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Bearing the wrong identity: A case study of an Indo-Pacific common shallow water sponge of the genus *Neopetrosia* (Haplosclerida; Petrosiidae)

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Abstract

Sponges of the order Haplosclerida are often abundant and characteristic components of Indo-Pacific reefs, but are often misidentified, because of the lack of clear distinctive morphological characters. *Neopetrosia exigua* is an example of a haplosclerid sponge that is very common in Indonesian shallow coral reef environments but bears several different names. In the present study we investigated type material of several Indo-Pacific *Neopetrosia* species with a similar morphology and examined freshly collected specimen materials including specimens that are deposited at several institutions. In addition, we used molecular phylogenetic methods for assisting the morphological examinations. We conclude that the true identity of *Neopetrosia exigua* should be *Neopetrosia chaliniformis.* Likewise, *N. exigua* and *N. pacifica* should be considered as junior synonyms of *N. chaliniformis.* In conclusion, we advocate that molecular barcoding could significantly aid on sponge species' delimitation that possess limited morphological characters.

Key words: Porifera, *Neopetrosia*, type specimen, Indonesia, DNA barcoding

Introduction

Sponges are common and important elements of reef and mangrove communities in Indonesian marine ecosystems, where haplosclerid sponges are categorised as one of the most conspicuous orders due to their abundance in terms of diversity and quantity (Amir 1992, de Voogd 2004, de Voogd *et al*. 2004, de Voogd & Cleary 2008, de Voogd & van Soest 2002, van Soest 1989). However, the published knowledge of haplosclerids is far from complete (de Weerdt & van Soest 2001). Taxonomically, haplosclerid sponges are recognised as one of the most complicated and unreliable groups in demosponges (Borchiellini *et al* 2004), and "a sound classification of the order is a long way from being established" (Redmond *et al*. 2011). For this reason, the use of classical taxonomy based on spicule dimensions, type and arrangement of skeletal meshes often fails in delimiting lower taxa and creates nonmonophyletic groups because of "vague and elusive morphological synapomorphies" within the suborders Haplosclerina and Petrosina (van Soest & Hooper 2002). Moreover, current taxonomical rank of suborder Haplosclerina and Petrosina is abandoned, and Haplosclerida order rank is retained. In addition, a revision of Haplosclerida using added characters data is urged (Morrow & Cardenas, 2015)

The genus *Neopetrosia* (family Petrosiidae) is an example of a haplosclerid genus that is very difficult to recognise and identify. De Laubenfels (1949) erected this genus for the previously described species *Haliclona longleyi*, although he did not define this new genus. The genus was further discussed by de Laubenfels (1954), Bergquist (1965), Wiedenmayer (1977), and van Soest (1980), but was only properly defined in 2002 (see Systema

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Porifera, Hooper & van Soest 2002). The genus differs from other genera within the family Petrosiidae by its relatively small size of spicules and a recognisable anisotropic pattern in its skeleton (Desqueyroux-Faúndez & Valentine 2002). So far, 32 nominal species have been recognised within *Neopetrosia* (World Porifera Database, van Soest *et al*. 2017).

Neopetrosia exigua (Kirkpatrick, 1900) is a very common species in many Indo-Pacific shallow water reefs and has extensively been mentioned in the literature, primarily for its interesting bioactive properties (Abdillah *et al*. 2013a,b, de Almeida Leone *et al*. 2008; Liu *et al*. 2004; Orabi *et al*. 2002). *Neopetrosia exigua* was originally described as *Petrosia exigua* from Christmas Island, Indian Ocean (south of Java, Indonesia) by Kirkpatrick (1900). He noted that the species possessed very small oxeas as opposed to its most closely allied species, *Petrosia similis,* Ridley & Dendy 1886. Currently, two sponge taxa are accepted as junior synonyms of *N. exigua,* namely *Neopetrosia pandora* de Laubenfels, 1954 from Ponape (de Laubenfels 1954), Micronesia, and *Xestospongia pacifica* (Kelly-Borges & Bergquist, 1988) from Motupore Island, Papua New Guinea (World Porifera Database, van Soest *et al*. 2017).

During our phylogeographic study, we observed a striking similarity between freshly collected specimens and *Neopetrosia chaliniformis* (Thiele, 1899) originally described as *Petrosia chaliniformis*. This species from Sulawesi, Indonesia was described one year before *N. exigua*, however, this name was never used subsequently after its original description. Here, we aim to unravel the true identity of these common shallow water Indo-Pacific sponges. We investigate type material and examine freshly collected specimen materials, including additional specimens that are deposited at several institutions. Main morphological characters such as spicule measurements and other skeleton features were recorded. In addition, molecular phylogenetic methods employing fragments of the mitochondrial cytochrome oxidase subunit 2 (cox2) and 28S ribosomal DNA (28S rDNA) sequences are compared to supplement morphological examinations, since haplosclerids possess a reduced suite of phylogenetic informative characters (de Weerdt 1985).

Materials and methods

Data collection. Fourteen samples were freshly collected from several localities (West Java, North and South Sulawesi) in Indonesia. Directly after being collected, the samples were cut, rinsed and soaked in 98% ethanol before being preserved in 99% ethanol. Additional 18 samples were received from the Naturalis Biodiversity Center, Leiden, The Netherlands (samples coded with ZMAPOR, are at present the Zoological Museum Amsterdam collection housed in the Naturalis Biodiversity Center in Leiden), and further specimens were provided by the Queensland Museum (QM) Brisbane, Australia (G prefix). Holotype specimens were retrieved from the British Museum of Natural History, London, UK (BMNH, *Neopetrosia exigua*), the Zoological Museum Berlin, Germany (ZMB, *Neopetrosia chaliniformis*), the Smithsonian Museum, Washington DC, USA (USNM, *Neopetrosia pandora*), and the Australian Museum, Sydney, Australia (AM Z, *Xestospongia pacifica*) (see Table 1).

The descriptions presented below are based on external morphology, skeletal architecture and shape and size of the spicules. Spicule dimensions are given as the minimum-**mean**-maximum of length measurements x minimum-**mean**-maximum of width measurements from 25 spicule measurements. For study of the skeletal architecture, hand-cut perpendicular sections were made. These sections were air-dried, mounted in Ultrabed on a microscope slide, and studied under a Leica high power light microscope. Spicule preparations were made by dissolving a small piece of the specimen in commercial bleach, after which the residue was rinsed four times with water and once with 96% ethanol. The spicules were air-dried on microscopic slides and prepared for study with the light microscope by mounting them in Ultrabed.

Data Analyses. The statistical analyses were carried out using PASW 20.00 (SPSS Inc. 2012). The statistical significance of phenotypic differences among selected localities was tested by the Univariate analysis of variance (ANOVA, with Duncan's post hoc test, $p < 0.05$). These analyses were only conducted for some localities where at least three specimens were sampled (Thailand, West Java, North Sulawesi, South Sulawesi, Solomon Islands and the Great Barrier Reef Australia, see Table 1).

DNA extraction and sequencing. DNA extraction based on the previously published and established methods in sponge barcoding (Vargas *et al*. 2012) was performed for all specimens except the holotypes. *Neopetrosia*

pandora and X. pacifica were extracted separately using the DNeasy Blood & Tissue kit (Qiagen) following the instructions of the manufacturer. Because first attempts to amplify two >100-year-old holotypes (*N. chaliniformis and N. exigua*) failed, DNA extractions for those two holotypes were repeated using a modification from the CTAB phenol-chloroform method of Porebski *et al*. (1997). In this modified method, the phenol-octanol and RNase solutions steps were skipped. With this modified method we also gained amplifiable DNA for the two 100-year-old holotypes as opposed to the spin column method. The Polymerase Chain Reaction (PCR) using primers CO2F Por, 5'- TTTTTCACGATCAGATTATGTTTA-3' and CO2R Por, 5'-ATACTCGCACTGAGTTTGAATAGG-3' (Rua *et al*. 2011), was performed to amplify a fragment of the cox2 gene, and with primer 28S-C2-fwd, 5'-GAAAAGAACTTTGRARAGAGAGT-3' and 28S-D2-rev and 5'- TCCGTGTTTCAAGACGGG-3' (Chombard *et al*. 1998) for a fragment of 28S rDNA. The 25 µL PCR mix consisted of 5 µL 5x green Go*Taq*® PCR Buffer (Promega Corp, Madison, WI), 4µL 25mM $MgCl_2$ (Promega Corp, Madison, WI), 2 µL 10mM dNTPs, 1 µL each primer (5µM), 9.8 µL water, 2 µL DNA template, and 0.2 µL Go*Taq*® DNA polymerase (5u/µl) (Promega Corp, Madison, WI). The PCR regime comprised an initial denaturation at 94° C for 3 minutes, 35 cycles of 30 seconds denaturation at 94° C, 20 s annealing at 40° C and 60 s elongation at 72° C each, followed by a final elongation at 72° C for 5 min. For the holotypes of *N. chaliniformis and N. exigua* 2 µL Bovine Serum Albumin (BSA 10 mg/ml) were added in the PCR mix and PCR regime was modified with 45 s annealing at 40° C, 45 s elongation at 72° C each and a final elongation at 72° C for 7.5 min. All of the PCR products were cleaned with the ammonium acetate precipitation method. Sequencing of the forward and reverse strand was performed with the ABI BigDye v3.1 (Applied Biosystems, California USA) chemistry and the amplification primers following the manufacturer's protocol on an ABI 3730 Automated Sequencer in the Genomic Sequencing Unit of LMU Munich (http://www.gi.bio.lmu.de/sequencing/). All sequences are deposited at NCBI GenBank under accession numbers KM030095-KM030106, KM030107-KM030118 (cox2 mtDNA) and KM030120-KM030128, KM030130-KM030145 (28S rDNA fragment C2-D2).

Molecular Phylogenetic Analysis. Geneious version 6.1.7, (http://www.geneious.com) was used for assembling, trimming and analysing the sequences. Additionally, sequences were checked with BLAST against GenBank (http://www.ncbi.nlm.nih.gov/) for contaminations. Sequences were aligned with MUSCLE version 3.5 program (Edgar 2004) as implemented in Geneious under default settings. Phylogenetic reconstructions with cladistic analyses under probabilistic criteria were inferred for cox2 sequences using Bayesian Inferences (BI) and Maximum Likelihood (ML). The HKY+ I model of evolution as suggested by jModeltest v. 2.1.3 (Darriba *et al*. 2012) under the Akaike Information Criterion (Akaike 1974) was selected. BI was performed in MrBayes v. 3.2.1 (Ronquist *et al*. 2012). Each analysis consisted of two independent runs of four Metropolis-coupled Markov-chains under default temperature with trees sampled at every 1000th generation. Analyses were terminated automatically when the chains converged significantly as indicated by an average standard deviation of split frequencies <0.01. Similarly ML analyses were inferred using RAxML v. 7.0.4 in the raxmlGUI v. 1.3 program (Silvestro & Michalak 2012) with a rapid bootstrap of 1000 replications (Stamatakis *et al*. 2008). As the HKY+ I model is not implemented in RAxML, the model utilised in RAxML was changed to GTR+ I (Stamatakis 2008).

Cladistic analyses were not performed for the 28S rDNA sequences because an unambiguous final alignment was not possible due to the abundance of highly variable sites. Therefore, a simple phenetic analysis was carried out instead to display the grade of similarities between the sequences. For this purpose a data set of the 28S rDNA sequences was approached using MUSCLE (Edgar 2004) under default settings and analyzed with Neighbour-Joining (NJ) analysis with observed distances in SeaView version 4.4.2 (Gouy *et al*. 2010).

A sample of *X. testudinaria* (Lamarck, 1815) (GW1341: Indonesia, Central Java, Kep Karimun Jawa, Pulau Sintok, South side, 5° 47' 06" S, 110° 30' 18" E, 27 May 2011, coll. E. Setiawan) was sequenced (KM 030119) and utilised only for the molecular analysis as additional non-*Neopetrosia* sequence in cox2 mtDNA phylogenetic analysis. We excluded molecular phylogeny analyses of samples from localities and sites that consist of less than three specimens, i.e., Northern Territory, Singapore, Palau (see details Table. 1).

Results

Systematics

Phylum Porifera Grant, 1835
Class Demospongiae Sollas, 1885

Order Haplosclerida Topsent, 1928

Family Petrosiidae van Soest, 1980

Genus *Neopetrosia* de Laubenfels, 1949

Syn. *Densa* de Laubenfels, 1934

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 fLm long (Desqueyroux-Faundez & Valentine 2002).

Neopetrosia chaliniformis (Thiele, 1899)
Figure 1A, 2A, 3A, Table 1

Petrosia chaliniformis Thiele, 1899: 21, Pl. 2, Fig. 9; Pl. 5, Fig. 15

Material examined: Holotype: ZMB2889, Indonesia, North Sulawesi, Kema, Minahasa.

Description (amended from Thiele 1899): Sponge consists of several fragments. Frequently found in the form like plates with branches. Branches have a diameter of 5- 6 mm. Size of osculum is measured 1-2 mm. Height is measured in average of 12 cm.

Consistency. Hard to touch, crumbly.

Colour. Chocolate brown and light brown in alcohol.

Skeleton. Skeleton has structure of isodictyal tangential spicule network with one size of spicule.

Spicules. Oxeas in a range of 100-**140.8**-165 µm x 7.5-**9.9** -12.5 µm.

Remarks: Thiele (1899) remarked that the specimen was more properly related to the genus *Petrosia* rather than in *Pellina* because it has an easy removable epidermal layer, however he was not certain about placing the specimen in the genus *Petrosia* because its consistency was more elastic than in typical *Petrosia*. Likewise, Thiele observed that the skeletal structure of the specimen is similar to that of *Reniera* or *Rhizochalina*. Nevertheless, similarities between genera mentioned by Thiele are no longer relevant given that *Reniera* and *Pellina* were transferred to several genera within the family Petrosiidae e.g., *Petrosia*, *Haliclona*, *Rhizochalina* (the latter renamed *Oceanapia,* World Porifera Database, van Soest *et al*. 2017).

Neopetrosia exigua (Kirkpatrick, 1900)
Figure 1B, 2B, 3B, Table 1

Petrosia exigua Kirkpatrick, 1900: 139, Pl, XII, Fig. 7, Pl. XIII, Fig. 4

Material examined: Holotype: BMNH1898.12.20.49, Australia, Christmas Island, Indian Ocean.

Description (amended from Kirkpatrick 1900): Sponge forms a hard, thick, nodulated crust, smooth surface, showing an irregular reticulate pattern formed by pore-areas, oscules are measured in a range of 1-5 mm in diameter.

Colour. Pale gray brown in the dry condition.

Skeleton. Forms of slender main lines of fibres passing vertically to the surface and connected at right angles to this plane by closely packed single spicules, so as to form circular or obscurely polygonal tubes about 70 mm in diameter. The skeletal network is dense and irregularly oriented.

Spicules. Oxeas in a size range of 70-**113.8**-130 x 5-**7.3**-10 µm.

Remarks: Kirkpatrick (1900) remarked that spicules are considerably smaller than the allied species *Petrosia similis* (less than half the size). We also discovered that only a very small fragment of the type material is left in the BMNH collections.

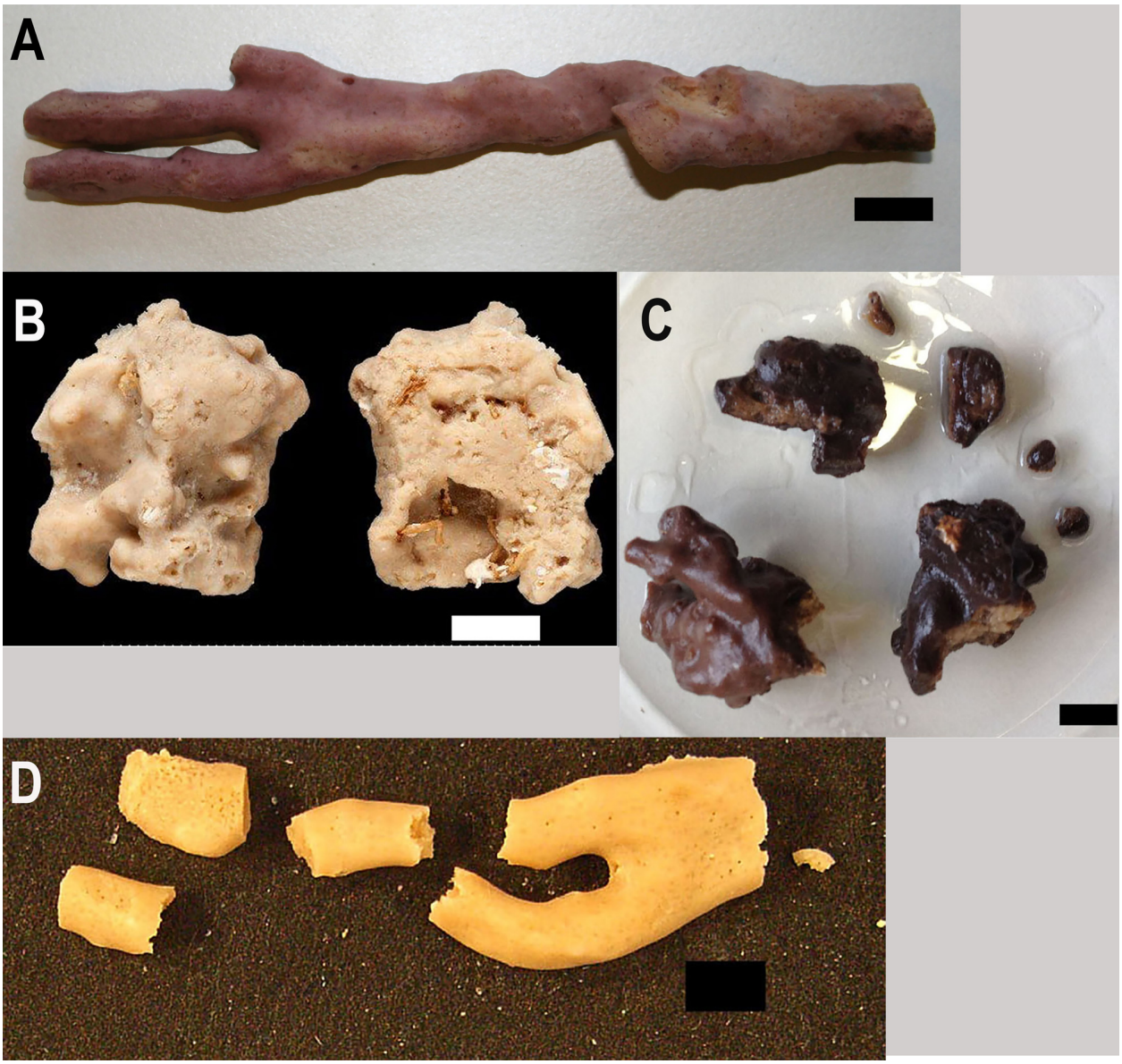

FIGURE 1. A *Neopetrosia chaliniformis* (Thiele, 1899) holotype ZMB2889, **B** *Neopetrosia exigua* (Kirkpatrick, 1901), holotype BMNH1898.12.20.49, **C** *Xestospongia pacifica* Kelly-Borges & Bergquist, holotype AM Z4999, and **D** *Neopetrosia pandora* de Laubenfels, 1954, holotype USNM4806. Scale bar= 1 cm.

Xestospongia pacifica Kelly-Borges & Bergquist, 1988

Figure 1C, 2C, 3C, Table 1;

Xestospongia pacifica Kelly-Borges & Bergquist, 1988: 155, P. 6c, 6d.

Material examined: Holotype: AM Z4999, Papua New Guinea, Buna Motu Reef, Bootless Bay, South Papua New Guinea.

Description (amended from Kelly-Borges & Bergquist 1988): The sponge grows as an encrustation of 5-10 mm thickness, frequently with papillae 20-60 mm high and 5-10 wide. It has small oscules of 1-3 mm width.

Colour. In life, the colour is brownish orange to olive. In alcohol, the colour changes into olive black and slightly browner.

Consistency. Tough, compressible and extremely brittle.

Spicules. Oxeas in a size range of 100-**149.6**-175 x 5-**9.2**-10 µm

Skeleton. Dense and an isodictyal tangential spicule network with one size of spicules.

Remarks: This species was synonymised with *Neopetrosia exigua* by Van Soest (2008) based on morphological similarities. These include the spicule consisting only of oxeas smaller than 200 µm and the skeletal meshes are more compact in structure than those of *Xestospongia* (Desqueyroux-Faundez & Valentine 2002).

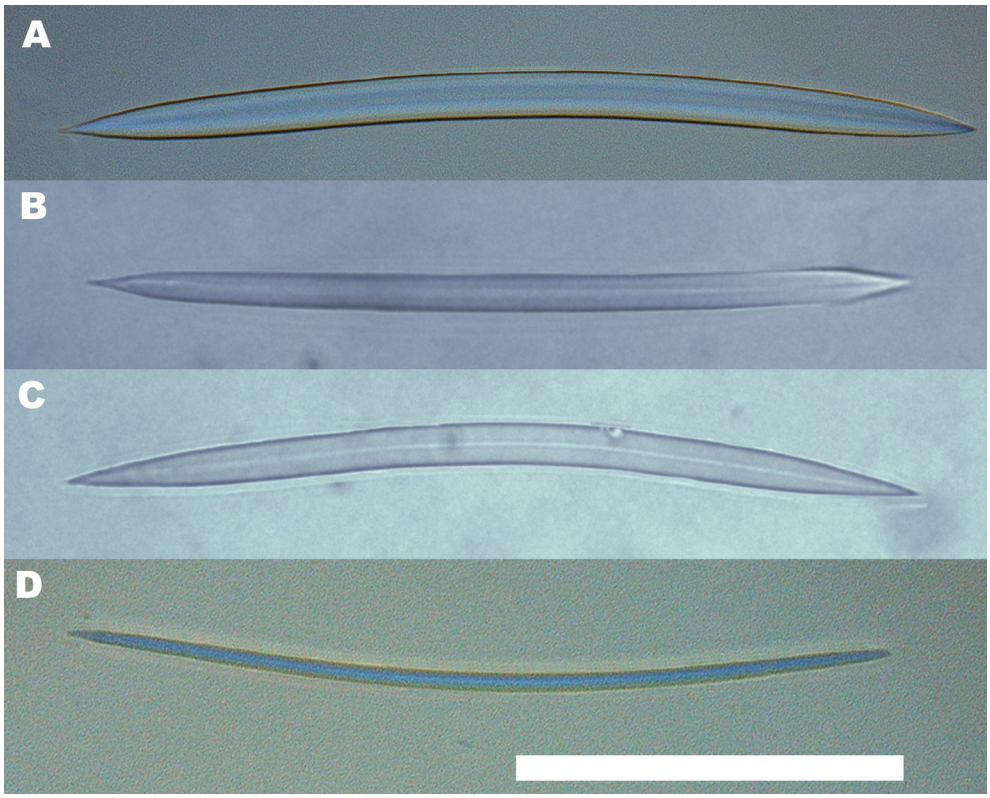

FIGURE 2. Oxea from the holotypes of **A** *Neopetrosia chaliniformis* (Thiele, 1899) ZMB2889, **B** *Neopetrosia exigua* (Kirkpatrick, 1901), BMNH1898.12.20.49, **C** *Xestospongia pacifica* Kelly-Borges & Bergquist, AMS Z4999, and **D** *Neopetrosia pandora* de Laubenfels, 1954, USNM4806. Scale bar= 50 µm

Neopetrosia pandora de Laubenfels, 1954

Figure 1D, 2D, 3D, Table 1

Neopetrosia pandora de Laubenfels, 1954: 81, Fig. 49.

Material examined: Holotype: USNM 23046, Palau, Matalim, East Ponape, 1 August 1949, 5m depth, coll. M.W. de Laubenfels.

Description (amended from de Laubenfels 1954): A repent or sprawling ramose sponge, reaching a maximum length of at least 13 cm. The diameter of the branches is 9 mm; oscules are about 2 mm in diameter and 2 cm apart.

Colour. Colour in life is dull olive drab.

Consistency. Slightly spongy but also somewhat stiff, tearing very easily.

Spicules. Oxeas in a range of 100-**119.8**-150 x 2.5-**2.9**-5 µm.

Skeleton. Less dense, more irregular isodictyal spicule network with one size of spicule.

Remarks: De Laubenfels (1954) described that the consistency of the type specimen was slightly spongy, somewhat stiff and tearing very easily. The surface of the type specimen does not possess an ectosomal specialisation, which is a typical characteristic of sponges from Family Chalinidae. Little or lack of spongin in

skeleton was observed. The spicules are also much thinner than the other spicules of type specimens and samples analysed in this study.

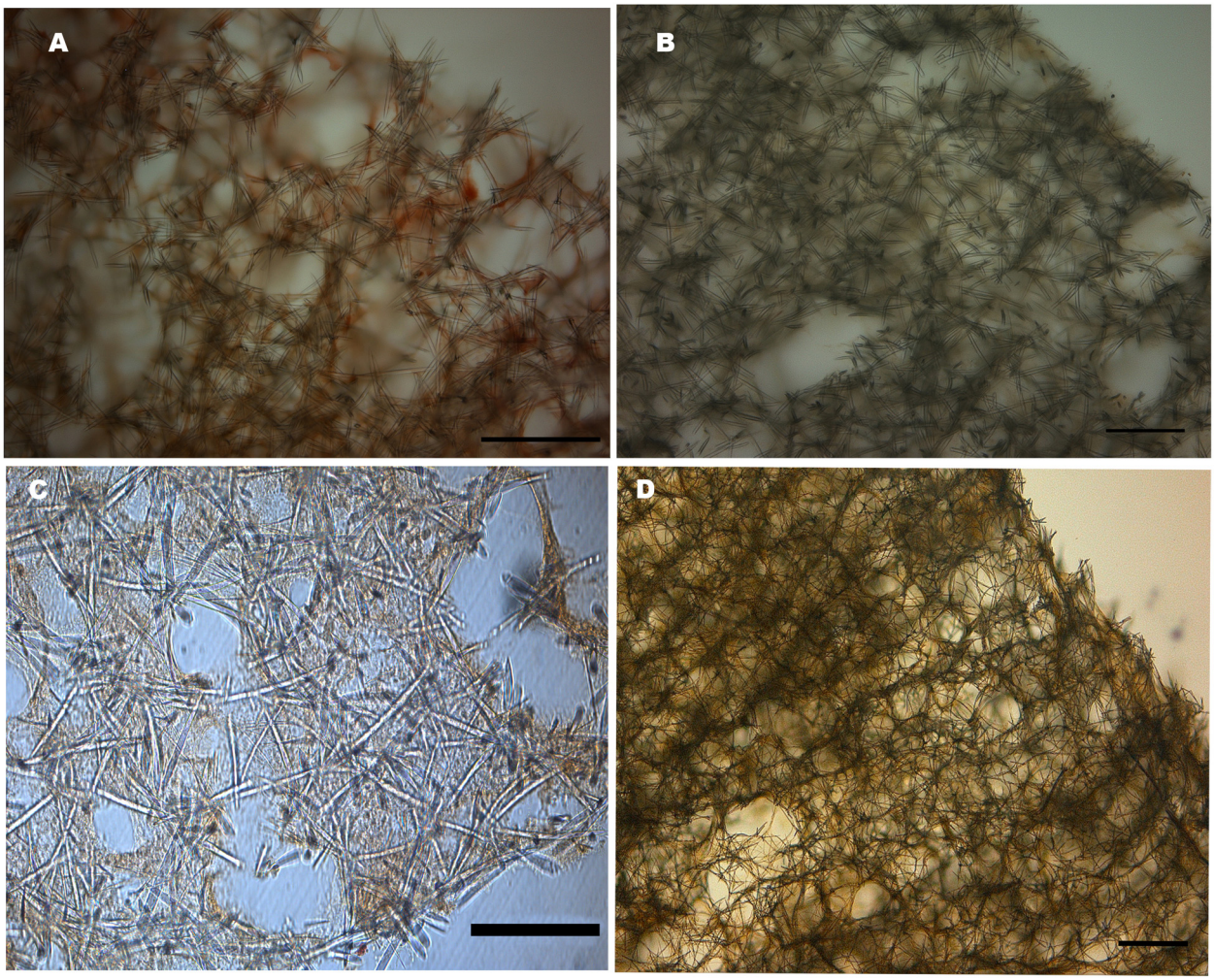

FIGURE 3. Spicule arrangement in holotypes of **A** *Neopetrosia chaliniformis* (Thiele, 1899) ZMB2889, **B** *Neopetrosia exigua* (Kirkpatrick, 1901), BMNH1898.12.20.49, **C** *Xestospongia pacifica* Kelly-Borges & Bergquist, AMS Z4999, and **D** *Neopetrosia pandora* de Laubenfels, 1954, USNM4806. Scale bar= 150 µm.

Additional material examined

We examined additional material identified as *Neopetrosia exigua*) and freshly collected specimens identified as *Neopetrosia chaliniformis* from various location in Mauritius, Thailand, Japan, the Philippines, Singapore, Indonesia, Palau, Papua New Guinea, Australia, Solomon Islands, Vanuatu, Palau, and Tonga (see detail below, Table 1 and Figure 4 A-C).

QM G303302 (GW18429): Australia, Northern Territory, Dudley Point Reef, East Point, Darwin, intertidal reef, 12° 25' 03" S, 130° 49' 00" E, 20 September 1993, coll. JNA Hooper; QM G313113 (GW18478): Singapore, Pulau Tembakul (Kusu I), Freyberg Channel, very silty patchy coral reef, 1° 13' 05" N, 103° 51' 07" E, 2 May 1995, 18.7m depth, coll. JNA Hooper; QM G313297 (GW18491): Tonga, Vaipuua, at end of channel, highly silted fringing reef, *Porites* spires, 18° 37' 55" S, 173° 58' 47" W, 14 November 1997, 15 m depth, coll. JNA Hooper; QM G306321 (GW18598); Palau, Ongingiang, W of, W. Palau, fringing coral reef surrounding channel in outer barrier reef, strong current, spur and grooves, 7° 16' 05" N, 134° 14' 05" E, 10 December 1995, 31m depth, coll. JNA Hooper; QM G311804 (GW18777): Papua New Guinea, North of Lion Island, S/w Of Motupore Island; Near Port

Moresby, PNG, top ridge or reef, dead coral, 19 September 1990, 6-18m depths, coll. JNA Hooper; QM G315226 (GW18793): Australia, Queensland, Hook Reef lagoon, coral reef lagoon, 19° 45' 14" S, 149° 10' 45" E, 5 June 1999, 9.4m depth, coll. JNA Hooper; QM G312397 (GW 18804): Australia, Western Australia, North Head,

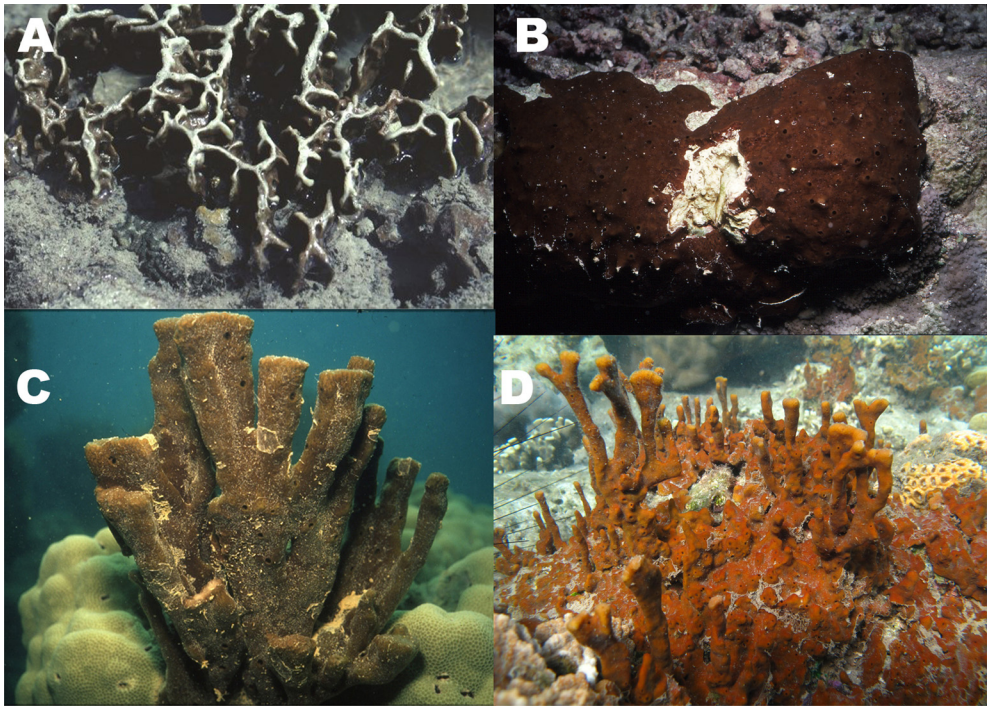

FIGURE 4. In situ photo of sponges identified as *N. exigua*, that were collected in (A) Northern Territory Australia, QM G303302, (B) Palau, QM G306321, (C) Tonga, QM G313297 and (D) a sponge identified as *N. chaliniformis* that was photographed in Probolinggo, East Java, Indonesia.

Beagle Bay, NW WA, rock salt , 16° 30' 00" S, 122° 19' 12" E, 12 August 1991, coll. JNA Hooper; QM G315374 (GW18806): Australia, Queensland, Stevens Reef, GBR, back reef, 20° 32' 34" S, 150° 6' 26" E, 7 June 1999, 30m depth, coll. JNA Hooper; QM G322668 (GW19027): Solomon Islands, Rendova Island Tetepare, Fringing reef, inshore slope northwest side, 8° 42' 04" S, 157° 28' 14" E, 6 July 2009, 20-50m depth, coll. JNA Hooper; QM G322710 (GW19041): Solomon Islands, Rendova Island Tetepare, Fringing reef, inshore slope northwest side, 8° 42' 04" S, 157° 28' 14" E, 6 July 2009, 40-50m depth, coll. JNA Hooper; QM G322696 (GW19052): Solomon Islands, Vangunu Island, Barrier reef, external slope of slope facing northwest vertical wall, richly covered with various organisms, 8° 40' 19" S, 157° 50' 15" E, 5 July 2009, coll. JNA Hooper; GW2037: Indonesia, West Java, Thousand Island, JAK01- Pulau Air, 5° 45' 35" S, 106° 34' 44" E, 26 July 2011, coll. N.J de Voogd; GW2112: Indonesia, West Java, Thousand Island, JAK03 - Semak Daun NW, 5° 43' 40" S, 106° 33' 57" E, 26 July 2011, 16m depth, coll. N.J de Voogd; GW2113: Indonesia, West Java, Thousand Island, JAK03 - Semak Daun NW, 5° 43' 40" S, 106° 33' 57" E, 26 July 2011, 16m depth, coll. N.J de Voogd; QM G315299 (GW18758): Australia, Queensland, Edgell Reef, back reef, 20° 8' 53" S, 149° 55' 09" E, 6 June 1999, 18m depth, coll. JNA Hooper; ZMA POR16482 (GW4870): Japan, Ryukyu Islands, Saki-shima Islands, Hatoma Island, coral reef, 24° 26' 60" N, 123° 49' 60" E, coll. K. Watanabe; GW4782: Indonesia, N Sulawesi, Lembeh, W Sarena Kecil, 1° 27' 25.5234"N, 125° 13' 31.1874"S, 17 Feb 2012, coll. N.J de Voogd; GW4783: Indonesia, N Sulawesi, Lembeh, Tanjung Nanas I, 1° 27' 40.212"N, 125° 13' 36.408"S, 3 Feb 2012, coll. N.J de Voogd; GW4784: Indonesia, N Sulawesi, Lembeh, Tanjung Kelapasatu, 1° 25' 38.568"N, 125° 11' 0.7794"S, 15 Feb 2012, coll. N.J de Voogd; GW4785: Indonesia, N Sulawesi, Lembeh, N Tanjung Pandean, 1° 24' 21.7074"N, 125° 10' 4.5114"S, 14 Feb 2012, coll. N.J de Voogd; GW4788: Indonesia, N Sulawesi, Lembeh, S Pulau Dua, 1° 23' 17.016"N, 125° 12' 43.1274"S, 13 Feb 2012, coll. N.J de Voogd; ZMA POR16473 (GW4811): Palau, Koror Island, Abe's Traverse, smooth; ZMA POR21753 (GW4828): Philippines, Calamian Group: Busuanga Island; ZMA POR21752 (GW4829): Philippines, Calamian Group: Busuanga Island; ZMA POR18793 (GW4843): Thailand, Laem Tum-Pung, South of Ko Kram, Sattahip, Chonburi, rock, 25 February 2007, coll. Sumaitt Putchakarn; ZMA POR18754 (GW4845): Thailand, South of Ko Mark, Chang Islands, Trad, rock, 11° 47' 10" N, 102° 29' 14" E, 8 August 2012, coll. Sumaitt Putchakarn; ZMA POR18737 (GW4849): Thailand, West side of Ko Klum, Chang Islands, Trad, rock, 11° 55' 02" N, 102° 21' 43" E, 8 August 2012, coll. Sumaitt Putchakarn; ZMA POR17251 (GW4866): Mauritius, 25 June 2006, identified by D.

Marie; ZMA POR17229 (GW4867): Indonesia, South Sulawesi, Makassar, 8 May 2012, identified by R.A. Edrada; GW7173: Indonesia, South Sulawesi, Barangbaringan, 5° 2' 59.60"S, 119° 25' 12"E, 5 Aug 2012; GW7174: Indonesia, South Sulawesi, Barangbaringan, 5° 2' 59.60"S, 119° 25' 12"E, 5 Aug 2012; GW7175: Indonesia, South Sulawesi, Lankai, 5° 1' 44.7"S, 119° 5' 8.8" E, 8 Aug 2012.

Comments: The length of oxeas overlap among the type specimens of *N. chaliniformis, N. exigua, X. pacifica* and most of the additional analysed material. Conversely, the oxeas from the type of *N. pandora* and specimens identified as *N. exigua* from the Great Barrier Reef, Australia are much thinner (Table 1). Further statistical tests based on the 20 collected samples from six localities (Thailand, West Java, North Sulawesi, Southeast Sulawesi, Great Barrier Reef and Solomon Islands) revealed that there was a significant difference in spicule lengths (0.005, $p < 0.05$) and widths (0.001, $p < 0.05$) from each locality.

The spicule arrangement of *Neopetrosia chaliniformis*, *N. exigua,* and *Xestospongia pacifica* types is similar (Figure 3A, B, C), sharing a high degree of skeletal density and possessing an isodictyal tangential skeleton. On the other hand, the type specimen of *N. pandora* exhibited a less dense spicular network and more irregular spicule arrangement despite its similarity to an isodictyal tangential skeleton (Figure 3D).

Molecular phylogenetic analyses

Only the 28S rDNA sequences from one type specimen (*N. pacifica*) could be generated.). Furthermore, the cox2 mtDNA fragments were successfully amplified in three type specimens; *N. chaliniformis*, *N. exigua* and *N. pacifica* (see Table 1). The cox2 alignment length with all taxa included was 350 base pairs with 129 variable sites.

The sequences number in the 28S rDNA phylogenetic tree increased because three individuals (G315229, GW2037, and GW2113) consist of two sequence types. In order to resolve the sequence type ambiguities, SeqPHASE (Flot 2010) were implemented. The alignment length for the 28S sequences was 606 characters. It comprises only 69 % alignable characters from the one holotype, collected specimens, and outgroups.

The resulting trees are shown in Figures 5 and 6. The cox2 holotype sequences from *N chaliniformis*, *N. exigua*, and *X. pacifica* were identical. This sequence type is shared with several other samples collected in the field, which is corroborated in the 28S rDNA analyses.

Proposed Nomenclatural Acts

Synonymise *Petrosia chaliniformis* Thiele, 1899, *Petrosia exigua* Kirkpatrick, 1900, and *Xestospongia pacifica* Kelly-Borges & Bergquist, 1988, with the former being the senior available name for the taxon *Neopetrosia chaliniformis* (Thiele, 1899).

Discussion

The morphology is important for distinguishing sponge species within the family Petrosiidae. In general, all holotypes and other collected samples examined in this study have an overall encrusting appearance and occasionally possessing branches. Likewise, all of those specimens possess a dark to light brown colouration, a sticky texture, and a brittle and compressible consistency that relate to the field characteristics of *Neopetrosia* species. We conclude that *N. chaliniformis* and *N. exigua* are the same species based on both morphological and genetic analyses. The former species name was described only one year before *N. exigua* (Kirkpatrick 1900, Thiele 1899, Van Soest *et al*. 2014). Thus, based on the principle of priority of the ICZN (2015), *N. chaliniformis* is the valid name and *N. exigua* is its junior synonym. The type material of *N. chaliniformis* is well recognizable and conforms to recent freshly collected material of this species, whereas the material of *N. exigua* is only a small fragment of a crust. It is difficult to identify haplosclerid sponges and misidentifications are frequent, further exacerbated by incomplete descriptions or inadequate illustrations depicting key characters, leading to the plethora of synonyms amongst many nominal species.

Growth forms are frequently regarded as being static (only encrusting or only branching species), but in situ observations revealed that encrusting species (assumed as *N. exigua*) forms branches under certain circumstances (cf. *N. chaliniformis*). This species is encrusting most of the time but forms branches occasionally (Figure 4 A–D).

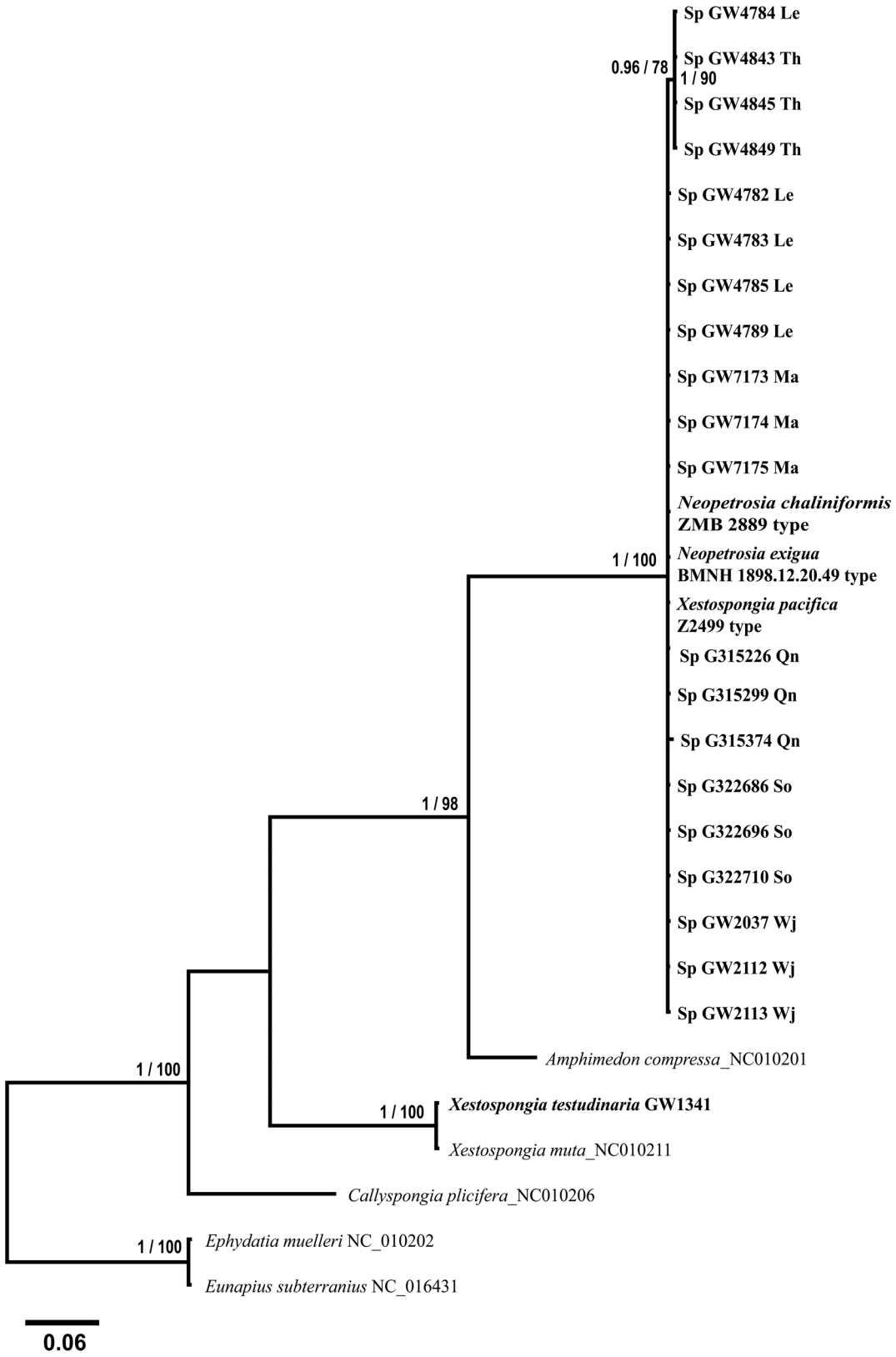

FIGURE 5. Bayesian cox2 phylogram of *Neopetrosia* spp. Numbers on the branches represent posterior probabilities (PP) / bootstrap proportions (BP) of maximum likelihood analyses. Scale bar indicates the number of substitutions per site. Bold is sequences, which are obtained from this study. Wj= Pulau Seribu, West Java, Indonesia, Indonesia Le= Lembeh, North Sulawesi, Indonesia, Ma = Makassar, South Sulawesi, Indonesia Th= Southeast Thailand, Qn= The Great Barrier Reef, Queensland, Australia, So= Solomon Islands

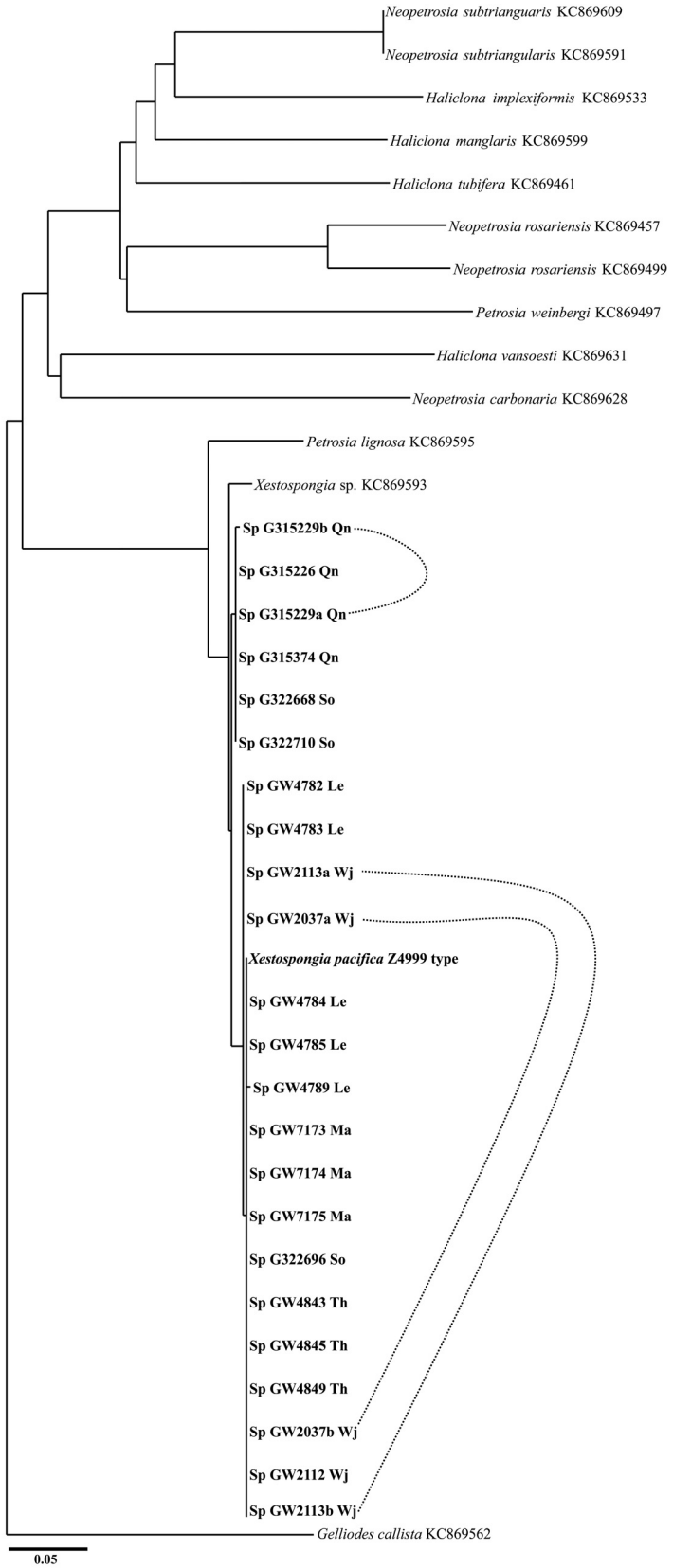

FIGURE 6. Neighbor Joining phylogram of 28S rDNA sequences from *Neopetrosia* spp. The scale bar indicates the number of substitutions per site and dashed lines indicate connectivity between two sequence types from one individual. Bold are sequences, which are obtained from this study. Wj= Pulau Seribu, West Java, Indonesia, Le= Lembeh, North Sulawesi, Indonesia, Ma = Makassar, South Sulawesi, Indonesia Th= Southeast Thailand, Qn= The Great Barrier Reef, Queensland, Australia, So= Solomon Islands.

TABLE 1. TABLE 1. Neopetrosia spp. examined in this study (X=could not be amplified its DNA, *= two sequence types, XX= excluded from Molecular Phylogeny Analyses). GW vouchers numbers are also official Museum Numbers of the Bavarian State collection for Paleontology and Geology as SNSB-BSPG.GWXXXXX.

Voucher Numbers	Museum code	Length (µm)	Width (µm)	Localities	Cox2 Genbank accession numbers	28S rDNA Genbank accession numbers
GW7107	ZMB2889 (Holotype *N. chaliniformis*)	100–**140.8**–165	7.5–**9.9**–12.5	North Sulawesi, Indonesia	KM030103	X
GW7185	BMNH1898.12.20.49 (Holotype *N. exigua*)	70–**113.8**–130	5–**7.3**–10	Christmas Island, Australia	KM030104	X
GW4805	Z4999 (Holotype *N. pacifica*)	100–**149.6**–175	5–**9.2**–10	Bootless Bay Papua New Guinea	KM030105	KM030128
GW4806	USNM23046 (Holotype *N. pandora*)	100–**119.8**–150	2.5–**2.9**–5	East Ponape, Palau	X	X
GW4843		110–**136.8**–155	5–**6.1**–7.5	Southeast Thailand	KM030113	KM030137
GW4845		100–**134.4**–155	5–**6.3**–7.5		KM030114	KM030138
GW4849		100–**137.2**–160	5–**7.6**–10		KM030115	KM030139
GW2037		100–**138.8**–170	5–**7.4**–7.5	Thousand Island, West Java	KM030116	KM030140 KM030141*
GW2112		110–**151.8**–170	5–**7.3**–10	Indonesia	KM030117	KM030142
GW2113		100–**140.2**–175	5–**7.3**–7.5		KM030118	KM030143 KM030144*
GW4782		110–**141**–160	5 –**8**– 10	Lembeh, North Sulawesi	KM030095	KM030120
GW4783		120–**146.4**–175	7.5–**7.8**–10		KM030096	KM030121
GW4784		105–**148**–175	5–7**.6**–10		KM030097	KM030122
GW4785		100–**144.2**–175	7.5–**8.2**–10		KM030098	KM030123
GW4788		110–**141.6**–155	5–**5.4**–7.5		KM030099	KM030124
GW7173		125–**151.8**–175	5–**7.3**–10	Makassar, South Sulawesi	KM030100	KM030125
GW7174		100–**134.8**–175	5–**7.5**–10		KM030101	KM030126
GW7175		80–**141.6**–155	5–**5.4**–7.5		KM030102	KM030127
GW18793	G315226	95–**112.8**–130	2.5–**4**.9–5	GBR, Australia	KM030107	KM030130
GW18758	G315299	80–**119.8**–150	2.5–**4.9**–5		KM030108	KM030131 KM030132 *
GW18806	G315374	100–**128.6**–150	2.5–**4**.5–5		KM030109	KM030133
GW19027	G322668	100–**137.4**–150	5–**5.6**–7.5	Solomon Islands	KM030110	KM030134
GW19052	G322696	100–**129.8**–150	2.5–**5**–7.5		KM030111	KM030135
GW19041	G322710	100–**133.8**–150	2.5–**3.8**–5		KM030112	KM030136
GW18429	G303302	100–**135.4**–155	5–**5.8**–7.5	Northern Territory, Australia	XX	XX
GW18478	G313113	105–**143**–185	5–**6.5**–7.5	Pulau Tembakul Singapore	XX	XX

...Continued on next page

TABLE 1. (Continued)

Voucher Numbers	Museum code	Length (µm)	Width (µm)	Localities	Cox2 Genbank accession numbers	28S rDNA Genbank accession numbers
GW18491	G313297	105–**126.4**–150	5–**6.4**–7.5	Vaipuaa, Tonga	XX	XX
GW18598	G306321	74–**104**–130	5–**6**–7.5	Ongiangiang, Palau	XX	XX
GW18777	G311804	105–**128.6**–155	5–**7.4**–7.5	Motupure Island Papua New Guinea	XX	XX
GW18804	G312397	105–**123.4**–150	5–**5.5**–7.5	Western Australia	XX	XX
GW4870	ZMA POR16482	100–**113.8**–170	5–**5.2**–7.5	Ryukyu Island, Japan	XX	XX
GW4811	ZMA POR16473	100–**121.8**–145	2.5–**4.9**–5	Koror Islands, Palau	XX	XX
GW4828	ZMA POR21753	100–**143**–175	5–**5.38**–7.5	Busuanga Island, the Philippines	XX	XX
GW4829	ZMA POR21752	105–**145.4**–175	5–**6.1**–7.5	Busuanga Island, the Philippines	XX	XX
GW4866	ZMA POR17251	115–**158.8**–190	7.5–**8.1**–10	Mauritius	XX	XX
GW4867	ZMA POR17229	100–**132.2**–165	5–**5.7**–7.5	Makassar, South Sulawesi	XX	XX

The difference between spicule widths of the type specimen of *N. pandora* and collected samples from the GBR localities might be affected by silica influxes as recognized for other demosponges (e.g., Bavestrello *et al*. 1993, Maldonado *et al*. 1999, Stone 1970, Uriz *et al*. 2003, Zea and van Soest, 1986). Currently, we still need an additional support from molecular analyses for resurrecting *N. pandora* as a distinct species from the others *Neopetrosia* spp., and as a valid species with a distinctive skeleton and thinner oxeas. Conversely, the GBR samples, which possess a similar skeleton structure and sequence types (partial cox2 mtDNA and 28S rDNA genes) to the other type specimens of *N. chaliniformis*, *N. exigua*, and *N. pacifica* could not be defined and raised as a new species, and are retained within this species-group. This finding also contradicts previous studies, which placed *N. chaliniformis* as a different species to *N. exigua* with *X. pacifica* as its junior synonym due to similarity of morphological characters, particularly spicules (Van Soest *et al*. 2017).

Identifying and recognizing the different haplosclerid sponges in the field is less problematic than the identification of small, preserved museum samples and the link to existing species based on incomplete and old descriptions like *N. chaliniformis* and *N. exigua*. Molecular markers are indispensable for this purpose and also in this case facilitated the identification of a common species.

Further attempts are required to obtain 28S rDNA sequences from the *N. chaliniformis*, *N. exigua* and *N. pandora* holotypes in addition to a cox2 sequence from *N. pandora* holotype. This will contribute to "the bottom up strategy" (see review in Cárdenas *et al*. 2012) in the phylogenetic study of haplosclerids since the genus *Neopetrosia* is still recovered as a polyphyletic group (Redmond *et al*. 2013, Redmond *et al*. 2011, Thacker *et al*. 2013). The bottom up strategy helps taxonomists with the revision or "re-evaluation (as the best alternative) of morphological characters under the light of molecular results" (studying type species of each haplosclerid sponge genus first) to resolve the discrepancies between current morphology and phylogeny in haplosclerids.

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