.3777° N, 82.1265° W) 8 m in depth, coll. Jan Vicente, May 8, 2015; UF 3858 and UF 3860 Dolphin Rock (9.35076° N, 82.1863° W), 14 m in depth coll. Jan Vicente and Arcadio Castillo May 20, 2015. Uncatalogued fragments PPA 35, 37 and 38, Isla Colón, Aeropuerto (9.3339° N, 82.2548° W), on rubble and sand, fringing reef, 7 m in depth, coll. Sven Zea, August 9, 2012. Colombia: INV POR1304, Bahía de Santa Marta, El Morro (11.2494° N, 74.2302° W) reef base, 30-36 m in depth, coll. S. Zea, February 10, 1988. INV POR1306, San Andrés Archipelago Old Providence, north of Low

Cay (Pallat Bank, 13.5525° N, 81.3245° W), fore reef terrace, 25 m in depth, coll. Sven Zea, 19 October 1994. (Further Colombian material is described in Zea 1987.)

Description (Fig. 1, Table 1). The external morphology vary from cylindrical (Fig. 2A) or flat branching individuals (from 5 × 15 cm by 5 cm thick), to thickly, encrusting (2 cm thick) mounds (Fig. 1B, C); encrusting specimens often fill cavities and appear level with the substratum. Oscule size ~~vary~~ between 2–7 mm in diameter and are either randomly scattered along the body of the sponge (Fig. 1A, C) or ~~arranged in-line~~ along elevated ridges (Fig. 1B). A white membrane collar surrounding the oscules was observed in some individuals (Fig. 1C). Consistency is toughly compressible but difficult to cut with a scalpel or a knife. The surface texture is velvety, from even and smooth (Fig. 1A) to rugged (Fig. 1B, C), often knobby from conical or blunt elevations around oscules; massive specimens often have keyhole to irregular grooves. All individuals produced a sticky substance when cut or squeezed. Surface color across individuals from Panama varied from yellow (Fig. 1A), dark brown (Fig. 1B), to light purple (Fig. 1C); Santa Marta specimens in Colombia are characteristically violet to pink (see Zea 1987); in other areas color is predominantly yellowish to purplish dark brown. Internal coloration across all specimens is cream.

Skeleton. The skeleton consist of a fasciculated reticulation of isotropic multispicular tracts that form circular to irregularly elongated meshes. In the ectosome, a paratangential reticulation of tracts (20–200 µm) make meshes that vary between 120 and 400 µm in diameter (Fig. 1Ai–Ci). Depending on the individual [(180–300 µm (Fig. 1Ai), 80–240 µm (Fig. 1Bi), and 280–390 µm (Fig. 1Ci)]. Smaller circular meshes in the ectosome seem to be the result of thicker spicule tracts (80–170 µm Fig. 1Bi), when compared to individuals with thinner spicule tracts (50–100 µm Fig. 1Ai-1Ci). Dark purple pigments from cyanobacteria penetrate about 750 µm into the choanosome (Fig. 1Ai, 1Bi). In some individuals, pigments were not observed from the surface but 500 µm below the ectosome (Fig. 1Cii). The ectosome can also be distinguished by the presence of large (500 µm) subectosomal spaces clearly visible in some individuals (Fig. 1Aii, 1Cii) but in others it forms smaller (250 µm) openings (Fig. 1Bii) as a result of denser and thicker spicule tracts. Erect ascending spicule brushes radiate the ectosome surface. The choanosome also show a large number of circular meshes that vary in abundance and size (200–700 µm) according to the thickness of spicule tracts.

Spicules. Most spicules are slightly curved, symmetric oxeas with very few strongyloxeas present (Fig 1Aiii, 1Biii, 1Ciii); some are more curved and there is variation in size with developmental stage. Oxea endings vary between hastate and conical shapes. Size 92–205 µm long by 1.7–12 µm wide (Table 1).

Habitat and ecology. This species is found living from shallow rocky shores and reefs, to deep reef habitats in a variety of wave-exposures (Zea 1987, Zea *et al*. 2014); also in caves (Pérez *et*

al. 2017). Specimens UF 3858 and UF 3860 were collected in a highly exposed reef (Dolphin Rock) with strong wave energy, while specimen UF 3856 was collected inside Almirante Bay (Punta Caracol) with very low wave exposure. Strong wave energy is known to influence the appearance of aligned oscula ~~morphology~~ (observed in *Xestospongia deweerdtae* collected in the same site, see Fig. 7B of Vicente *et al.* 2016) and is apparent in specimen UF 3856. Brooding larvae were not observed in any specimens; zoanthids were also absent.

Distribution. Bahamas (Zea *et al*. 2014). Caribbean: Puerto Rico, U.S. Virgin Islands, Jamaica, Martinique, Barbados, Panama, Colombia, Belize, (Zea, 1987, Van Soest & Stentoft 1988, Lehnert & van Soest 1996; Rützler *et al.* 2000, Díaz 2005, Collin *et al*. 2005, Zea *et al.* 2014, Pérez *et al.* 2017). Guyana (Van Soest 2017). Brazil: North to North East Regions (Amapa, Maranhao, Rio Grande do Norte and Sergipe states) (Campos *et al*. 2005, Muricy *et al*. 2011, Santos *et al.* 2016).

Taxonomic remarks. All *Neopetrosia proxima* specimens collected in this study exhibited varied morphologies (physical appearance, color, thickness of fiber tracts, circular meshes). These differences initially lead us to think that these were heterospecific. Upon closer examination, spicule sizes, spicule shapes, the skeletal arrangement of the choanosome and ectosome are all in agreement with previous descriptions (e.g., Zea 1987, Diaz 2005, Zea *et al*. 2014).

***Neopetrosia dendrocrevacea* sp. nov.**
(Fig. 2; Table 1)

Haplosclerida unident. sp. 1; Zea 2001, Table 1 (unpublished addendum).
Neopetrosia sp. –"soft"; Zea *et al.* 2014 (field guide).
?*Neopetrosia proxima*; Zea *et al.* 2014 (field guide, in part, only two images of partly branching and knobby individuals, taken in Panama, Bocas del Toro, Isla Solarte, Punta Hospital, March 3, 2012, identified from fresh spicule preparations).

Type material and type locality. *Holotype*: UF 3854, Panama, Bocas del Toro, STRI point (9.3429° N, 82.1258 ° W), 2 m in depth, coll. Jan Vicente, June 10, 2015. *Paratypes*: Colombia: ICN-MHN(Po) 0269, Bahía de Nenguange, playa del Manglar, Santa Marta (11.2494° N, 74.2301° W), 1.5 m in depth, coll. Sven Zea, March 18, 1999. INV POR1335, Bahía de Chengue (11.3200° N, 74.1267 ° W), 1.5 m in depth, coll. Sven Zea, May 19, 1982. INV POR1336, Bancos de Salmedina, Cartagena (10.3735° N, 75.6663° W), 24 m in depth, coll. Sven Zea, August 19, 1980. INV POR1337, Islas del Rosario, Isla Rosario (10.1583° N, 75.8050° W), 8 m in depth, coll. Sven Zea, March 7, 1998. INV POR0535, Cabo Tiburón, Golfo de Urabá (8.6840° N, 77.3710 ° W), 9 m in depth, coll. Sven Zea, September 28, 1995. INV POR1333, Isla de Providencia, San Andrés Archipelago (13.5058° N, 81.3558 ° W), 16 m in depth, coll. Sven Zea,

October 21, 1994. INV POR 1334, Banco Serrana, leeward terrace, San Andrés Archipelago (14.4592° N, 80.2740 ° W), 16 m in depth, coll. Sven Zea, May 14, 1995.

Additional material. Bocas del Toro, Panama: uncatalogued sample PPA 07, Isla Bastimentos, Adriana's reef (9.2419° N, 82.1736 ° W), 5 m in depth, coll. Sven Zea, March 2, 2012.

Description. Thin to thick (1 cm) encrustations growing up to 30 cm in diameter; or made up of coalescing, 1–2 mm thick branches, elevating to 10–15 cm from the base (Fig. 2B).The surface has densely reticulated or scattered characteristic grooves that converge at the rim of the oscules, cutting through them and making them appear lumpy or incomplete (Fig. 2A–C); sometimes the grooves surround smooth knobs of varied sizes. Oscular diameter from 1–2 mm in encrusting individuals to 0.5 cm in branching ones. A translucent membrane surrounds the oscules, sometimes closing them. Consistency from slightly soft to firm, but crumbly. Texture is particularly velvety and when squeezed the sponge produces a sticky substance. External color is golden yellow to reddish brown to dark purple with ochre yellow tinges; cream in ethanol. Interior color cream;

Skeleton. Ectosome as a paratangential reticulation, composed of rather confused, loose, uni to paucispicular tracts, up to 4–10 spicules and 25–70 µm across, forming polygonal meshes 100–200 µm in diameter (Fig. 2Ai-Ci). Single spicules and spicule brushes from the end of choanosomal ascending tracts ~~hispidate~~ the surface. Pigments from cyanobacteria penetrate about 600 µm inside the choanosome. The choanosome consists of an anisotropic reticulation with distinguishable, but loose primary tracts, 6–13 spicules and 10–50 µm across, separated by 50–200 µm. Tracts are interconnected by solitary spicules or loose paucispicular tracts, forming confused meshes measuring 80–300 µm in diameter (Fig. 2Aii-Cii).

Spicules. Symmetric oxeas, curved, with hastate endings (short but thick pointed ends, 86–198 µm long by 2.8–10.5 µm wide (Table 1). Spicule sizes are influenced by geographic location. For example, spicules from specimens collected closer to the continental shelf (i.e. Urabá, Bocas del Toro) are generally longer and thicker than those collected on the insular shelf (San Andrés Archipelago) (Table 1, Fig. 2D).

Habitat and ecology. This species is found on shallow ~~(1.5 m)~~ rocky substrates and deep reefs (16 m) living on dead coral rubble or over other sponges. This species is a common sponge of the leeward fore reef terrace of Banco Serrana in the San Andrés Archipelago with an average density of 0.56 individuals per 20 m^2 (Zea 2001; unpubl. addendum data).

Distribution. Panama (Bocas del Toro), Colombia (Urabá, Cartagena, Santa Marta, San Andrés Archipelago, cf. Zea 2001), Puerto Rico (Zea *et al.* 2014). S.Z. examined a dried fragment from the Bay of Honduras which belongs to this species (courtesy of J.C. Lang).

Taxonomic remarks. Although some specimens initially analyzed showed different characteristics from *Neopetrosia proxima* (Zea 2001; unpubl. data like *Haplosclerida* unidet. sp. 1, y Zea *et al.* 2014 as *Neopetrosia* sp.-"soft"), others were thought to be *N. proxima* [e.g., ICN-MHN(Po) 0269 and INV POR1335]. Accordingly, a more detailed morphological and molecular analysis was pursued to detect less obvious differences. Some obvious morphological differences between these species lie in the consistency of individuals, where *N. proxima* is generally firmer and tougher to cut than *N. dendrocrevacea* **sp. nov.** *Neopetrosia proxima* also exudes a stickier mucus when cut. Oscules are larger in *N. proxima* and the surface lack the grooves that seem to be a diagnostic morphological character of *N. dendrocrevacea* **sp. nov.** The arrangement of the choanosomal and ectosomal skeleton show very distinct morphologies from *N. proxima*, with reticulation being more isotropic in *N. proxima*. Meshes are also larger in diameter and better organized in *N. proxima*; multispicular tracts are thicker, more dense and fasciculated as described by Campos *et al.* (2005) and Zea (1987). In the field, *N. dendrocrevacea* **sp. nov.** can be easily confused with *Svenzea cristinae* Alvarez, van Soest & Rützler, 2002 which is also a crumbly, thin to thicker encrustation with yellow tinges, but its spicules are long styles (Zea *et al*. 2014). *N. dendrocrevacea* **sp. nov.** also share some similar external features ~~to~~ *Haliclona* (*Soestella*) *walentinae* Diaz, Thacker, Rützler & Piantoni, 2007 including the sometimes bumpy surface between ~~less deep~~ grooves and the similar~~ly sized and shaped~~ oxea (100–180 x 3–9 μm); the latter ~~is~~ more thinly encrusting and soft, has a looser and more unispicular skeleton, and the tissue is crisscrossed by purple filamentous cyanobactera.

Etymology. The given species name is an adjective derived from the Greek word *dendron* that refers to tree, and *crevace* from the old French word referring to groove (Brown 1956) which denotes the presence of branching and meandering grooves along the surface of the sponge. We use the feminine *dendrocrevacea* assuming that *Neopetrosia* is feminine, following Article 31.2 of the International Code for Zoological Nomenclature (http://www.iczn.org/, accessed on October 1, 2018).

***Neopetrosia cristata* sp. nov.**
(Fig. 3; Table 1)

Type material and type locality. *Holotype*: UF 3859, Panama, Bocas del Toro, Dolphin Rock (9.35076° N, 82.1863° W), 14 m in depth, coll. Jan Vicente and Arcadio Castillo, May 20, 2015.

Description. The holotype is a thickly (up to 1 cm) encrusting sponge with an irregular shape, 10 cm in diameter. Surface with scattered pointy conulose ends or smooth ridges. Oscules aligned on ridges along the sponge body, sometimes on top of conical elevations, <1mm in diameter. There are also sometimes narrow grooves that converge around ~~some~~ oscules (Fig. 3A, arrow). Consistency is firm but crumbly when torn. Surface texture is smooth and velvety. Specimens

exude a sticky substance when squeezed. External color is reddish brown to dark purple and the interior is cream; cream in ethanol.

Skeleton. The ectosome is composed of a rather confused reticulation of loose multispicular tracts, 3–15 spicules and 40–120 µm across, forming circular to polygonal meshes, 150–250 µm in diameter (Fig. 4B). Cyanobacterial pigments penetrate to 700 µm inside the choanosome (Fig. 3C). The choanosome consists of a confused reticulation of loose multispicular tracts, 5–20 spicules and 60–100 µm across (Fig. 3D), forming circular meshes, 100–150 µm in diameter (Fig. 3E).

Spicules. Slightly curved oxeas, 121–160 ~~µm long and~~ 2.1–9.6 µm ~~wide~~ (Fig. 3F; Table 1).

Habitat and ecology. The holotypewas found in a spur and groove, high wave energy environment, growing on a dead coral skeleton.

Distribution. Bocas del Toro, Panama.

Taxonomic remarks. This species shares many external morphological characters with *Neopetrosia dendrocrevacea* **sp. nov.**. These characters are 1) the appearance of grooves along the sponge's surface that converge at the oscules, 2) the velvety texture of the sponge surface, and 3) the disorganized reticulation of the choanosome and ectosome. Nevertheless, both of these species are distinguishable based on the morphology of the grooves along the surface of the sponge which are a lot less pronounced and few in number in *Neopetrosia cristata* **sp. nov.** (Fig. 3A). In *N. dendrocrevacea* **sp. nov.** up to 7 grooves converge around the oscules in both branching (Fig. 2B) and encrusting (Fig. 2A and 2C) individuals, forming a star-like pattern around the oscules. The appearance of a crown or irregular mounds around the oscules is also missing in *Neopetrosia cristata* **sp. nov.**. The diameter of the oscules is less than 1 mm in *Neopetrosia cristata* **sp. nov.**, being larger than 1mm in *N. dendrocrevacea* **sp. nov.**. The surface of *Neopetrosia cristata* **sp. nov.** is also smoother and lacks the rounded knobs surrounded by grooves found in *N. dendrocrevacea* **sp. nov.**, while those are pointed and dispersed in *Neopetrosia cristata* **sp. nov.**. Spicules in Panama are also somewhat smaller and straighter in *Neopetrosia cristata* **sp. nov.** [holotype UF 3859: 121–*142.1* (±9.8)–163.2] than in *N. dendrocrevacea* **sp. nov.** [PPA 07: 111–*156.5* (±14.6)–181; holotype UF 3854: 91–*165.2* (± 15.9)–188].

Etymology. The given species name is an adjective derived from the Latin word *crista*, referring to the surface ridges of the holotype (Brown 1956). We use the feminine *cristata*, assuming that *Neopetrosia* is feminine, following Article 31.2 of the International Code for Zoological Nomenclature (http://www.iczn.org/, accessed on October 1, 2018).

***Neopetrosia sigmafera* sp. nov.**
(Fig. 4; Table 1, Table 2)

Type material and type locality. *Holotype*: UF 3857, Bocas del Toro, Panama, Punta Caracol (9.3777° N, 82.1863° W), 3 m in depth, coll. Jan Vicente, May 8, 2015. *Paratypes:* Cartagena, Colombia: ICN-MHN(Po) 270, Islas del Rosario, Pajarales, close to Yohmara islet (10.1779° N, 75.7750° W), 5 m in depth, coll. Sven Zea, March 10, 2002. INV POR1338, 1339, Isla del del Rosario, Pajarales (lagoon) (10.1780° N, 75.7750° W), 4–5 m in depth coll. Sven Zea, August 13, 2014.

Additional material. Bocas del Toro, Panamá: uncatalogued samples PPA 36, Isla Colón, Aeropuerto (9.3339° N, 82.2548° W), 7 m in depth, coll. Sven Zea, August 9, 2012; PPA 48, Isla Cristobal, Buoy 19 (9.3018° N, 82.2943° W), 8 m in depth, coll. Sven Zea, August 15, 2012. Martinique: uncatalogued samples SZ-20, SZ-21, Les Anses d´Arlet, Le Grande Anse, Salomon´s Garden, Northeast point (14.5053° N, 61.0947° W), 11–18 m, coll. Sven Zea, December 5, 2013. Uncatalogued fragment SZ-23, Les Anses d´Arlet, Le Grande Anse, Pointe Legarde, Southeast point (14.4969° N; 65.0897° W), 24 m, coll. Sven Zea, December, 2013.

Description. Group of tubes or chimneys or ramified, erect, anastomosed mounds, reaching 10–30 cm in width and 10–30 cm in height (Fig. 4A, B, C). Sponge surface is smooth, but sometimes with horizontal crests (sinuous channels) (Fig. 4C). Surface is also quite porous (0.5–1 mm diameter pores) and in some specimens can be reticulated. Oscules are generally apical and measure 2–5 mm in some individuals (Fig. 3A) and up to ~~a~~ 1–2 cm in others (Fig. 4C). A translucent membrane surrounding the oscules was obvious in some individuals. Consistency is firm, rigid and tough to cut with scalpel but brittle once squeezed with considerable force. Unlike the other congeneric species described in this study, the sponge did not exude a sticky substance when squeezed. The exterior color varies between brownish amber, yellow with sporadic brownish-green blotches (Fig. 4A) to crimson with light and dark tones (Fig. 4C). Color at the base and in the interior of the sponge is cream.

Skeleton. The ectosome is partially tangential, isodictyal, with unispicular or paucispicular tracts (1–6 spicules and 13–75 µm across) (Fig. 4Bi, 4Ci). Spongin was detected in some nodal points where ascending choanosomal tracts traversed the ectosome (Fig. 4Bi). Pigments from cyanobacteria penetrate about 1 mm into the choanosome (Fig. 4Bii, 4Cii). The choanosome is an anisotropic reticulation with ascending multispicular tracts (4–8 spicules and 1–80 µm across) and occasional 130–250 µm openings, interconnected by single or loosely arranged spicules (Fig. 4Bii, 4Cii). The choanosomal tracts have a larger number of free spicules, are thicker, more confused and become harder to depict deeper into the choanosome. Tracts become thinner as they ascend towards the ectosome, eventually becoming almost unispicular. Channels in the choanosome have a diameter ~~300 µm–2 mm~~.

Spicules. Slightly curved oxeas, hastate, with conical to sharp ends, 130–260 ~~µm long by~~ 5–18 µm ~~wide~~ (Table 1). The mean of oxea sizes vary according to the location where specimens were collected (Fig. 5Di-Diii). C-shaped ~~microsclere~~ sigmas are present in the ectosome and choanosome, 8–*21*–33 µm in length, showing no variation in size among geographic locations but varied abundance across specimens (Table 2).

Habitat and ecology. This species is found on shallow ~~(3 m)~~ patch reefs and sand flats in Bocas del Toro and Islas del Rosario (Cartagena), and deeper ~~(11–24 m)~~ reef habitats in Martinique. This species is viviparous, being the only congener observed to brood larvae in the summer months in Bocas del Toro, Panama (Fig. 4Aii). A detailed description of the morphology and phototactic swimming behavior of *Neopetrosia sigmafera* **sp. nov.** larvae are described by Collin *et al.* (2010). At the time, *N. sigmafera* **sp. nov.** was misidentified as *N. proxima*, but the morphological assessment in this study clearly shows a different spicule composition, and skeletal arrangement of the choanosome and ectosome ~~to~~ *N. proxima*. The presence of larvae seems to be a diagnostic character that also helps distinguish it from *N. proxima* when both are found living in the same habitat. Zooanthids are occasionally found growing on *N. sigmafera* **sp. nov.** but not on *N. proxima* in shallow reef habitats of Bocas del Toro (Fig. 4Aii). This sponge is known to harbor a host specific community of *Synechococcus spongiarum* cyanobacteria which produce high amounts of chlorophyll-a (Erwin & Thacker 2007, 2008).

Distribution. Panama (Bocas del Toro), Colombia (Cartagena), Martinique (Les Anses d' Arlet). S. Zea observed specimens in Belize (Carrie Bow Cay and Pelican Cays).

Taxonomic remarks. Similar *in situ* characters shared between *Neopetrosia sigmafera* **sp. nov.** and *Neopetrosia proxima* have made their classification difficult over the last decade; particularly in Bocas del Toro, Panama, where they are sympatric (Fig. 1A and 4A). However, a closer look at the spicules of each species revealed that oxeas are longer, thicker, and have more hastate endings in *N. sigmafera* **sp. nov.** (130–260 × 5–18 µm) compared to *N. proxima* (85–223 × 2.4–10 µm). *Neopetrosia sigmafera* **sp. nov.** also has sigmas as microscleres, which are never present in *N. proxima* or any other *Neopetrosia* spp. The skeleton is also less dense with less massive spicule tracts in *N. sigmafera* **sp. nov.** ~~.~~ Oxeas are also smaller and thinner than *Neopetrosia dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** ~~.~~ Grooves, which are a diagnostic character of *N. dendrocrevacea* **sp. nov.** are also absent in *N. sigmafera* **sp. nov.** ~~.~~ From all congeners, larvae were only found in *Neopetrosia sigmafera* **sp. nov.**, suggesting that viviparity seems to be a diagnostic character of this species.

The external morphology of this species is also similar to *Neopetrosia dominicana* (Pulitzer-Finali 1986) but ~~*N. dominicana*~~ has strongyles instead of oxeas and also lacks sigmas. There are also some similarities with *Xestospongia caminata* (Pulitzer-Finali 1986) although the oscules

are much larger (5–10 mm) and spicules are larger (200–260 × 5–14 µm) in the latter species. In addition to oxeas, the spicule composition of *X. caminata* also includes strongyles while sigmas are absent. Additionally, although *N. sigmafera* **sp. nov.** also shares a similar branching morphology with ~~*Neopetrosia*~~ *subtriangularis* (Duchassaing & Michelotti 1864) the skeleton of *N. subtriangularis* is much more defined with numerous circular channels and denser multispicular tracts and also has smaller oxeas (131–181 × 1.6–11.7 µm). *Xestospongia bocatorensis* (Diaz *et al*. 2007) also has hastate oxeas and sigmas but oxeas reach greater lengths (230–320 × 8–15 µm), and sigmas have a smaller length range (10–26 vs. 8–33 µm in *N. sigmafera* **sp. nov.**). In addition, *X. bocatorensis* is a thinly encrusting sponge with a purple signature color from associated *Oscillatoria* filamentous cyanobacteria throughout the ectosome and choanosome (Diaz *et al*. 2007). In contrast, color patterns in *N. sigmafera* **sp. nov.** are similar to other congeners, having two distinct colors ~~throughout~~ the body, brown ectosome from cyanobacteria and cream choanosome.

Etymology. The given species name is an adjective that combines the name of the *sigma* microsclere with the Greek suffix *phero*, which translates to "carrying" or "bearing" (Brown, 1956). We use the feminine *sigmafera*, assuming that *Neopetrosia* is feminine, following Article 31.2 of the International Code for Zoological Nomenclature (http://www.iczn.org/, accessed on October 1, 2018). Add your results here.

Phylogenetic analysis. The phylogenetic relationship between novel *Neopetrosia* spp. using mitochondrial (COI) and nuclear genes (28S rRNA/18S rRNA) reconfirmed the polyphyletic nature of this genus (Erpenbeck *et al.* 2007; Redmond *et al.* 2011; Setiawan 2014~~; Setiawan~~ 2018) (Fig. 5). Nevertheless, the use of different markers allowed us to detect enough genetic differences across all new *Neopetrosia* spp. and confirmed their morphological differences. In particular, COI showed the highest resolution of sequence dissimilarity between all new congeners, confirming morphological differences as diagnostic characters (Fig. 5A). For example, *Neopetrosia proxima*, *Neopetrosia dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** were all closely related and formed a divergent clade that was closely related (87 % identical) to *Amphimedon queenslandica* sequence EU237474.1 (Hooper & Van Soest 2006; Kayal & Lavrov 2008). Within this clade all *N. proxima* morphotypes were 100 % identical to each other, 96 % identical to *N. dendrocrevacea* **sp. nov.**, 95 % identical to *Neopetrosia cristata* **sp. nov.**, and 81% identical to *N. sigmafera* **sp. nov.**. As predicted by similarities in their morphological characters, *N. dendrocrevacea* **sp. nov.** was more closely related to *Neopetrosia cristata* **sp. nov.** than to any other congeneri~~c~~, with 98 % identity, and diverged from *N. proxima* with strong bootstrap support. Additional sequence data spanning the COI extension (700 bp product alignment) showed that *N. dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** were 96 % identical which further supports their heterospecificity. As expected, *N. sigmafera* **sp. nov.** was the most distantly related (<85 % sequence similarity) congener with a well-supported and deeply divergent clade. The closest relative to *N. sigmafera* **sp. nov.** was *Gelliodes wilsoni*

(Carballo *et al.* 2013) with 99 % identity. Additional congeners like *Neopetrosia exigua* (Kirkpatrick 1900) sequence KX454496.1 and *Neopetrosia seriata* (Hentschel 1912) sequence JN242213.1, were distantly related (<85 %) from all new congeners in this study.

The phylogenetic tree of the 28S rRNA gene showed a very similar topology to COI. For example, as in COI, a well-supported clade (1/100) with all specimens of *N. proxima*, *N. dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.**, were also deeply divergent from the rest of the congeners in the tree (Fig. 5B). Nonetheless, 28S rRNA did show a lower resolution of sequence dissimilarity, with no sequence differences between *Neopetrosia cristata* **sp. nov.** and *N. dendrocrevacea* **sp. nov.** Both sequences of these species were 97 % identical, with strong support (1/100), to all *N. proxima* morphotypes in the clade. All congeneric sequences in this clade were 93–94 % identical to *Petrosia lignosa* (Wilson 1925) KC869595.1. In addition, *N. sigmafera* **sp. nov.** showed <85 % sequence identity to all congeners in this clade. The closest relatives to *N. sigmafera* **sp. nov.** were *Gelliodes callista* (De Laubenfels 1954) KC869562.1 (89 % identical) and *Xestospongia deweerdtae* (Lehnert & Van Soest 1999) KX668524.1 (90 % identical). Additional sequences from congeners like *Neopetrosia rosariensis* (Zea & Rutzler 1983) KC869457.1 and *Neopetrosia subtriangularis* (Duchassaing 1850) KC869591.1, were <85 % identical to all new species. The only other congener with the closest sequence similarity to any of the new species was *Neopetrosia carbonaria* (Lamarck 1814) with 88 % sequence identity to *N. sigmafera* **sp. nov.**.

The phylogeny of *Neopetrosia* spp. using 18S rRNA resulted in the lowest resolution of sequence dissimilarity with four congeners being 100 % identical and grouping into Clade C (Redmond *et al.* 2011) (Fig. 5C). Identical congeners include *Neopetrosia cristata* **sp. nov.**, *N. proxima*, *N. exigua*, and *N. dendrocrevacea* **sp. nov.** The sequence from *N. sigmafera* **sp. nov.** was 93 % identical to congeners in Clade C and grouped into Clade B, which also included *N. carbonaria*. *N. rosariensis* grouped into Clade A and was 95 % identical to congeners in Clade C and 97 % identical to *N. sigmafera* **sp. nov.**

Discussion

Morphological and molecular assessments of putative *Neopetrosia proxima* and close relatives sampled throughout the lesser Antilles and the Southern Caribbean revealed three new species with a variety of new morphological characters and a new reproductive strategy for the genus. *Neopetrosia* was originally defined by the presence of a hispid surface produced by the rise of subectosomal tracts above a compact choanosomal skeleton composed of circular meshes with anisotropic reticulation of oxeas that are less than 200 µm in length (De Laubenfels 1949). In a closer examination of the new congeners we have found that *Neopetrosia dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov**. have a more confused ectosomal and choanosomal skeleton with less obvious circular meshes. The recently discovered congener from mesophotic reefs in Curacao, *Neopetrosia ovata,* also shows a similar confused skeleton organization as *N. dendrocrevacea* **sp. nov.** and *N. cristata* **sp. nov.** (Van Soest *et al.* 2014). In addition, *Neopetrosia sigmafera* **sp. nov.** further complicates this definition by the presence of sigmas

(microscleres), oxeas >200 µm in length and being the only congener so far known to brood larvae.

Our study also highlights the effect that environmental factors may have on the size of oxeas. Previous studies have shown that spicule morphology can be influenced by hypersilicification as a result of high silica concentrations (Maldonado *et al.* 1999) and by living in association with other sponges (Vicente *et al.* 2014). In this study, higher silica concentrations from terrestrial runoff in habitats closer to the continental shelfs are likely the cause of larger oxeas in both *N. dendrocrevacea* **sp. nov.** and *N. sigmafera* **sp. nov.** collected in Bocas del Toro, Urabá, Cartagena and Santa Marta (continental shelf) than specimens collected in San Andres or Martinique (oceanic islands). Similar variations in spicule size have been reported for other species collected in sites with low/high terrestrial runoff (Zea 1987, Debiasse & Hellberg 2015, Vicente *et al.* 2016, Silva & Zea 2017).

Differences in morphological characters were mostly resolved by partial sequences of the mitochondrial gene (COI) but less so by nuclear genes (28S rRNA and 18S rRNA). These results are in agreement with the phylogeny of other Haplosclerida where mitochondrial genes (including the COI extension) resolved up to 12 well supported subclades of *Haliclona* spp. while ribosomal sequences only resolve six (Knapp *et al.* 2015). Similar results were also observed in *Tethya* spp. where mitochondrial genes resolved up to five supported subclades, while ribosomal sequences supported four (Schaffer *et al.* 2018). In all phylogenetic trees, *N. proxima*, *N. dendrocrevacea* **sp. nov.** and *Neopetrosia cristata* **sp. nov.** formed a well supported clade with deep divergence from *N. sigmafera* **sp. nov.** These results are congruent with multiple diagnostic morphological characters present in *N. sigmafera* **sp. nov.** that are absent in all other congeners (i.e. presence of sigmas and brooding larvae). Despite these striking differences, and distant genetic relatedness to other congeners, it is difficult to place *N. sigmafera* **sp. nov.** in a different genus on the basis of its viviparous nature or presence of sigmas. Other than *Xestospongia bocatorensis, N. sigmafera* **sp. nov.** is the only other larval brooding Petrosiidae (Collin *et al.* 2010), which rejects the hypothesis that all Petrosiidae are oviparous (Fromont & Bergquist 1994; Maldonado & Riesgo 2009), and shows that viviparity is not a good synapomorphic character (Van Soest & Hooper 2002). In addition, the only other Petrosiidae with sigmas is also *X. bocatorensis* (Diaz *et al.* 2007) and shows that sigmas can be shared across different genera within Petrosiidae. Furthermore, neither of these characters are monophyletic across different taxa by mitochondrial or ribosomal molecular markers (Redmond *et al*. 2011).

Despite highlighting the polyphyletic nature of Haplosclerida, applying a multi-locus based approach using ribosomal and mitochondrial markers continues to prove as a useful tool in resolving the taxonomy between congeneric species. Recently this approach has been used across a wide taxonomic range of sponges (Erpenbeck *et al.* 2016; Yang *et al.* 2017). These methods are useful in assessing a "first pass" classification for a wide range diversity of sponges and integration of morphological systematics. However, in order understand the evolutionary relationship within Haplosclerida we must continue to focus our research efforts towards finding

monophyletic markers. ~~Future research to fill the monophyly knowledge gap of Haplosclerida should continue to make more genomes from species within different orders of Haplosclerida available.~~

Conclusions

We report molecular and morphological congruence of three new *Neopetrosia* spp. in the Caribbean. Molecular congruence was mostly revealed at the highest resolution by partial sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) and less by nuclear ones (18S rRNA and 28S rRNA). The most distantly related new congener based on partial COI sequences was *N~~eopetrosia~~ sigmafera* **sp. nov.** which adds the presence of sigma microscleres, significantly wider/longer oxeas (>200 µm), and the presence of parenchymella larvae to the genus. *Neopetrosia dendrocrevacea* **sp. nov.** and *N~~eopetrosia~~ cristata* **sp. nov.** were confirmed as sister species based on partial COI sequences ~~which shared the appearance~~ of a more confused skeletal arrangement and the presence of grooves on the surface of the sponge body ~~that converge at the~~ oscula. Differences in morphological characters from *N~~eopetrosia~~ proxima* were also confirmed by differences in COI sequences. Despite being a polyphyletic genetic fragment in *Neopetrosia* spp., our study shows that the partial COI gene fragment continue to be a useful marker in resolving cryptic species belonging to highly diverse orders with variable growth forms.

Acknowledgements

We thank Cristina Díaz and Robert W. Thacker for providing helpful discussion points on the taxonomy of *Neopetrosia* spp. We are indebted to Rachel Collin and Plinio Gondola for hosting J.V. at the Bocas del Toro STRI research station. Arcadio Castillo and Micah J. Marty, are thanked for SCUBA diving assistance. We thank Laszlo Takacs at the NanoImaging Facility, University of Maryland Baltimore, and Inga Conti-Jerpe at the University of North Carolina Wilmington, for help with SEM images. This is HIMB contribution Nr. XXX, SOEST contribution Nr. XXX, CECIMAR contribution Nr. XXX and INVEMAR contribution Nr. XXX.

References

Abràmofff, M.D., Magalhães, P.J. & Ram, S.J. (2005) Image processing with ImageJ Part II. *Biophotonics International*, 11, 36–43.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
Alvarez, B., van Soest, R.W.M. & Rützler, K. (2002) *Svenzea*, a new genus of Dictyonellidae (Porifera: Demospongiae) from the tropical reef environments, with description of two new species. *Contributions to Zoology*, 71 (4), 171—176.
Borchiellini, C., Chombard, C., Manuel, M., Alivon, E., Vacelet, J. & Boury-Esnault, N. (2004) Molecular phylogeny of Demospongiae: Implications for classification and scenarios of character evolution. *Molecular Phylogenetics and Evolution*, 32, 823–837.
Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., Ingram, K.K. and Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends in ecology & evolution*, *22*(3), pp.148–155.
Brown, R.W. (1956) *Composition of scientific words. A manual of methods and lexicon of materials for the practice of logetchnics,* revised edition. Smithsonian Instituion Press, Wahington, D.C., 882 pp.
Campos, M., Mothes, B., Eckert, R. & Van Soest, R.W.M. (2005) Haplosclerida (Porifera: Demospongiae) from the coast of Maranhão State, Brazil, Southwestern Atlantic. *Zootaxa*, 963, 1–22.
Carballo, J.L., Aguilar-Camacho, J.M., Knapp, I.S. & Bell, J.J. (2013) Wide distributional range of marine sponges along the Pacific Ocean. *Marine Biology Research*, 9 (8), 768—775.
Cárdenas, P., Pérez, T. & Boury-Esnault, N. (2012) Sponge Systematics Facing New Challenges. *Advances in Marine Biology* 61, 79–209.
Chombard, C., Boury-Esnault, N. & Tillier, S. (1998) Reassessment of Homology of Morphological Characters in Tetractinellid Sponges Based on Molecular Data. *Systematic Biology*, 47 (3), 351–66.
Collin, R., Díaz, M.C., Norenburg, J., Rocha, R.M., Sánchez, J.A., Schulze, A., Schwartz, M. & Valdés, A. (2005) Photographic Identification Guide to Some Common Marine Invertebrates of Bocas Del Toro, Panama. *Caribbean Journal of Science*, 41 (3), 638–707,
Collin, R., Mobley, A.S., Lopez, L.B., Leys, S.P., Diaz, M.C. & Thacker, R.W. (2010) Phototactic responses of larvae from the marine sponges *Neopetrosia proxima* and *Xestospongia bocatorensis* (Haplosclerida: Petrosiidae). *Invertebrate Biology,* 129, 121–128.
Concepcion, G.T., Crepeau, M.W., Wagner, D., Kahng, S.E. and Toonen, R.J., 2008. An alternative to ITS, a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus Carijoa. *Coral Reefs*, *27*(2), pp.323–336.
Debiasse, M.B. & Hellberg, M.E. (2015) Discordance between morphological and molecular species boundaries among Caribbean species of the reef sponge *Callyspongia*. *Ecology and Evolution*, 5 (3), 663–75.
Díaz, M.C. (2005) Common sponges from shallow marine habitats from Bocas del Toro region, Panama. *Caribbean Journal of Science*, 41, 465–475.

Díaz, M.C., Thacker, R.W., Rützler, K., Piantoni, C. (2007) Two new haplosclerid sponges from Caribbean Panama with symbiotic filamentous cyanobacteria , and an overview of sponge-cyanobacteria associations. *In:* Custódio, M.R., Lôbo-Hajdu, G., Hajdu, E. & Muricy, G. (Eds.), *Porifera Research. Biodiversity, Innovation and Sustainability*. Livros de Museu Nacional 28, Rio de Janeiro, pp. 31–39.

Duchassaing de Fonbressin, P. (1850) *Animaux radiaires des Antilles*. Plon Frères, Paris, 35 pp.

Duchassaing de Fonbressin, P. & Michelotti, G. (1864) Spongiaires de la mer Caraibe. *Natuurkundige verhandelingen van de Hollandsche maatschappij der wetenschappen te Haarlem*, 21, 1–124.

Erpenbeck, D., Duran, S., Rützler, K., Paul, V., Hooper, J.N.A. & Wörheide, G. (2007) Towards a DNA taxonomy of Caribbean demosponges: A gene tree reconstructed from partial mitochondrial CO1 gene sequences supports previous rDNA phylogenies and provides a new perspective on the systematics of Demospongiae. *Journal of the Marine Biological Association of the United Kingdom*, 87 (6), 1563–1570.Erpenbeck, D., Voigt, O., Al-Aidaroos, A.M., Berumen, M.L., Büttner, G., Catania, D., Guirguis, A.N., Paulay, G., Schätzle, S. & Wörheide, G. (2016) Molecular biodiversity of Red Sea demosponges. *Marine Pollution Bulletin*, 105 (2), 507–514.

Erwin, P.M. & Thacker, R.W. (2007) Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. *Journal of the Marine Biological Association of the United Kingdom*, 87 (6), 1683–1692.

Erwin, P.M. & Thacker, R.W. (2008) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. *Molecular Ecology*, 17 (12), 2937–2947.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.

Fromont, J. & Bergquist, P.R. (1994) Reproductive biology of three sponge species of the genus *Xestospongia* (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef. *Coral Reefs*, 13, 119–126.

Forsman, Z.H., Barshis, D.J., Hunter, C.L. and Toonen, R.J., 2009. Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC evolutionary biology*, *9*(1), p.45.

Hentschel, E. (1912) Kiesel- und Hornschwämme der Aru- und Kei-Inseln. *Abhandlungen herausgegeben von der Senckenbergischen naturforschenden Gesellschaft*, 34, 293–448.

Holmes, B. & Blanch, H. (2007) Genus-specific associations of marine sponges with group I crenarchaeotes. *Marine Biology*, 150, (5), 759–772.

Hooper, J.N.A. (1984) A new genus and two new species of haplosclerid sponges (Porifera: Demospongiae) from the Timor Sea, Northwest Australia. *Proceedings of the Royal Society of Victoria* 96, 55–60.

754 Hooper, J.N.A. & Van Soest, R.W.M. (2006) A new species of *Amphimedon* (Porifera,
755 Demospongiae, Haplosclerida, Niphatidae) from the Capricorn-Bunker Group of Islands, Great
756 Barrier Reef, Australia: Target species for the "sponge genome project." *Zootaxa*, 1314, 31–39.
757 Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees.
758 *Bioinformatics*, 17, 754–755.
759 Kayal, E. & Lavrov, D. V. (2008) The mitochondrial genome of *Hydra oligactis* (Cnidaria,
760 Hydrozoa) sheds new light on animal mtDNA evolution and cnidarian phylogeny. *Gene*, 410 (1),
761 177–186.
762 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S.,
763 Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A.
764 (2012) Geneious Basic: An integrated and extendable desktop software platform for the
765 organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
766 Kelly-Borges, M. & Pomponi, S.A. (1994) Phylogeny and classification of lithistid sponges
767 (Porifera: Demospongiae): a preliminary assessment using ribosomal DNA sequence
768 comparisons. *Molecular Marine Biology and Biotechnology*, 3, 87–103.
769 Kirkpatrick, R. (1900) On the Sponges of Christmas Island. *Proceedings of the Zoological*
770 *Society of London*, 127–141.
771 Knapp, I.S., Forsman, Z.H., Williams, G.J., Toonen, R.J. & Bell, J.J. (2015) Cryptic species
772 obscure introduction pathway of the blue Caribbean sponge (*Haliclona* (*Soestella*) *caerulea*),
773 (order: Haplosclerida) to Palmyra Atoll, Central Pacific. *PeerJ*, 3, e1170.
774 Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics
775 Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
776 Lamarck, J. (1814) Sur les polypiers empâtés. Suite du mémoire intitulé: Sur les polypiers
777 empâtés. Suite des éponges. *Annales du Muséum national d'histoire naturelle Paris*, 20, 294–
778 312.
779 De Laubenfels, M.W. (1932) The marine and fresh-water sponges of California. *Proceedings of*
780 *the United States National Museum*, 81, 1–140.
781 De Laubenfels, M.W. (1934) New sponges from the Puerto Rican deep. *Smithsonian*
782 *Miscellaneous Collections*, 91, 1–28.
783 De Laubenfels, M.W. (1949) Sponges of the western Bahamas. *American Museum Novitates*,
784 1431, 1–25.
785 De Laubenfels, M.W. (1954) *The sponges of the West-Central Pacific*. *Oregon State*
786 *Monographs. Studies in Zoology*, 7, 1–306.
787 Lavrov, D. V., Wang, X. & Kelly, M. (2008) Reconstructing ordinal relationships in the
788 Demospongiae using mitochondrial genomic data. *Molecular Phylogenetics and Evolution*, 49
789 (1), 111–124.
790 Lehnert, H. & Soest, R.W. Van (1996) Noth Jamaican Deep Fore-reef Sponges. *Beaufortia*, 46,
791 53–81.
792 Lehnert, H. & Van Soest, R.W.M. (1999) More North Jamaican deep fore-reef sponges.
793 *Beaufortia*, 49, 141–169.

794 Maldonado, M., Carmona, M.C., Uriz, M.J. & Cruzado, A. (1999) Decline in Mesozoic reef-
795 building sponges explained by silicon limitation. *Nature*, 401, 785–788.
796 Maldonado, M. & Riesgo, A. (2009) Gametogenesis, embryogenesis, and larval features of the
797 oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *Marine Biology*, 156 (10),
798 2181–2197.
799 Morrow, C. & Cárdenas, P. (2015) Proposal for a revised classification of the Demospongiae
800 (Porifera). *Frontiers in Zoology*, 12, 7.
801 Muricy, G., Lopez, D.A., Hajdu, E., Carvalho, M.S., Morae, F.C., Klautau, M., Menegola, C. &
802 Pinheiro, U. (2011) *Catalogue of Brazilian Porifera.* Museu Nacional, Río de Janeiro, Serie
803 Livros, 46, 299 pp.
804 Pérez, T., Díaz, M.C., Ruiz, C., Cóndor-Luján, B., Klautau, M., Hajdu, E., Lobo-Hajdu, G., Zea,
805 S., Pomponi, S.A., Thacker, R.W., Carteron, S., Tollu, G., Pouget-Cuvelier, A., Thélamon, P.,
806 Marechal, J.P., Thomas, O.P., Ereskovsky, A. V., Vacelet, J. & Boury-Esnault, N. (2017) How a
807 collaborative integrated taxonomic effort has trained new spongiologists and improved
808 knowledge of Martinique Island (French Antilles, eastern Caribbean Sea) marine biodiversity.
809 *PLoS ONE*, 12 (3), e0173859.
810 Pulitzer-Finali (1986) A collection of West Indian Demospongiae (Porifera). In appendix, a list
811 of the Demospongiae hitherto recorded from the West Indies. *Annali del Museo civico di storia*
812 *naturale Giacomo Doria*, 86, 154–158.
813 Redmond, N.E., Morrow, C.C., Thacker, R.W., Diaz, M.C., Boury-Esnault, N., Cárdenas, P.,
814 Hajdu, E., Lôbo-Hajdu, G., Picton, B.E., Pomponi, S.A., Kayal, E. & Collins, A.G. (2013)
815 Phylogeny and systematics of demospongiae in light of new small-subunit ribosomal DNA (18S)
816 sequences. In: *Integrative and Comparative Biology*, 53 (3), 388–415.
817 Redmond, N.E., Raleigh, J., van Soest, R.W.M., Kelly, M., Travers, S.A.A., Bradshaw, B.,
818 Vartia, S., Stephens, K.M. & McCormack, G.P. (2011) Phylogenetic relationships of the marine
819 Haplosclerida (Phylum Porifera) employing ribosomal (28s rRNA) and mitochondrial (COI,
820 nad1) gene sequence data. *PLoS ONE*, 6 (9), e24344.
821 Rützler, K., Diaz, M.C., Van Soest, R.W.M., Zea, S., Smith, K.P., Alvarez, B. & Wulff, J. (2000)
822 Diversity of sponge fauna in mangrove ponds, Pelican Cays, Belize. *Atoll Research Bulletin*,
823 476, 229–248.
824 Santos, G.G., Sandes, J., Cabral, A. & Pinheiro, U. (2016) *Neopetrosia* de Laubenfels, 1949 from
825 Brazil: Description of a new species and a review of records (Haplosclerida: Demospongiae:
826 Porifera). *Zootaxa*, 4114, 331–340.
827 Schaffer, M.R., Dave, S.K. & Bell, J.J. (2018) Hidden diversity in the genus *Tethya*: comparing
828 molecular and morphological techniques for species identification. *Heredity*, 1, doi:
829 10.1038/s41437-018-0134-6.
830 Setiawan, E. (2014) *Genetic diversity of selected petrosiid sponges*. Doctoral dissertation,
831 Ludwig-Maximillians University, Munich, 166 pp.

Setiawan, E., E. Erpenbeck, D., Wörheide, G., & De Voogd, N. J. (2018). Bearing the wrong identity: A case study of an Indo-Pacific common shallow water sponge of the genus *Neopetrosia* (Haplosclerida; Petrosiidae). *Zootaxa*, *4500*(1), 43-58.

Silva, J.A. & Zea, S. (2017) New records of sponges of the genera *Petrosia* and *Xestospongia* (Demospongiae: Haplosclerida: Petrosiidae) from the Colombian Caribbean. *Boletín de Investigaciones Marinas y Costeras*-, 46, 113–136.

Stat, M., Baker, A.C., Bourne, D.G., Correa, A.M., Forsman, Z., Huggett, M.J., Pochon, X., Skillings, D., Toonen, R.J., van Oppen, M.J. and Gates, R.D., 2012. Molecular delineation of species in the coral holobiont. *Advances in marine biology*, 63, 1–65.

Van Soest, R.W.M. (1980) Marine sponges from Curaçao and other Caribbean localities Part II. Haplosclerida. *Studies on the Fauna of Curaçao and Other Caribbean Islands*, 62, 1–173.

Van Soest, R.W.M. (1984) Marine sponges from Curaçao and other Caribbean localities. Part III. Poecilosclerida. *Studies on the Fauna of Curaçao and other Caribbean Islands*. 66 (199): 1–167.

Van Soest, R.W.M. (2017) Sponges of the Guyana Shelf. *Zootaxa*, 4217, 66 (199), 1–167.

Van Soest, R.W., Boury-Esnault, N., Hooper, J.N.A., Rützler, K., de Voogd, N.J., Alvarez de Glasby, B., Hajdu, E., Pisera, A.B., Manconi, R., Schoenberg, C., Klautau, M., Picton, B., Kelly, M., Vacelet, J., Dohrmann, M., Díaz, M.-C., Cárdenas, P. & Carballo, J.L. (2018) World Porifera database. *Accessed at http://www.marinespecies.org/porifera on 2018–9–10*.

Van Soest, R.W. M. & Hooper, J.N.A. (2002) Suborder Petrosina Boury-Esnault & Van Beveren, 1982. *In*: Hooper, J.N.A., Van Soest, R.W.M. & P. Willenz (Eds.), *Systema Porifera*, Volume 1. Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow, pp. 891–892.

Van Soest, R.W.M., Meesters, E.H.W.G. & Becking, L.E. (2014) Deep-water sponges (Porifera) from Bonaire and Klein Curaçao, Southern Caribbean. *Zootaxa*, 3878 (5), 401–443

Van Soest, R.W.M. & Stentoft, N. (1988) Barbados Deep-Water Sponges. In: Hummelinck, P.W. & Van der Steen, L.J. (Eds), Uitgaven van de Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen. *Studies on the Fauna of Curaçao and other Caribbean Islands*, 70, 1–175.

Van Soest, R.W.M., Stone, S.M., Boury-Esnault, N. & Rutzler, K. (1983) Catalogue of the Duchassing & Michelotti (1864) Collection of West Indian Sponges (Porifera). *Bulletin Zoologisch Museum, Universiteit van Amsterdam*, 9, 189–205.

Sollas, W.J. (1885) A classification of the sponges. *Annals and Magazine of Natural History*, (5) 16 (95), 395.

Sperling, E.A., Peterson, K.J. & Pisani, D. (2009) Phylogenetic-signal dissection of nuclear housekeeping genes supports the paraphyly of sponges and the monophyly of eumetazoa. *Molecular Biology and Evolution*, 26 (10), 2261–2274.

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.

Thacker, R.W., Hill, A.L., Hill, M.S., Redmond, N.E., Collins, A.G., Morrow, C.C., Spicer, L., Carmack, C.A., Zappe, M.E., Pohlmann, D., Hall, C., Diaz, M.C. & Bangalore, P. V. (2013)

Nearly complete 28S rRNA gene sequences confirm new hypotheses of sponge evolution. In: *Integrative and Comparative Biology*, 53 (3), 373–387.
Topsent, E. (1928) Spongiaires de l'Atlantique et de la Méditerranée provenant des croisières du Prince Albert ler de Monaco. Résultats des campagnes scientifiques accomplies par le Prince Albert I, Monaco. *Imprimerie de Monaco*, 74, 1–376.
Vicente, J., Zea, S. & Hill, R.T. (2016) Sponge epizoism in the Caribbean and the discovery of new *Plakortis* and *Haliclona* species, and polymorphism of *Xestospongia deweerdtae* (Porifera). *Zootaxa*, 4178 (2), 209–233.
Vicente, J., Zea, S., Powell, R.J., Pawlik, J.R. & Hill, R.T. (2014) New epizooic symbioses between sponges of the genera *Plakortis* and *Xestospongia* in cryptic habitats of the Caribbean. *Marine Biology* 161 (12), 2803–2818.
Vosmaer, G.C.J. (1885) Porifera, Parts Vll-XI. *In*: H. G. Bronn (Ed.), *Die Klassen und Ordnungen des Thier-Reichs*. , pp. 1. Leipzig & Heidelberg, pp. 177–368.
Wiedenmayer, F. (1977) 1 Experientia: Suppl.;28 *Shallow-water sponges of the western Bahamas*. Experientia. Supplementum 28. Birkhäuser Verlag, Basel and Stuttgart, 287 pp.
Wilson, H.V.P. (1925) Silicious and horny sponges collected by the US Fisheries steamer "Albatross" during the Philippine Expedition. Bulletin of the United States National Museum, 100 (2, 4), 273–532.
Wörheide, G. and Erpenbeck, D., 2007. DNA taxonomy of sponges—progress and perspectives. *Journal of the Marine Biological Association of the United Kingdom*, *87*(6), 1629–1633.
Yang, Q., Franco, C.M.M., Sorokin, S.J. & Zhang, W. (2017) Development of a multilocus-based approach for sponge (phylum Porifera) identification: Refinement and limitations. *Scientific Reports* 7, 41422.
Zea, S. (1987) *Esponjas del Caribe Colombiano*. Catálogo Científico, Bogotá, 286 pp.
Zea, S., Henkel, T. & Pawlik, J.R. (2014) The Sponge Guide: a picture guide to Caribbean sponges. Available online at *www.spongeguide.org* (accessed 22–10– 2018).
Zea, S. & Rutzler, K. (1983) A new species of *Xestospongia* (Porifera, Demospongea) from the Colombian Caribbean. *Caldasia*, 817–831.

Figure 1

Neopetrosia proxima (Duchassaing & Michelotti, 1864)

In-situ images of Panama specimens (A) UF 3856 (B) UF 3858 (C) UF 3860, with corresponding (from top to bottom) images of (i) tangential sections of the ectosome (LM); (ii) perpendicular sections through the ectosome and choanosome (LM); (iii) size and morphological variations of oxeas (SEM).

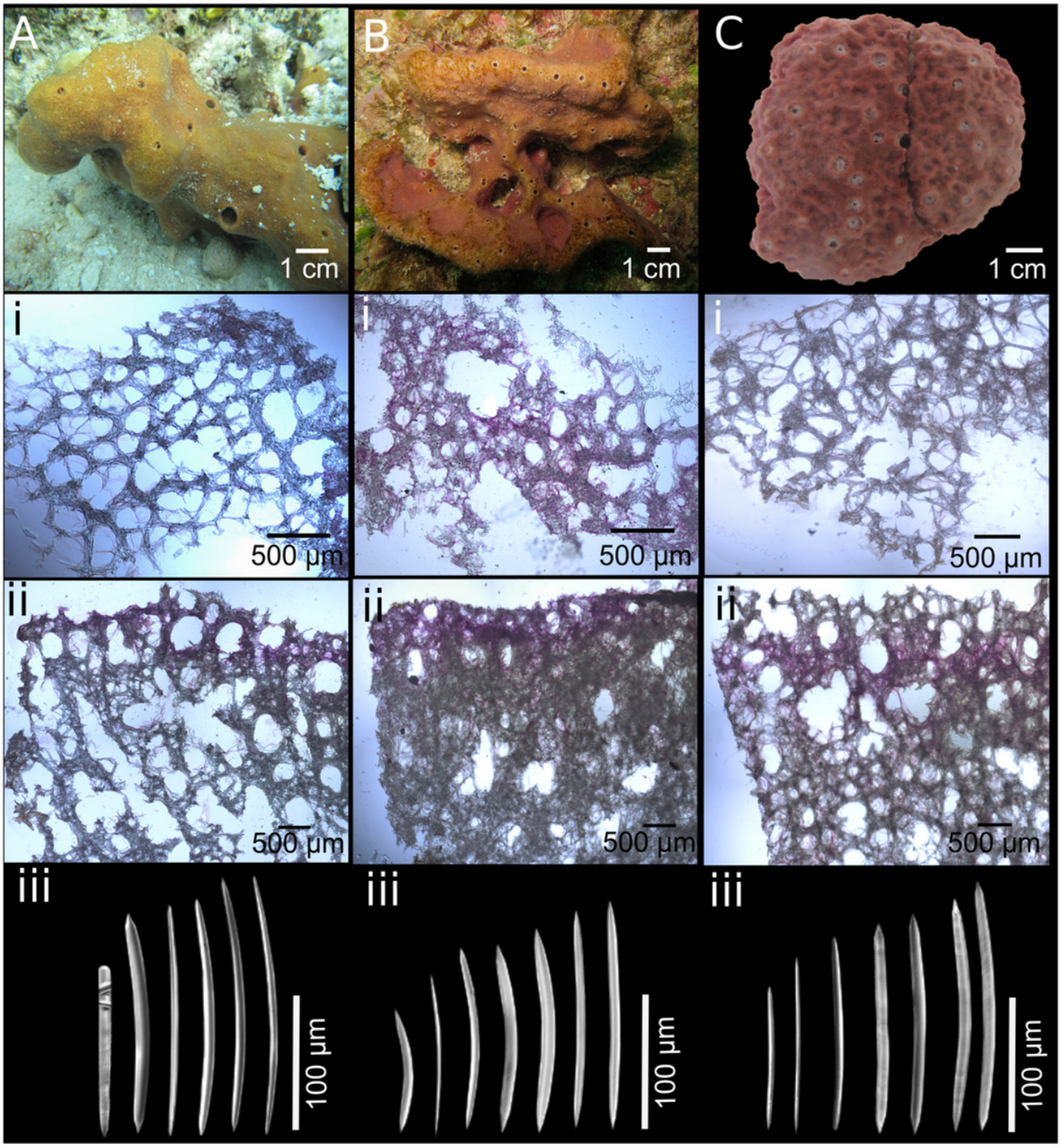
A
1 cm
B
1 cm
C
1 cm
i
500 µm
i
500 µm
i
500 µm
ii
500 µm
ii
500 µm
ii
500 µm
iii
100 µm
iii
100 µm
iii
100 µm

Figure 2(on next page)

Neopetrosia dendrocrevacea sp. nov.

In-situ images of individual PPA 07 (A) and the holotype UF 3854 (B), both from Panama, and of the paratype ICN-MHN(Po) 0269 (C) from Santa Marta, Colombia, with corresponding (second and third rows) images of (i) tangential sections of the ectosome (LM); (ii) perpendicular sections through the ectosome and choanosome (LM); (D) size and morphological variations of oxeas from specimens collected in (i) Uraba, (ii) Panama, (iii) Cartagena, (iv) Santa Marta, and (v) San Andrés Archipelago (LM). Scale bar of panel D is 100 µm.

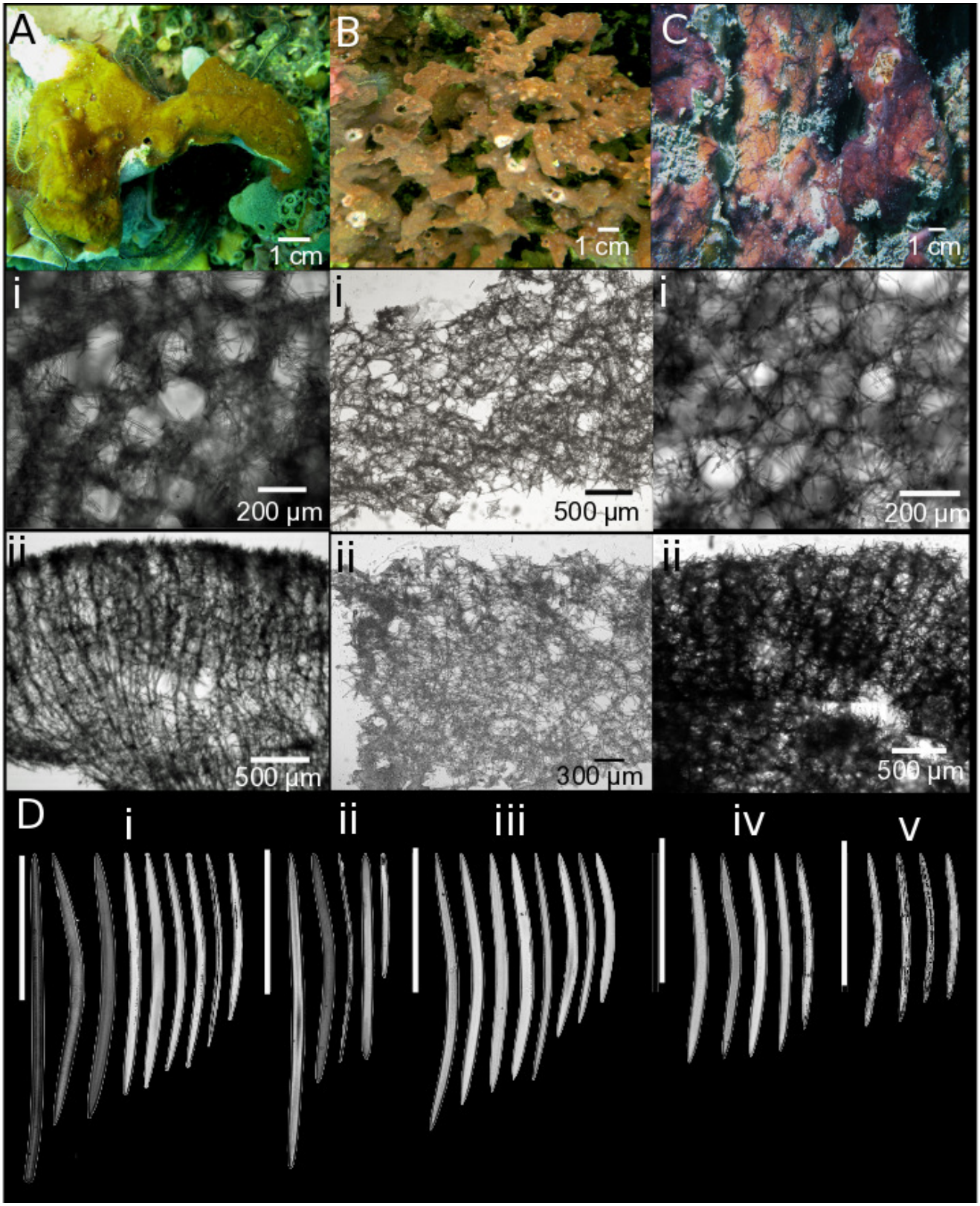
A
1 cm
B
1 cm
C
1 cm
i
200 µm
i
500 µm
i
200 µm
ii
500 µm
ii
300 µm
ii
500 µm
D
i
ii
iii
iv
v

Figure 3(on next page)

Neopetrosia cristata sp. nov.

Holotype (UF3859) (A) Ex-situ image; (B) tangential section of the ectosome (LM); (C) perpendicular section through the ectosome and choanosome (LM); (D) close-up of perpendicular section through the ectosome (LM); (E) close-up of perpendicular section through the choanosome (LM); (F) variation of oxeas (SEM)

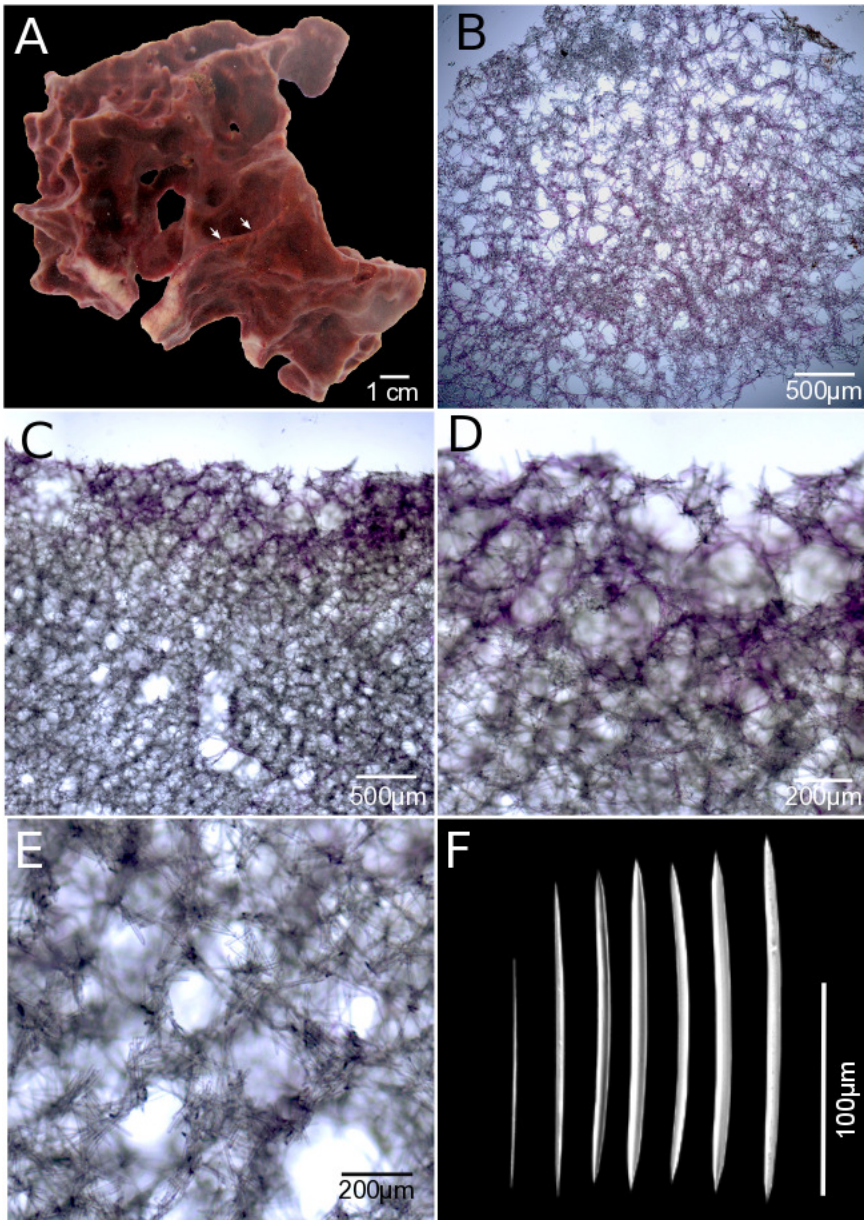
A
1 cm
B
500µm
C
500µm
D
200µm
E
200µm
F
100µm

Figure 4

Neopetrosia sigmafera sp. nov.

In-situ images of the holotype UF 3857 (A) and individuals PPA 38 (B), both from Panama, and SZ-21 from Martinique (C), with corresponding (second and third rows) images of (Ai) zoanthids; (Aii) brooding larvae (arrows); (Bi-Ci) tangential sections of the ectosome (LM); (Bii-Cii) perpendicular sections through the ectosome and choanosome (LM); (D) Size and morphological variations of oxeas and sigmas from (i) Panama, (ii) Cartagena, (iii) Martinique (LM).

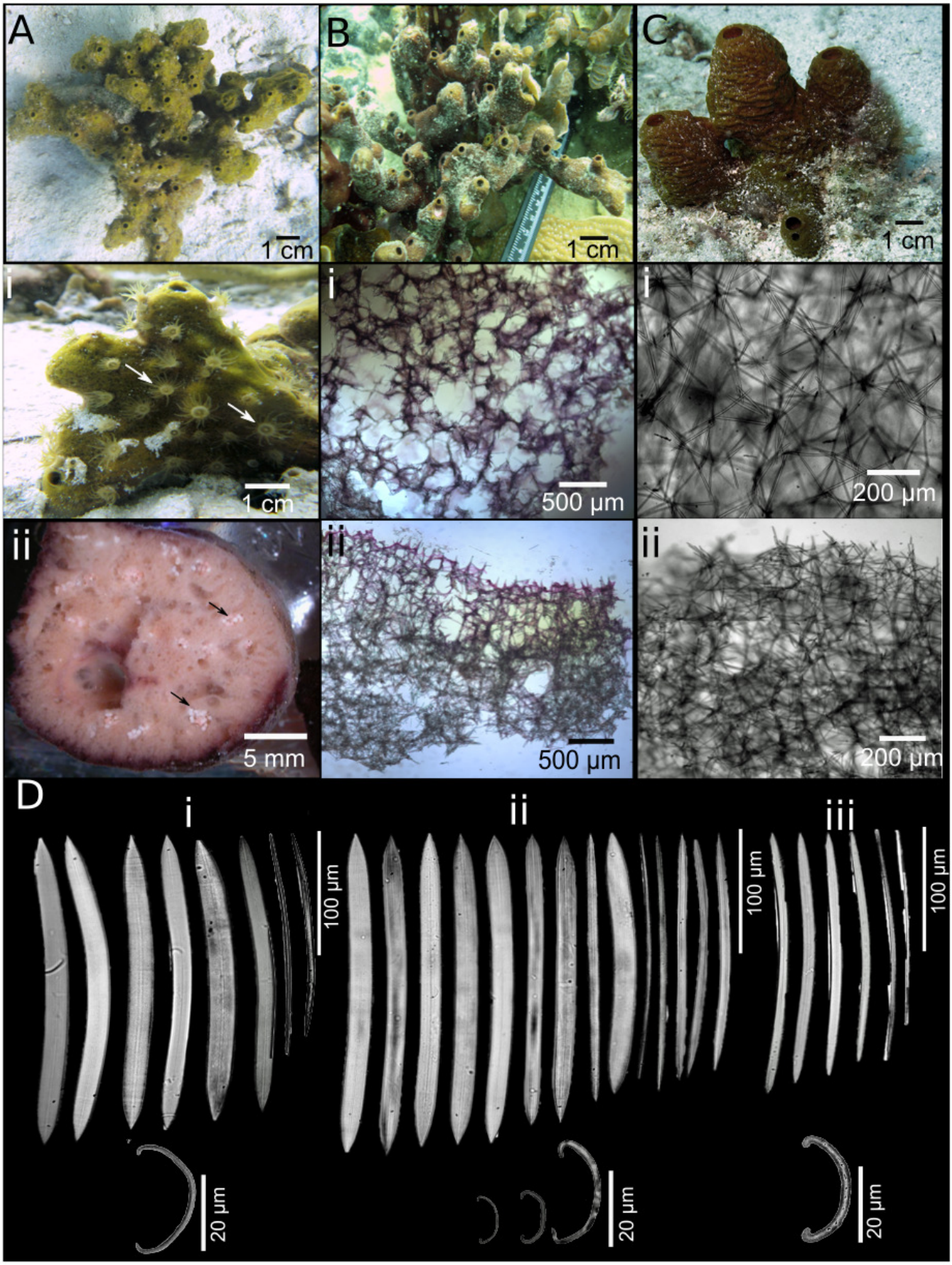
A
1 cm
B
1 cm
C
1 cm
i
1 cm
i
500 µm
i
200 µm
ii
5 mm
ii
500 µm
ii
200 µm
D
i
ii
iii
100 µm
100 µm
100 µm
20 µm
20 µm
20 µm

Figure 5(on next page)

Phylogenetic trees

Bayesian and Maximum Likelihood topology generated from partial sequences spanning the (A) Folmer (5') region of the cox1 gene, (B) D1-D2 region of the 28S rRNA gene and (C) 18S rRNA gene, from Haplosclerida taxa generated in this study (blue) and sequences downloaded from GenBank. Clades in panel C correspond to clades assigned by Redmond et al. (2013). Sequences in bold highlight other *Neopetrosia* species. Bootstrap values less than 50 % have been omitted from the trees. Numerical values at nodes show Bayesian posterior probabilities followed by RAxML bootstrap values. Nodes with '--' refer to the absence of the node generated by RAxML.

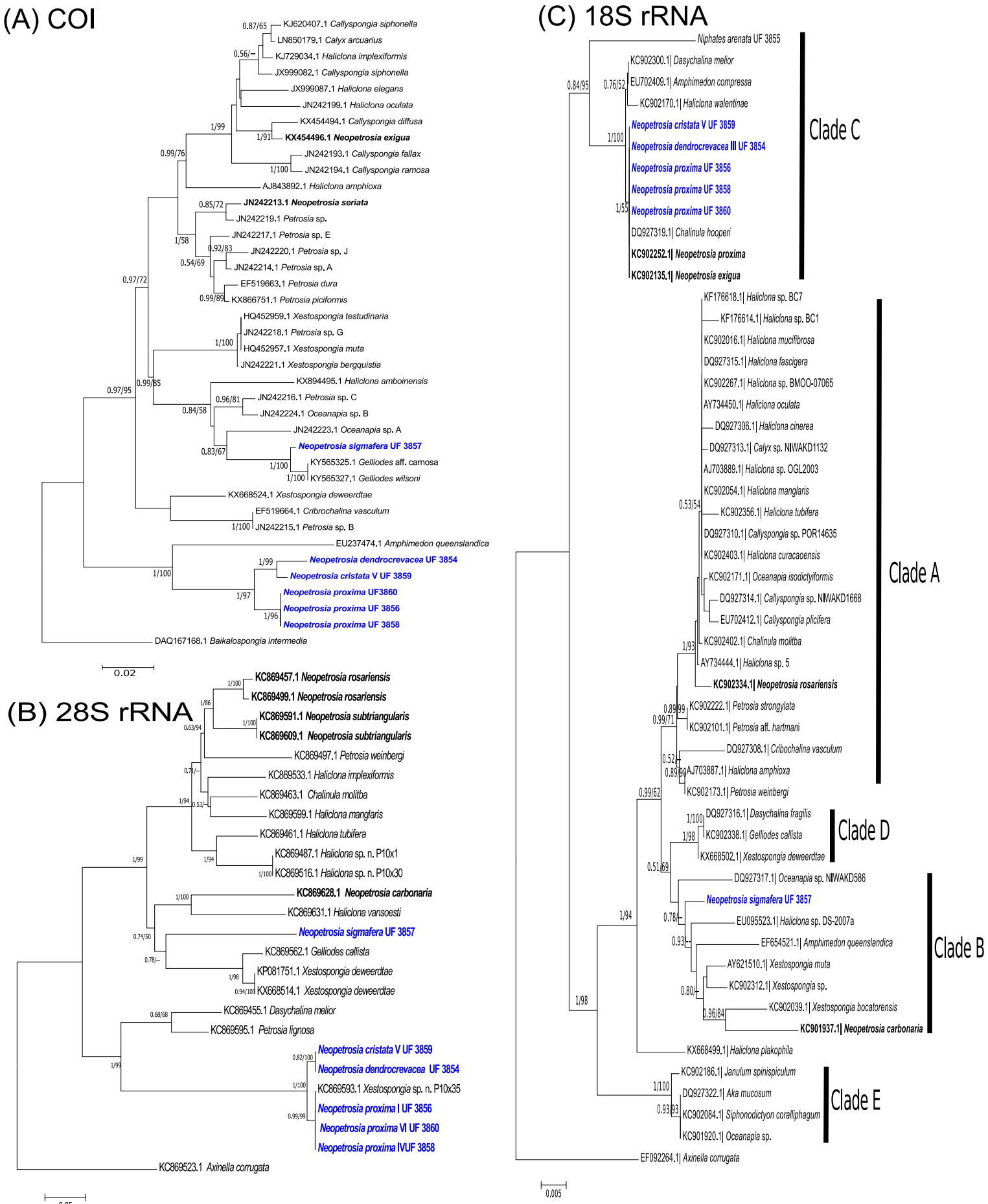
(A) COI
KJ620407.1 Callyspongia siphonella
LN850179.1 Calyx arcuarius
KJ729034.1 Haliclona implexiformis
JX999082.1 Callyspongia siphonella
JX999087.1 Haliclona elegans
JN242199.1 Haliclona oculata
KX454494.1 Callyspongia diffusa
KX454496.1 Neopetrosia exigua
JN242193.1 Callyspongia fallax
JN242194.1 Callyspongia ramosa
AJ843892.1 Haliclona amphioxa
JN242213.1 Neopetrosia seriata
JN242219.1 Petrosia sp.
JN242217.1 Petrosia sp. E
JN242220.1 Petrosia sp. J
JN242214.1 Petrosia sp. A
EF519663.1 Petrosia dura
KX866751.1 Petrosia piciformis
HQ452959.1 Xestospongia testudinaria
JN242218.1 Petrosia sp. G
HQ452957.1 Xestospongia muta
JN242221.1 Xestospongia bergquistia
KX894495.1 Haliclona amboinensis
JN242216.1 Petrosia sp. C
JN242224.1 Oceanapia sp. B
JN242223.1 Oceanapia sp. A
Neopetrosia sigmafera UF 3857
KY565325.1 Gelliodes aff. carnosa
KY565327.1 Gelliodes wilsoni
KX668524.1 Xestospongia deweerdtae
EF519664.1 Cribrochalina vasculum
JN242215.1 Petrosia sp. B
EU237474.1 Amphimedon queenslandica
Neopetrosia dendrocrevacea UF 3854
Neopetrosia cristata V UF 3859
Neopetrosia proxima UF3860
Neopetrosia proxima UF 3856
Neopetrosia proxima UF 3858
DAQ167168.1 Baikalospongia intermedia
0.02
(B) 28S rRNA
KC869457.1 Neopetrosia rosariensis
KC869499.1 Neopetrosia rosariensis
KC869591.1 Neopetrosia subtriangularis
KC869609.1 Neopetrosia subtriangularis
KC869497.1 Petrosia weinbergi
KC869533.1 Haliclona implexiformis
KC869463.1 Chalinula molitba
KC869599.1 Haliclona manglaris
KC869461.1 Haliclona tubifera
KC869487.1 Haliclona sp. n. P10x1
KC869516.1 Haliclona sp. n. P10x30
KC869628.1 Neopetrosia carbonaria
KC869631.1 Haliclona vansoesti
Neopetrosia sigmafera UF 3857
KC869562.1 Gelliodes callista
KP081751.1 Xestospongia deweerdtae
KX668514.1 Xestospongia deweerdtae
KC869455.1 Dasychalina melior
KC869595.1 Petrosia lignosa
Neopetrosia cristata V UF 3859
Neopetrosia dendrocrevacea UF 3854
KC869593.1 Xestospongia sp. n. P10x35
Neopetrosia proxima I UF 3856
Neopetrosia proxima VI UF 3860
Neopetrosia proxima IVUF 3858
KC869523.1 Axinella corrugata
0.05
(C) 18S rRNA
Niphates arenata UF 3855
KC902300.1| Dasychalina melior
EU702409.1| Amphimedon compressa
KC902170.1| Haliclona valentinae
Neopetrosia cristata V UF 3859
Neopetrosia dendrocrevacea III UF 3854
Neopetrosia proxima UF 3856
Neopetrosia proxima UF 3858
Neopetrosia proxima UF 3860
DQ927319.1| Chalinula hooperi
KC902252.1| Neopetrosia proxima
KC902135.1| Neopetrosia exigua
Clade C
KF176618.1| Haliclona sp. BC7
KF176614.1| Haliclona sp. BC1
KC902016.1| Haliclona mucifibrosa
DQ927315.1| Haliclona fascigera
KC902267.1| Haliclona sp. BMOO-07065
AY734450.1| Haliclona oculata
DQ927306.1| Haliclona cinerea
DQ927313.1| Calyx sp. NIWAKD1132
AJ703889.1| Haliclona sp. OGL2003
KC902054.1| Haliclona manglaris
KC902356.1| Haliclona tubifera
DQ927310.1| Callyspongia sp. POR14635
KC902403.1| Haliclona curacaoensis
KC902171.1| Oceanapia isodictyiformis
DQ927314.1| Callyspongia sp. NIWAKD1668
EU702412.1| Callyspongia plicifera
KC902402.1| Chalinula molitba
AY734444.1| Haliclona sp. 5
KC902334.1| Neopetrosia rosariensis
KC902222.1| Petrosia strongylata
KC902101.1| Petrosia aff. hartmani
DQ927308.1| Cribochalina vasculum
AJ703887.1| Haliclona amphioxa
KC902173.1| Petrosia weinbergi
Clade A
DQ927316.1| Dasychalina fragilis
KC902338.1| Gelliodes callista
KX668502.1| Xestospongia deweerdtae
Clade D
DQ927317.1| Oceanapia sp. NIWAKD586
Neopetrosia sigmafera UF 3857
EU095523.1| Haliclona sp. DS-2007a
EF654521.1| Amphimedon queenslandica
AY621510.1| Xestospongia muta
KC902312.1| Xestospongia sp.
KC902039.1| Xestospongia bocatorensis
KC901937.1| Neopetrosia carbonaria
Clade B
KX668499.1| Haliclona plakophila
KC902186.1| Janulum spinispiculum
DQ927322.1| Aka mucosum
KC902084.1| Siphonodictyon coralliphagum
KC901920.1| Oceanapia sp.
Clade E
EF092264.1| Axinella corrugata
0.005

Table 1(on next page)

Lengths of sigma of *Neopetrosia sigmafera* sp. nov.

Measurements are expressed as minimum–*mean*–maximum

Specimen	Location	Number of sigmas	Length (μm)
UF 3857, holotype	Bocas del Toro, Panama	20	7.6–*22.0*–27.0
Uncat PPA 36	Bocas del Toro, Panama	15	12.7–*22.6*–29.3
Uncat PPA 38	Bocas del Toro, Panama	13	20.6–*24.4*–29.5
INV POR 1338	Cartagena, Colombia	10	11.0–*20.2*–28.7
INV POR 1339	Cartagena, Colombia	10	8.7–*15.1*–30.2
ICN-MHN(Po) 270	Cartagena, Colombia	10	11.2–*19.2*–31.4
Uncat SZ-20	Martinique	10	20.6–*24.4*–29.5
Uncat SZ-21	Martinique	6	10.0–*21.2*–28.7
Uncat SZ-23	Martinique	10	9.3–*19.8*–31.0

1

Table 2(on next page)

Spicule measurements of oxeas (length and width) of *Neopetrosia* spp. described in this study.

Measurements are expressed as minimum–*mean* (±1 standard deviation) – maximum. N=50.

NA=not available. Uncat=uncatalogued sample

Species	Specimen	Location	Length (µm)	Width (µm)
Neopetrosia proxima	UF 3856	Bocas del Toro, Panama	98–*158.7* (± 19.9)–193	3–*10.9* (± 1.7)–11
Duchassaing & Michelotti	UF 3858	Bocas del Toro, Panama	92–*146.6* (± 14.0)–168	3–*9.0* (± 1.7)–12
(1864)	UF 3860	Bocas del Toro, Panama	117–*159.3* (± 14.0)–181	6–*9.3* (± 1.1)–12
	Uncat. PPA 35	Bocas del Toro, Panama	75–*NA* (± NA)–205	2.7–*NA* (± NA)–10.7
	Uncat. PPA 37	Bocas del Toro, Panama	85–*NA* (± NA)–167	1.7–*NA* (± NA)–12.7
	INV POR1306	Old Providence, Colombia	110–*NA* (± NA)–150	2.5–*NA* (± NA)–5
Neopetrosia dendrocrevacea **sp. nov.**	UF 3854 Holotype	Bocas del Toro, Panama	91–*165.2* (± 15.9)–188	2.8–*7.4* (± 1.5)–10.5
	Uncat. PPA 07	Bocas del Toro, Panama	111—*156.5* (± 14.6)—181	4.5—*6.6* (± 0.9)—8.9
	INV POR0535	Urabá, Colombia	134–*171.4* (± 12.9)–198	4.4–7.*4* (± 1.0)–9.4
	UF 3854	Bocas del Toro, Panama	91–*165.2* (± 15.9)–188	2.8–*7.4* (± 1.5)–10.5
	INV POR 1336	Cartagena, Colombia	133–*165.1* (± 12.9)–189	4.6–*6.4* (± 0.6)–7.7
	INV POR1337	Cartagena, Colombia	139–*164.4* (± 12.9)–192	4.1–*7.3* (± 1.3)–9.8
	ICN-MHN(Po) 0269	Santa Marta, Colombia	130–*151.6* (± 9.4)–168	5.0–*6.5* (± 0.9)–9
	INV POR1335	Santa Marta, Colombia	103–*147.8* (± 12.3)–169	4–*6.5* (± 1.1)–9
	INV POR1333	San Andrés, Colombia	86–*119.3* (± 11.3)–150	3.5–*4.8* (± 0.6)–6
	INV POR1334	San Andrés, Colombia	114–*130.1* (±7.7)–149	5.1–*3.8* (± 0.6)–7
Neopetrosia cristata **sp. nov.**	UF 3859 Holotype	Bocas del Toro, Panama	121–*142.1* (±9.8)–163.2	2.1–7.2 (± 1.7)–9.6
Neopetrosia sigmafera **sp. nov.**	UF 3857 Holotype	Bocas del Toro, Panama	173–*235.9* (±14.1)–259	6.5–*13.6* (± 1.5)–15.9
	Uncat. PPA 36	Bocas del Toro, Panama	174–*226.5* (±12.4)–248	6.5–*14.2* (± 2.4)–17.6
	Uncat. PPA 48	Bocas del Toro, Panama	196–*232.7* (±10.4)–233	6.9–*13.5* (± 1.9)–15.5
	INV POR 1338	Cartagena, Colombia	203.5–*237.4* (±11.8)–255	5.8–*14.0* (± 2.8)–14.0
	INV POR 1339	Cartagena, Colombia	201.9–*240.2* (±14.5)–260	5.0–*17.0* (± 2.9)–17.7
	ICN-MHN(Po) 270	Cartagena, Colombia	201.9–*240.2* (±14.5)–260	5.0–*17.0* (± 2.9)–17.7
	Uncat. SZ-20	Martinique	153–*197.2* (±10.6)–219	6.9–*8.2* (± 0.6)–9.3
	Uncat. SZ-21	Martinique	115–*204.6* (±18.6)–230	6.8–*8.8* (± 0.7)–10.3
	Uncat. SZ-23	Martinique	130–*190.4* (±13.2)–190	4.9–*6.8* (± 1.0)–8.6

1