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Genetic data confirms the enigmatic demosponge *Janulum* as haplosclerid

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Historically, sponge classification is based on the interpretation of morphological characters, whose phylogenetic information content is frequently limited, subject to homoplasies, or prone to environmental plasticity (e.g., Chombard *et al.* 1998). Therefore, the currently accepted order-level classification of its largest class, Demospongiae, has been largely revised with molecular phylogenetic data (Morrow & Cárdenas 2015). Nevertheless, numerous sponge genera with ambiguous or provisoric phylogenetic placement still await definite classification.

Among those demosponge genera with uncertain placement is *Janulum* de Laubenfels, 1936, which was originally placed by de Laubenfels (1936) in Poecilosclerida, (Family Acarniidae, currently considered a junior synonym of Microcionidae). Hooper (2002) assigned *Janulum*, with question, to the plocamiform group of raspailiids (Family Raspailiidae Nardo, subfamily Plocamioninae Hooper [then Order Poecilosclerida, currently Order Axinellida]), in particular, to genus *Lithoplocamia* Dendy, due to the possession of "acanthostrongyles" in an end-to-end skeletal arrangement that Hooper considered to be "plocamiform". Species of *Lithoplocamia* also have styles and oxeas in radiating plumose tracts obscured by the dense secondary isodictyal reticulation of these acanthostrongyles (Hooper 2002). The so-called "acanthostrongyles" of *Janulum* (Hooper 2002) are more precisely, spined strongyles (Kelly *et al.* 2015), and are the only spicule type found in the various species of *Janulum*. Moreover, the morphology of the spined strongyles in *Janulum* differ from those of *Lithoplocamia* in that their ends are abruptly bent to the same or different sides of the spicule shaft, the spines are sharp and (unusually) concave, and are concentrated in the middle of the strongyle (Kelly *et al.* 2015) as opposed to the ends as in *Lithoplocamia* (Hooper 2002: 505, fig. 21 C, D).

Finally, the "end-to-end isodictyal reticulation" found in plocamiform sponges is also present in many haplosclerid genera, such as in family Chalinidae and Petrosiidae. Consequently, Vacelet (1969), Boury-Esnault *et al*. (1994), and Wiedenmayer (1994) considered haplosclerid origin for *Janulum* earlier (see Kelly *et al.* 2015 for a review). This found first support by molecular systematics in Redmond *et al*. (2013), who also redescribed the *Janulum* type species, *J. spinispiculum* (Carter), and recovered this species distant from Raspailiidae in a clade with *Siphonodictyon* Bergquist (including the type species *S. mucosum* Bergquist), currently assigned to the haplosclerid family Phloeodictyidae. The apparent close phylogenetic relationship with *Siphonodictyon* points towards a possible membership of *Janulum* to this family. However, Redmond *et al.* (2013) also sequenced a specimen of *Oceanapia isodictyiformis* (Carter), *Oceanapia* Norman being the type genus of Phloeodictyidae, and this appeared distantly related to *Siphonodictyon* and *Janulum*, lending no support for any familial allocation at present.

In addition to redescribing new material of *Janulum spinispiculum*, Kelly *et al.* (2015) described two species (one fossil) from New Zealand, strengthening the integrity of the genus and its diagnosis by including a detailed analysis of the spicule morphology, noting the presence of a tangential isodictyal skeleton and the resemblance of the skeleton and morphology to Chalinidae and Haplosclerida in general. While Kelly *et al.* (2015) indicated their support for *Janulum* species affinity with order Haplosclerida, they refrained from assigning *Janulum* to any family or order of Demospongiae in anticipation of supporting molecular data.

In the meantime, DNA sequence data has been obtained for the new *Janulum* species, *J. imago* Kelly & van Soest represented by Holotype NIWA 94196 and Paratype NIWA 93421 (TAN1402/31, Lot 224, Forde Guyot, Louisville

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Seamount Chain, International Waters, 35.317° S, 170.452° W, 1205–1600 m, 11 Feb 2014, collected by epibenthic sled from RV *Tangaroa*). Furthermore, three specimens of *J. spinispiculum* ZMA Por. 19460, 19461 (BIOSYS/HERMES 2005, boxcore 24, southeast Rockall Bank, 55.506° N, 15.786° W, 680 m, 27 Jun 2005, collected by 50 cm boxcore from RV *Pelagia*) and ZMA Por. 19579, (BIOSYS/HERMES 2005, boxcore 60, southeast Rockall Bank, 55.444° N, 16.076° W, 780 m, 30 Jun 2005, collected by 50 cm boxcore from RV *Pelagia*) (all morphologically examined in Kelly *et al.* 2015), could be sequenced. Comparative sequences obtained for the current study comprise *Siphonodictyon mucosum* RMNH Por.7253, and *S. viridescens* (Schmidt) RMNH Por.9238, which was examined by van Soest *et al.* (2014) in the course of reassignment this species from *Oceanapia* Norman. We sequenced the 28S rDNA C-region (459 bp), a DNA barcoding marker for Demospongiae (Erpenbeck *et al.* 2016b). DNA extraction and amplification of followed standard published protocols as successfully applied for demosponge type and other material (Erpenbeck *et al.* 2016b). Amplifications of the 28S C-Region were conducted with primers 28S-C2-fwd and 28S-D2-rev (Chombard *et al.* 1998). Raw sequences were basecalled, trimmed and checked in CodonCode Aligner 9.0.1. Sequences were aligned with MAFFT 7.4.50 (Katoh & Standley 2013) with other haplosclerid raspailiid and other heteroscleromorph sequences as published in NCBI Genbank under default settings. As the 28S C-Region possesses a high variability suitable for species-level analyses, which in turn hampers the alignment of more distantly related taxa, we used gblocks (Castresana 2000) under all setting combinations in order to verify that the high variability of the 28S C-region does not affect the resulting classification context of *Janulum*. To minimise the loss of phylogenetic signal we applied in our analysis a two-step approach, in which first the order-level classification of *Janulum* has been verified in a phylogenetic reconstruction with a representative suite of heterosleromorph sequences (Fig. 1) and a subsequent order-level phylogeny (Fig. 1 inset). Maximum-likelihood reconstructions were generated with RAxML version 8.2.11 (Stamatakis 2014) under the GTRGAMMA model and 100 rapid bootstrap replicates.

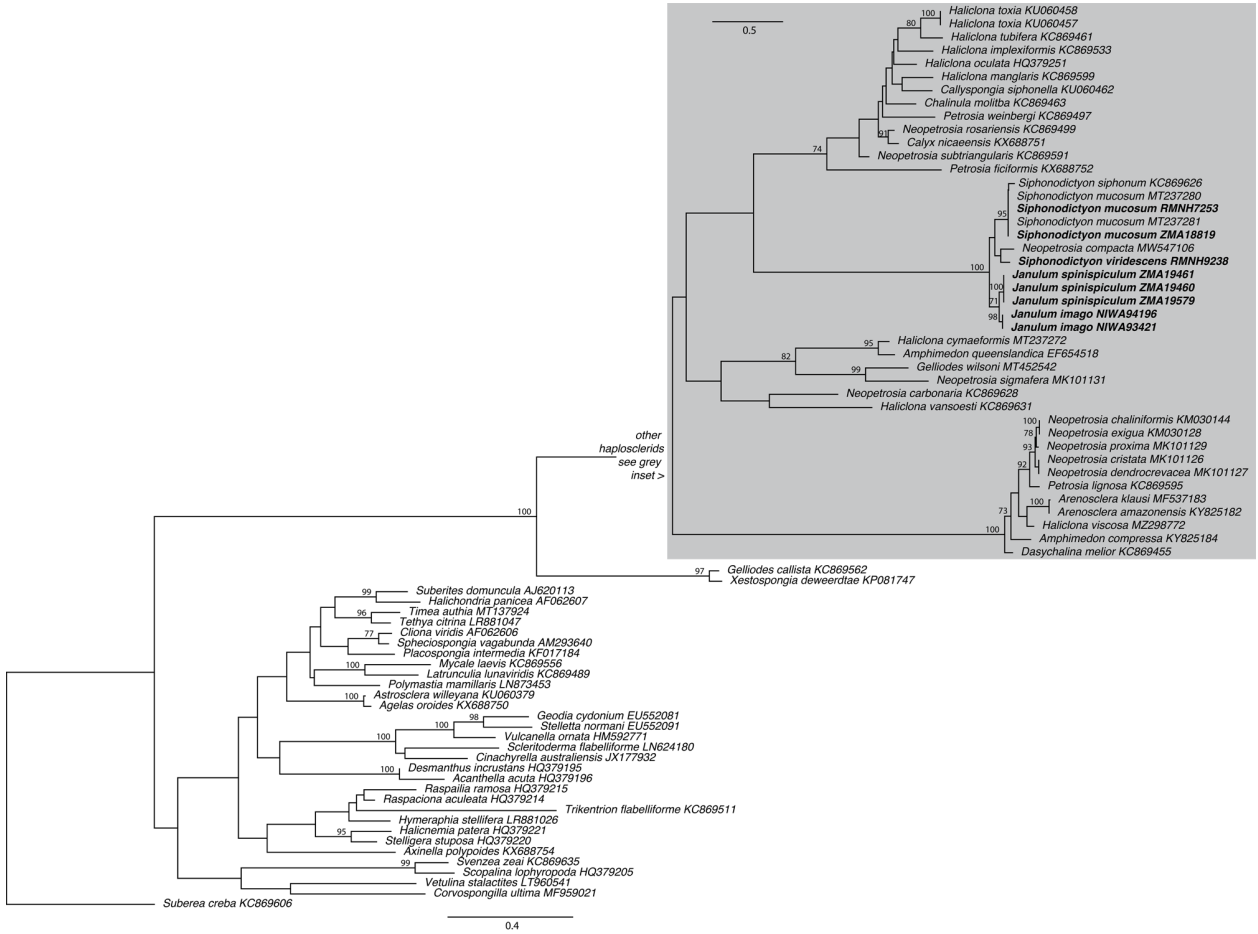

FIGURE 1. Maximum likelihood phylogram to highlight the phylogenetic position of *Janulum.* Newly sequenced taxa are in bold. Numbers following the species names are collection numbers (for the new sequences) or NCBI-Genbank accession numbers. The inset in grey is based on the separate, haplosclerid-only alignment of the same taxa (rooted with *Gelliodes callista* KC869562 and *Xestospongia deweerdtae* KP081747). Numbers above branches are RAxML rapid bootstrapping support values (if larger than 70). Scale bars indicate substitutions per site.

The phylogenetic reconstructions support the classification of *Janulum,* including the type species *J. spinispiculum*, and *J. imago*, to the Haplosclerida and distant from Raspailiidae, other Axinellida or Poecilosclerida, corroborating Vacelet (1969), Boury-Esnault *et al.* (1994), Wiedenmayer (1994) and the 18S rDNA results of Redmond *et al.* (2013) (Fig. 1). *Janulum* is recovered as monophyletic and forms a supported clade shared with several *Siphonodictyon mucosum*, in congruence to the findings of Redmond *et al*. (2013). However, support for the congeneric *S. viridescens* with *Siphonodictyon* is still lacking with the current data set. We also find *Neopetrosia compacta* (Ridley & Dendy) in this clade, despite being identified by one of the authors (M.K. in Posadas *et al.* 2022), and distant from other *Neopetrosia* de Laubenfels, reflecting the current, persistent challenges in haplosclerid systematics. Redmond *et al.* (2011) stated that, Haplosclerida constitutes the "most difficult and unstable group of Demospongiae" as molecular phylogenies unanimously demonstrate the non-monophyly of its (morphologically defined) six families, and many of its genera (e.g., McCormack *et al.* 2002; Redmond *et al.* 2011, 2013). All molecular systematics studies (including Figure 1) recover a puzzling patchwork of paraphyletic taxa, which consequently urges for the need of a molecular taxonomic bottom-up approach based on type species (and ideally type specimens, see Erpenbeck *et al.* 2016a) aiming for a robust phylogenetic framework (Cárdenas *et al.* 2012) that facilitates subsequent re-interpretation of morphological characters. Until this has been undergone, the *Janulum* can be confirmed as Haplosclerida, but remains for the time being Haplosclerida *incertae sedis*.

Conclusion

We hereby propose reassignment of *Janulum* to Order Haplosclerida *incertae sedis* in recognition of the weight of opinion in support of assignment to Order Haplosclerida based on skeletal architecture and the current DNA sequence data.

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Michelle Kelly's participation was funded by NIWA under Coasts and Oceans Research Programme 2 Marine Biological Resources: Discovery and definition of the marine biota of New Zealand (2020/2021 SCI). We thank Nicole Enghuber, Gabriele Büttner and Simone Schätzle for their support in the lab.

DNA deposition

Novel sequences are archived at the European Nucleotide Archive: OX431168-OX431175

Data availability

Data is available at the Sponge Barcoding Database: SBD#2328–SBD#2335
Data is available at the European Nucleotide Archive: OX431168-OX431175

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