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Sponge taxonomy in the -omics era: resolving Haplosclerida polytomies with phylogenetics and metabolomics

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ABSTRACT

With several new taxa described every day, we are still in the Age of Discovery of the biodiversity. On the other hand, the sixth mass extinction of biological species is underway, rapid, and aggressive, which renders the description of biodiversity to inform conservation, of high priority. Systematics allows a reliable assessment of biological diversity, facilitating conservation management. Unfortunately, marine invertebrates often present few morphological characters that help to define boundaries and classify taxa. Therefore, the integration of new approaches and data is necessary to obtain more robust diagnostic characters enabling a more accurate identification of the taxa. The sponge order Haplosclerida is no exception within the Metazoa in general when it comes to a sizeable mismatch between the growing phylogeny of the group and its accepted classification. The monophyly of marine haplosclerids is well supported, whereas many of the Haplosclerida families and even genera are paraphyletic. A deep morphological, molecular and metabolomic investigation of all species of the genus *Arenosclera* (Callyspongiidae, Haplosclerida) allowed a reassessment of the genus' diagnosis, a description of the new genus *Arenospicula* gen. nov., and a reorganization of species in the classification. Our results highlight the potential of an integrative approach to solve part of the extensive mismatch between phylogeny and classification for the Haplosclerida. Integration of morphology, molecular and metabolomic data is a robust way to face the challenge of widespread polyphyly in every non-monotypic higher taxon within the Haplosclerida. Integration of these data could also lead to a more accurate and natural view of genera and family boundaries of currently para- and polyphyletic taxa in marine invertebrates.

1. Introduction

The sixth mass extinction of biological species is underway (Ceballos et al., 2010) and has accelerated due to climate change and anthropogenic activities. Therefore, knowledge and description of biodiversity using new techniques such as the -omics sciences are of high priority (Reddy et al., 2021). Reliable assessment of biological diversity and accurate systematic classification is vital for conservation and management of biodiversity. For example, automated pipelines based on large DNA databases highlight the importance of taxonomy for fundamental knowledge and for more advanced evolutionary hypotheses (Pappalardo et al., 2021). Taxonomic studies based on morphological characters alone are difficult because diagnostic characters may not accurately reflect phylogenetic relationships (Komarek and Beutel, 2006). The

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misinterpretation of homologous features may arise from evolutionary convergence, and most often, from environmental factors (*e.g.* distance from main sources of silicates, seasonal climatic variations, physiological cycles such as the reproductive one; Zea, 1987; Schönberg, 2021). For marine invertebrates, accurate identification of taxa is challenging due to limited morphological characters for species diagnosis and classification. For instance, morphological characters used to classify sponges are often insufficient for accurate identification because of their simple body plan and intraspecific variability (Boury-Esnault et al., 2013; Erpenbeck et al., 2016a).

Data used for the description and classification of biological diversity has recently benefitted from the development of the -omics sciences (Reddy et al., 2021). Although morphology and cytology remain essential, current approaches require large sets of data for species delimitation, to enable improved identification of taxa (Criscione et al., 2021), and to reflect phylogenetic relationships more accurately (Marchán et al., 2018). The use of integrative approaches including molecular data has significantly improved the classification of sponges, and has highlighted diagnostic morphologic traits for higher taxa of various Linnean ranks (Boury-Esnault et al., 2013; Cárdenas et al., 2012; Pons et al., 2017; Vargas et al., 2012).

Biochemical characters can also be included for a more robust integrative approach in sponge taxonomy. For example, sterols and lipids were among the first biochemical characters used in chemotaxonomy, albeit with severe limitations (Erpenbeck and van Soest, 2005). Thereafter, some families of specialized metabolites (also referred to as secondary metabolites or natural products) that are produced by specific metabolic pathways have been used as additional taxonomic markers (Braekman et al., 1992; Hooper et al., 1992; Ivanišević et al., 2011a; Tribalat et al., 2016). However, sponge chemotaxonomy based solely on data obtained from the literature is often not reliable since most investigations on sponge metabolites have focused on the discovery of new compounds, and do not usually report known compounds (van Soest and Braekman, 1999). Additionally, reliable identification of species associated with metabolites are often questionable as sponge taxonomists are scarce and frequently not directly involved in the biodiscovery process (Reddy et al., 2021). More comprehensive metabolomic approaches have been proposed to complement taxonomic characters and guide sponge classification (Ivanišević et al., 2011b). We hypothesized that the targeted selection of specific and highly concentrated classes of metabolites with a likely contribution of the host should be more effective in retrieving clues to resolve the evolutionary history of selected sponge taxa and facilitating improved taxonomy (Boury-Esnault et al., 2013; Galitz et al., 2021; Ivanišević et al., 2011b).

An Achilles' heel in the classification of Demospongiae lies in the order Haplosclerida Topsent, 1928, one of the largest in Porifera Grant, 1836, with over 1100 species (de Voogd et al., 2024) (Table 1). The monophyly of marine haplosclerids is well supported (Borchiellini et al., 2004; Morrow and Cárdenas, 2015), whereas many of the Haplosclerida families and even genera are paraphyletic (Redmond et al., 2013). Spicule disparity in Haplosclerida mostly deviates very little from the simplest oxeas, and not all Haplosclerida families and genera can be confidently separated using morphological characters alone. Higher taxa relationships within this order imply parallelism of these characters (van Soest and Hooper, 2002; Redmond et al., 2011, 2013). The confusing taxonomic history of haplosclerids (Table 1) has not been resolved despite the re-examination of various type species in the order (Andersen et al., 1996; Desqueyroux-Faúndez & Valentine, 2002a, 2002b, 2002c, 2002d; de Weerdt, 2002; Tribalat et al., 2016; Leal et al., 2017). As such, the morphological classification of the order remains highly incongruent with phylogenetic relationships. Molecular data have uncovered five clades designated as Clades A–E (Redmond et al., 2011), each containing various genera and families delineated based on morphology and currently representing the accepted classification of the order (de Voogd et al., 2024). For example, Clade A includes the type species of several genera, such as *Arenosclera* Pulitzer-Finali, 1982, *Callyspongia* Duchassaing and Michelotti, 1864, *Cribrochalina* Schmidt, 1870, *Haliclona* Grant, 1841 and *Neopetrosia* de Laubenfels (1949), spanning several families, namely Callyspongiidae de Laubenfels (1936), Chalinidae Gray, 1867, Niphatidae van Soest (1980); Petrosiidae van Soest, 1980, as currently understood (de Voogd et al., 2024). Clade C includes the type species of *Amphimedon* Duchassaing and Michelotti, 1864, *Dactylia* Carter, 1885 and *Haliclona* (*Gellius*) Gray, 1867, classified in Niphatidae, Callyspongiidae and Chalinidae. Clade D includes the type species of *Dasychalina* Ridley and Dendy, 1886, currently classified in the Niphatidae. Finally, Clade E comprises the type species of *Janulum* de Laubenfels, 1936 and *Siphonodictyon* Bergquist, 1965, both currently classified in the Phloeodictyidae Carter, 1882. No type species has been resolved in Clade B, which includes representatives of *Amphimedon, Haliclona, Neopetrosia, Oceanapia* Norman, 1869, *Tabulocalyx* Pulitzer-Finali, 1993 and *Xestospongia* de Laubenfels, 1932, spanning families Chalinidae, Niphatidae, Petrosiidae and Phloeodictyidae. Clades A, B, C, and E were also retrieved in a phylogenomic study using 446 loci corroborating the proposal of Redmond and collaborators (2011; van der Sprong et al., 2024).

Table 1
Taxonomic history of the Haplosclerida Topsent, 1928.

Taxonomic Authority	Modifications in Haplos
Topsent (1928)	Erection of suborder Haplosclerina, with the single family Haploscleridae, subdivided in three subfamilies Chalininae, Geliinae, and Renierinae.
de Laubenfels (1936)	Proposed recognition of five families in the Haplosclerina, viz. Callyspongiidae, Desmacidonidae, Haliclonidae, Oscarellidae and Spongillidae. These include current members of the Homoscleromorpha, the Poecilosclerida, and the Spongillida.
Griessinger (1971)	Limited haplosclerid families to Geliidae, Haliclonidae, and Renieridae.
Lévi, 1973	Proposed a classification with two marine (Haliclonidae, Renieridae), and three freshwater families (Lubomirskiidae, Potamolepidae, Spongillidae).
Bergquist and Warne (1980)	Adopted Adociidae, Callyspongiidae and Haliclonidae, but also a second order, Nepheliospongida, including Nepheliospongiidae and Oceanapiidae, for sponges currently classified in the Haplosclerida.
van Soest (1980)	Recognized five families in the order: Callyspongiidae, Niphatidae fam. nov., Haliclonidae, Petrosiidae fam. nov. and Oceanapiidae fam. nov.
van Soest and Hooper (2002)	Recognized three suborders in Haplosclerida: Spongillina, including all freshwater families; Haplosclerina, composed by Callyspongiidae, Chalinidae and Niphatidae; and Petrosina composed by Calcifibrospongiidae, Phloedictyidae and Petrosiidae.
Morrow and Cárdenas (2015)	Restricted Haplosclerida to marine taxa, classified as in van Soest and Hooper (2002), abolishing all three suborders by van Soest and Hooper (2002).

Importantly, some Haplosclerida species are well known for the production of large quantities of specialized metabolites, therefore paving the way for a targeted metabolomic approach as a complement tool to an integrative approach combining morphological and molecular data (Almeida et al., 1997; van Soest and Braekman, 1999; Redmond et al., 2011; Tribalat et al., 2016). For instance, 3-alkylpyridinium alkaloids (3-APA) have been identified in *Arenosclera brasiliensis* Muricy and Ribeiro (1999); Torres et al. (2000), some *Haliclona* spp. (Andersen et al., 1996), as well as in *Pachychalina alcaloidifera* Pinheiro et al., 2005 (Almeida et al., 1997). This observation suggests the possibility of a shared evolutionary history for these species which are currently classified in distinct genera based on divergent aspects in their anatomy. It has been recently demonstrated that *A. brasiliensis* shares a clade with two other Brazilian *Arenosclera* spp., namely *A. amazonensis* Leal et al., 2017 and *A. klausi* Leal et al., 2017, and that this clade is distantly related to *A. heroni* Pulitzer-Finali (1982), the type species of *Arenosclera*. The lack of clear morphological characters to separate the Brazilian *Arenosclera* spp. from *A. heroni* and other Indo-west Pacific species hampered the designation of a new taxon by Leal et al. (2017). Nevertheless, they named the new clade according to the PhyloCode system (Cantino and de Queiroz, 2010), as *Arenospicula*^{P}. Since integrative efforts to build an acceptable haplosclerid classification are still largely insufficient, this work intends to integrate new sources of data, while revisiting classical morphology in order to revise the taxonomy of the group, and to better reflect evolutionary relationships among Haplosclerida sponges, many of which produce 3-APA compounds.

2. Material and methods

2.1. Sampling and taxonomy

Sponge samples were collected by snorkeling, SCUBA and dredging (Supplementary Material 1). Subsamples for metabolomics were frozen, freeze-dried and stored at −20 °C. Subsamples for molecular systematics were preserved in RNAlater. Subsamples for morphological assessment and vouchers specimens were preserved in 80% EtOH, with the voucher specimens deposited in the Porifera Collection of Museu Nacional/UFRJ (MNRJ). For the morphological analyses, subsamples were identified based on microscopic preparations of dissociated spicules and thick anatomical sections (Hajdu et al., 2011).

Specimens and/or slides of the type species of *Arenosclera heroni, A. parca* Pulitzer-Finali, 1982, *A. rosacea* Desqueyroux-Faúndez, 1984 and *A. arabica* (Keller, 1889) were obtained from the Museo Civico di Storia Naturale "G. Doria" (MSNG, Genova), Museum für Naturkunde (ZMB, Berlin), and Musée d'Histoire Naturelle de Genève (MHNG, Geneva).

2.2. Metabolomic analyses

Aliquots of 200 mg of freeze-dried sponge samples were extracted with MeOH/CH_2Cl_2 (1:1) (3 x 5 mL). Extracts of each specimen were combined, filtered, evaporated and fractionated by Solid Phase Extraction on a C_{18} cartridge (1 g, Agilent Technology Bond Elut), eluted with 100% H_2O (F1), 1:1 MeOH/H_2O (F2), 100% MeOH (F3) and 1:1 MeOH/CH_2Cl_2 (F4). Dry MeOH (F3) fractions were re-dissolved in 1 mL of MeOH and filtered through 0.22 μm syringe filters. UHPLC-HRMS and UHPLC-MS/MS analyses were run on a UHPLC (Agilent 1290) using a Nucleodur® C18 HTec column (100 mm × 2.0 mm, 1.8 μm). The gradient of elution was 90:10 H_2O/MeCN (with 0.1% of formic acid) during 2.0 min, then from 90:10 to 0:100 H_2O/MeCN (with 0.1% of formic acid) during 9.0 min, then maintained in 100% MeCN (with 0.1% of formic acid) during 3.0 min, and then back to 90:10 H_2O/MeCN (with 0.1% of formic acid) in 1.0 min. The flow rate was set at 0.45 mL min^{-1} and the volume of injection at 5.0 μL. The column was maintained at 40 °C and the samples at 5 °C. A quality control (QC) sample was prepared with 10 μL from each sample. Five injections of the QC were run at the beginning of the batch, one every five samples, and five at the end. Two injections of MeOH blanks were run at the beginning, and one injection after every 20 samples.Mass spectra were acquired on an Agilent 6540 Ultra High Definition (UHD) Accurate Mass Q-TOF with a Jet Stream dual ESI interface using the data-dependent analysis (DDA) mode, in positive mode within the range m/z 100–1700, scan time 0.2 s^{-1} and threshold of 200. Five precursor ions were fragmented per MS survey, using the same scan time and m/z range and a collision energy of 50 eV (Afoullouss et al., 2022).

Raw data were converted to.mzML files using peak picking (1-) filter on MSConvert software (ProteoWizard v. 3.0.11626). The Galaxy platform (Afgan et al., 2016) was used to generate a matrix (parameters: ppm = 15; centwave; min = 5; max = 12; bw = 10; minfrac = 0.1; mzwid = 0.015) with the features detected in the MS analysis. It is important to highlight that the sponges were treated here as holobionts and as such the metabolites analyzed could be part of the sponges' metabolism but also can be part of their microbiome metabolism.

The coefficient of variation was measured for each feature in QC samples. Features more than 20% variable were removed to ensure reliability of the data. For the metabolomic analyses, samples were split by species into 12 groups: (G1) Aren, *Arenospicula amazonensis* comb. nov. and *Arenospicula brasiliensis* comb. nov.; (G2) Pach (Previously *Pachychalina alcaloidifera*), *Arenospicula mammillaris* comb. nov.; (G3) Avir, *Amphimedon viridis* Duchassaing and Michelotti, 1864; (G4) Hcae, *Haliclona caerulea* (Hechtel, 1965); (G5) Himp, *Haliclona implexiformis* (Hechtel, 1965); (G6) Chal, *Chalinula molitba* (de Laubenfels, 1949); and *Chalinula* sp.; (G7) Clad, *Cladocroce caelum* Santos et al., 2014; (G8) Nere, *Niphates erecta* Duchassaing and Michelotti, 1864; (G9) Cally1, *Callyspongia* sp.; (G10) Cally2, *C. pseudotoxa* Muricy and Ribeiro, 1999; (G11) Niph, *Niphates* sp.; and (G12) Hsp (Previously *Haliclona* sp.), *Haliclona (Rhizoniera) fugidia* Muricy et al., 2015. Statistical analyses were performed using MetaboAnalyst (Xia and Wishart, 2016).

2.3. Molecular networking analyses

Three replicates of each target species and some external groups (*Amphimedon viridis, Callyspongia pseudotoxa, Chalinula molitba, Chalinula* sp., *Cladocroce caelum, Haliclona caerulea, H. implexiformis, Niphates erecta*, and Niphatidae) were selected to build a Molecular Network (MN; Wang et al., 2016). Processed UHPLC-HRMS/MS data were uploaded onto the UCSD GNPS FTP-server and investigated via Classical Molecular Network (METABOLOMICS-SNETS) workflow. All samples and blanks were organized into five groups (MNG1–MNG5) where the MNG1, MNG2 and MNG3 were the main groups identified in the Hierarchical Cluster Analysis (HCA). MNG4 was the group containing standard 3-APA isolated from *Haliclona sarai* (Clade C), and MNG5 the blank group. A minimum cosine score of 0.6 and a minimum number of matched fragments of 5 were chosen for these analyses. The mass tolerances were set to 0.02 Da for precursor and fragment ions. All other settings were left as default values. The resulting network was imported into Cytoscape 3.7.1 and visualized using the yFiles Organic layout. Data in GNPS can be accessed via the link (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=1cedb566ce6b4670b41ef055a9a01150). The resulting clustered spectra obtained by GNPS molecular networking was analyzed using the *In silico* Peptidic Natural Product Dereplicator and the Dereplicator+: Identification of Metabolites Through Database Search of Mass Spectra GNPS tools. The results and parameters for both analyses can be accessed via the links (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=87a29cb30ade43eda54818b48a70a323) and (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=75902a51c97b4b80b4d981d253722105), respectively.

Annotation of the metabolites fragmented by MS/MS was performed for the three species *Arenospicula brasiliensis*, *A. amazonensis* and *A. mammillaris* using the software Sirius 5 (Dührkop et al., 2019) on the major metabolites present in the window 4–8 min. The metabolites were first analyzed for their most likely molecular formulae (10 max). As 3-APA were evidenced as major metabolites for all species, fingerprint predictions using CFI:fingerID were only performed on molecular formulae matching 3-APA (number of carbons C_{28} to C_{33} and 2 nitrogens). Finally, the structure database search using the same software retrieved with high level of probability some key 3-APA. This targeted approach combined with manual identification and search in Marinlit (https://marinlit.rsc.org/marinlit) resulted in the identification of all major metabolites of these three species. Finally and in the absence of MS/MS data for this species, a manual search was performed on MS data from *H. (Rhizoniera) fugidia* which resulted in the annotation of the major compounds for this fourth species.

2.4. DNA extraction, amplification, sequencing

DNA was extracted using a modified phenol-$CHCl_3$ protocol (Sambrook and Russell, 2001), with a lysis buffer containing Tris-HCl 10 mM (pH 8.0), EDTA 50 mM, NaCl 0.1 M, 0.5% SDS and Proteinase K (20 mg/mL). The concentration and quality of the extracted DNA was verified using a nanospectrophotometer L-Quant Loccus (260/280 absorbance).

The fragment of nuclear 28S rRNA (C2–D2, ca. 480 bp) was PCR amplified using the primer pair 28S–C2–fwd (GAA AAG AAC TTT GRA RAG AGA GT) and 28S–D2–rev (TCC GTG TTT CAA GAC GGG) (Chombard et al., 1998; Erpenbeck et al., 2016b). PCR amplifications were performed in 25 μL reactions consisting of DNA (up to 50 μg/mL), 0.5 μL of each primer (10 μM), PCR SuperMix (Invitrogen™) supplemented with 200 μg of UltraPure™ BSA (Invitrogen™). For the PCR reactions the initial denaturation was performed at 94 °C for 3′, followed by 35 cycles of 30″ denaturation at 94 °C, 30″ annealing at 50 °C, 60″ elongation at 72 °C, and a final elongation at 72 °C for 5'. Amplicons of *Amphimedon viridis*, *Niphates erecta* and *Arenospicula mammillaris* comb. nov. repeatedly resulted in sequences of non-targeted organisms. Therefore, a new forward primer positioned downstream, 28S–C2–HaploC–fwd (GTC AAA GAG ACC GCG AAA CC), more specifically related to Clade C of Haplosclerida, was used together with the 28S–C2–rev primer to amplify a fragment of about 460 bp of the same region. A touchdown protocol with a greater denaturation temperature was performed to cope with the high GC content (>65%) in species from this Clade: initial denaturation step at 95 °C for 5′, followed by 10 cycles of 45″ denaturation at 95 °C, 30″ annealing at 60–55 °C (−0.5 °C by cycle), 45″ elongation at 72 °C; 25 cycles of 45″ denaturation at 95 °C, 30″ annealing at 55 °C, 45'' elongation at 72 °C and a final 8' elongation step at 72 °C.

Amplicons were purified using the Exonuclease I and Shrimp Alkaline Phosphatase and submitted to ACTGene (Porto Alegre, Brazil), Myleus Biotecnologia (Belo Horizonte, Brazil) or Labcen/Universidade Federal de Pernambuco (Recife, Brazil) sequencing facilities. Both strands were sequenced using ABI Genetic Analyzer 3500/3730 automated sequencers. Electropherograms were assembled and edited using Geneious Prime 2021. Ambiguous bases were coded with IUPAC ambiguity codes. Sequences with high quality were checked using the BLAST tool of NCBI (https://blast.ncbi.nlm.nih.gov). Sequences are deposited in NCBI GenBank under the accession numbers MZ366933–MZ366958; PQ144853-PQ144859 (Supplementary Material 1).

2.5. *Phylogenetic reconstruction and molecular taxonomy*

The 28S DNA sequences obtained in the present study together with relevant haplosclerid sequences available at GenBank were aligned with the MAFFT v.7 (Katoh et al., 2019) online service using the FFT–NS–i algorithm. Uncorrected *p*-distances (pairwise deletion and 500 bootstrap replicates) within and among species were calculated in MEGA 7 (Kumar et al., 2016). The phylogenetic analyses were conducted using the Maximum Likelihood (ML) framework in RAxML v.8.2.10 (Stamatakis, 2014) software implemented in the CIPRES Science Gateway (https://www.phylo.org/portal2), using the GTRGAMMA model and 1000 rapid bootstrap pseudoreplications (BST) to assess the confidence of the topology. Only bootstrap values above 70 are exhibited in the trees. The outgroup for 28S includes the sequences of *Svenzea zeai* (Alvarez et al., 1998) (KC869635) and *Scopalina ruetzleri* (Wiedenmayer, 1977) (KC869553) of the order Scopalinida, which nested close to Haplosclerida in molecular phylogenies based on large datasets (Thacker et al., 2013). The aligned dataset included 106 in-group taxa and 4541 bp as we opted to not trim longer sequences. For the ITS tree the sequences were recovered from the metagenome following the methodology explained in Leal et al. (2017). Sequences were aligned with MAFFT v.7.490 and ML phylogeny was conducted with FastTree 2.1.11 both in the Geneious platform.

3. Results

3.1. *Systematics of Haplosclerida: genus Arenospicula Leal, Bispo & Hajdu gen. nov*

Arenospicula gen. nov. is herein proposed with the same composition as the Phylocode clade *Arenospicula*^{p} (Leal et al., 2017), and, in addition, *Pachychalina alcaloidifera* (now designated as *Arenospicula mammillaris* comb. nov.) has been transferred to the new genus. We propose revised diagnoses for both genera *Arenosclera* and *Arenospicula*, as well as full re-descriptions of *Arenospicula mammillaris,* and *A. brasiliensis* in order to define and delimit both genera. Diagnosis and re-descriptions of *Arenosclera* spp. can be found in the Supplementary Material 2.

Class Demospongiae Sollas, 1885.

Subclass Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault (2012).

Order Haplosclerida Topsent, 1928.

Clade C *incertae sedis*.

Genus ***Arenospicula*** **Leal, Bispo & Hajdu gen. nov.**

Figs. 1 and 2.

Type species: *Arenospicula brasiliensis* (Muricy and Ribeiro, 1999).

3.1.1. *Diagnosis*

Haplosclerida - Clade C *incertae sedis* with non-detachable, paratangential ectosomal skeleton, forming meshes of irregular diameter. Choanosomal skeleton a subisotropic reticulation, with uni-to multispicular tracts or spongin fibres cored by oxeas, more confused towards the interior and more neatly organized closer to the surface, where primary and secondary tracts/fibres are discernible. Foreign debris may be present, dispersed into or among fibres, but never forming a reticulation. Colour light grey to beige in live material (Fig. 2). All species of this genus contain C-4/C-5' 3-alkylpiperidine markers.

3.1.2. *Remarks*

The new genus is similar to *Pachychalina* Schmidt, 1868 in having confused and irregular ectosomal and choanosomal skeletons. Both genera can be differentiated by *Pachychalina*'s stout, often ramified choanosomal primary tracts, where spongin is not observable (*e.g.* Desqueyroux-Faúndez, 1999). *Arenospicula* Leal, Bispo & Hajdu gen. nov., contrastingly, has delicate, unramified primary tracts, where enveloping spongin can be scarce to common, and frequently, sand into or among fibres. *Arenospicula brasiliensis* was selected as the type species because the species is well-described and can be easily found in the type locality.

3.1.3. *Arenospicula brasiliensis (Muricy and Ribeiro, 1999) comb. nov*

Fig. 1A, C; 2 A, B.

Arenosclera brasiliensis Muricy and Ribeiro, 1999(24): 97, Figs. 2D, 9–10.

3.1.4. *Material examined*

MNRJ 21064, 21065 – Ilhas Rasas, Guarapari, Espírito Santo state (20°40.533′S–040° 22.050′W), 9.5 m depth, coll. C.V. Leal, 27–III–2017; MNRJ 21090 – Ilhas Rasas, Guarapari, Espírito Santo state (20°40.533′S–040° 22.050′W), 12.3 m depth, coll. E. Hajdu, 27–III–2017.

3.1.5. *Comparative material*

MNRJ 28989 (formerly UFRJPOR 4627; Holotype) – João Fernandinho Beach, Búzios, Rio de Janeiro state, 3 m depth, coll. G. Muricy, 30–VIII–1997; UFRJPOR 3073 (Paratype), Armação de Búzios, Rio de Janeiro state, coll. E. Hajdu, 06–XII–1986.

3.1.6. *Diagnosis*

Massive, lobate, occasionally lamellate by fusion of lobes (2–7 cm high). Oscula (1–10 mm diam.). Ectosome not detachable, with spongin fibres cored by pauci-to multispicular tracts (5–40 μm diam.), and irregular to rounded meshes (40–280 μm diam.). Choanosome a subisotropic reticulation of uni-to multispicular lines (5–50 μm diam.), mostly enclosed by spongin and always cored by oxeas, creating mostly irregular, but also rounded or squared meshes (40–1385 μm diam.); confused at parts. Sand and foreign debris in moderate quantity

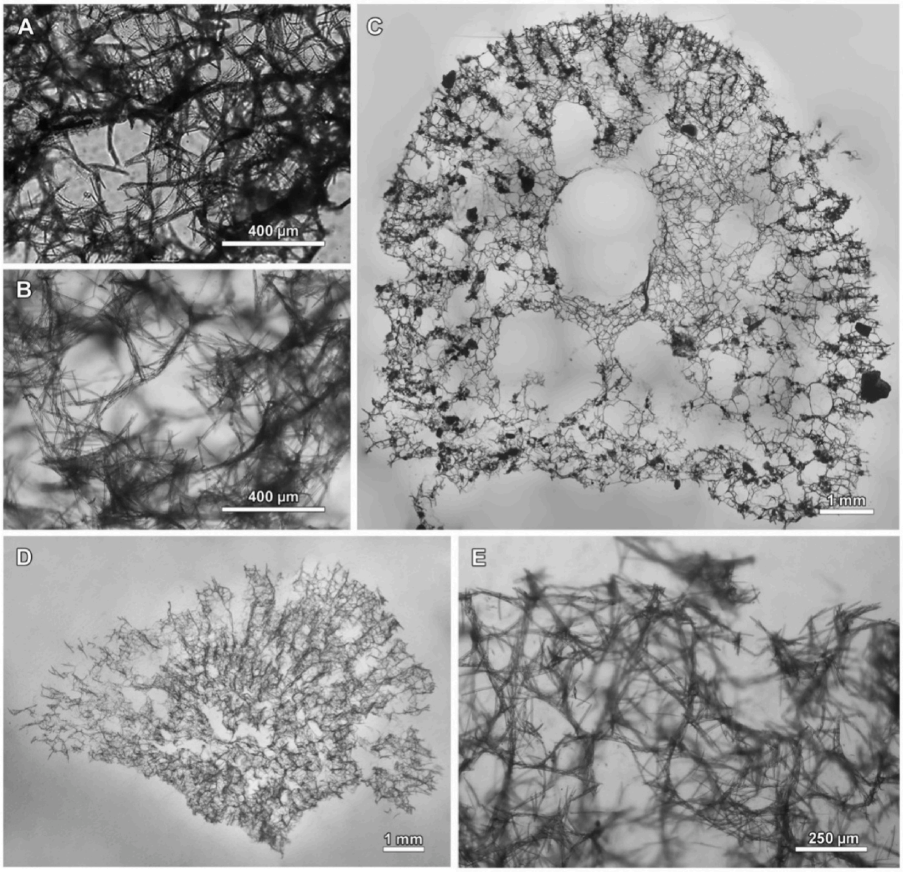

Fig. 1. *Arenospicula* gen. nov.: tangential sections of the ectosomal (A–B) and choanosomal skeletons (C–E). (A, C) *Arenospicula brasiliensis* comb. nov., MNRJ 21090. (B, D, E) *Arenospicula mammillaris* comb. nov., MNRJ 15568.

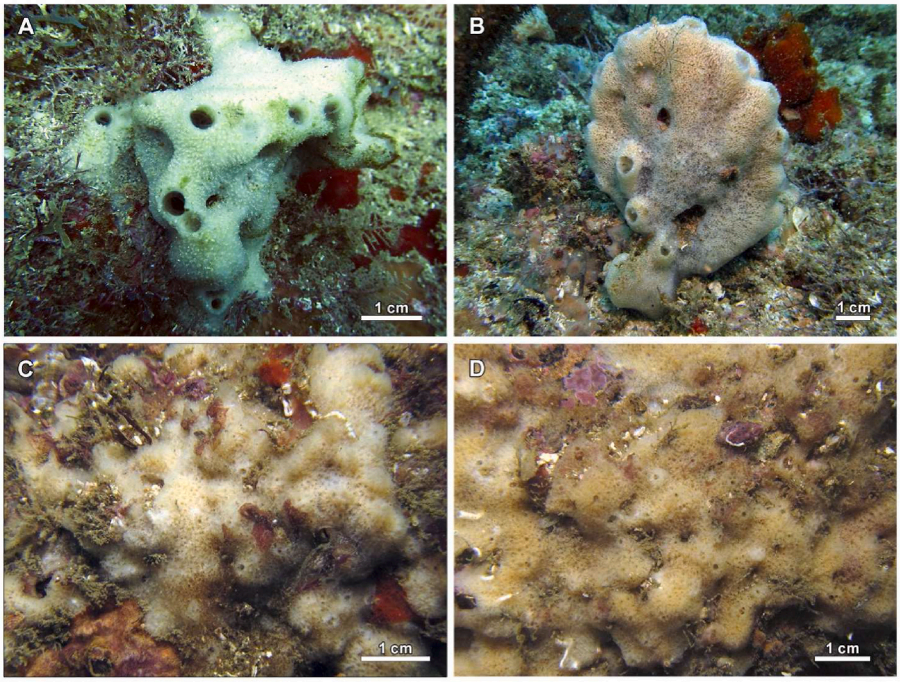

Fig. 2. Species of *Arenospicula* gen. nov. *in situ*: (A–B) *Arenospicula brasiliensis* comb. nov.; (A) MNRJ 21064; (B) MNRJ 21090. (C–D) *Arenospicula mammillaris* comb. nov.*;* (C) MNRJ 21133; (D) MNRJ 21170.

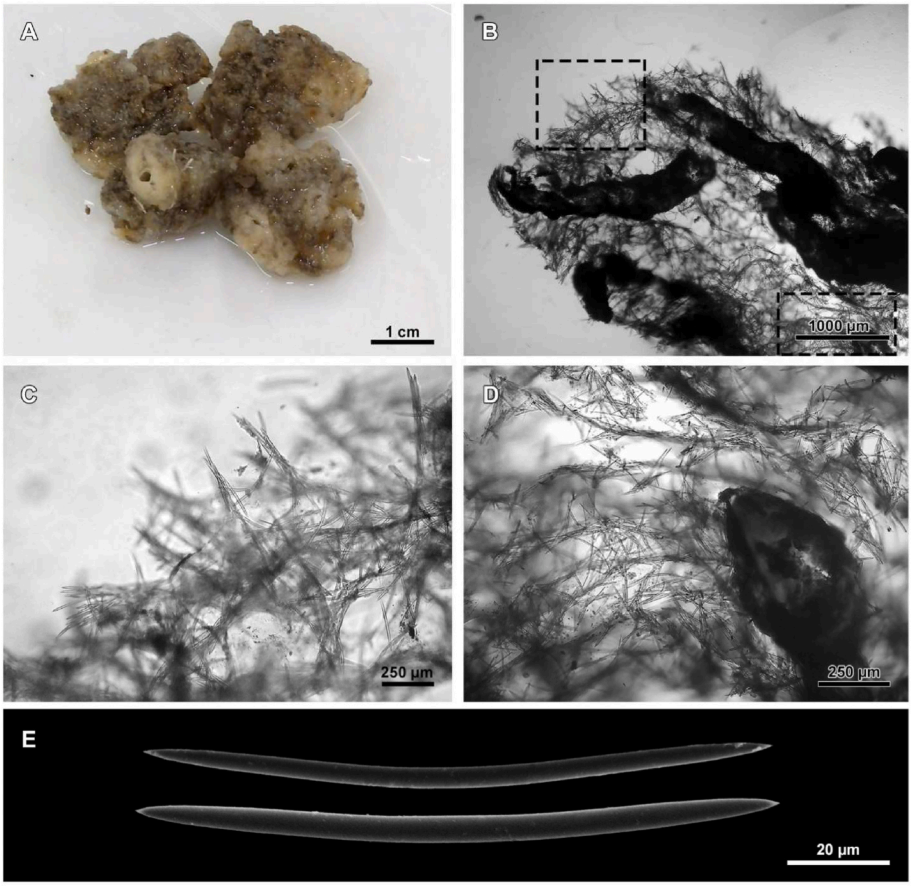

Fig. 3. Morphology, skeletal architecture and oxeas of the holotype of *Arenospicula mammillaris* (Mothes and Lerner, 1994) comb. nov. (A–E) MCNPOR 1482; (A) Preserved specimen; (B) Panoramic view of the choanosomal skeleton; (C) Detail of B, showing the skeletal architecture of the choanosome near the surface; (D) Choanosomal skeleton in the inner parts; (E) Oxeas.

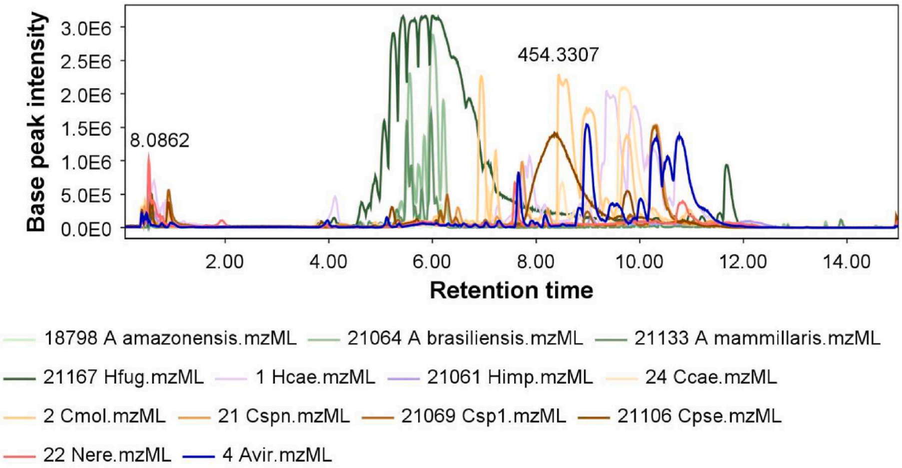

Fig. 4. Overlaid chromatograms for all samples analyzed in LC-MS/MS in this work.

dispersed throughout the skeleton, but mainly close to surface. Oxeas (41–108 x 1.5–6.5 µm), slightly curved, fusiform, acerate. In the 3-APA-region described above, this species contains mainly features at *m/z* 483, 465, 467, 469 identified in the MN as arenosclerins, tetradehydrohaliclonacyclamine, haliclonacyclamine E or F, and haliclonacyclamine A or B using Sirius 5. (see Supplementary Material 5).

3.1.7. Description

3.1.7.1. External morphology. Massive (Fig. 2A) to lamellate (Fig. 2B) (up to 7 cm high x 5 cm in diameter). Oscula circular (1.9–5.8 mm in diameter) flush with the surface or slightly elevated, usually aligned in rows (Fig. 2A,B), but also randomly distributed, surrounded by a transparent rim. Surface microconulose, slightly hispid to the touch, with clearly observable imbedded debris, and producing mucus upon

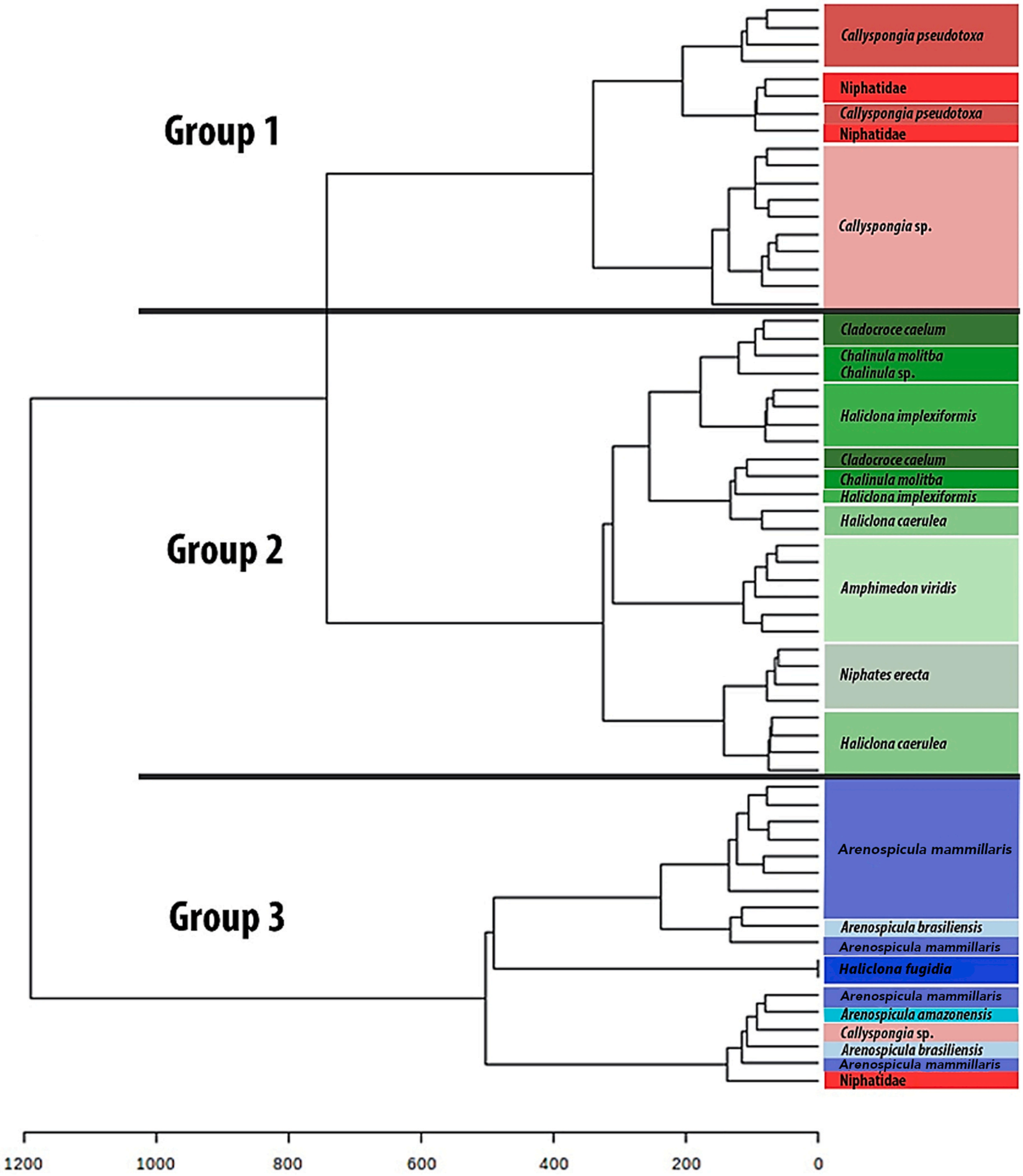

Fig. 5. Hierarchical Cluster Analysis (HCA) of herein investigated Brazilian Haplosclerida species metabolomes using Pearson distance. The metadata of the samples used here are in the Supplementary Material 1.

collection and handling. Consistency is soft, compressible, but resistant to tear. Colour alive whitish to beige, remaining nearly the same after preservation in ethanol.

3.1.7.2. Skeleton. Ectosome not detachable, with spongin fibres cored by uni-to multispicular tracts (5–36 μm diam.), forming irregular to rounded meshes (55–280 μm diam.) (Fig. 1A). Choanosome a sub-isotropic reticulation of uni-to multispicular lines (5–36 μm diam.), mostly enclosed by spongin and always cored by slender oxeas, creating mostly irregular, but also rounded or squared meshes (28–1385 μm in diameter) (Fig. 1C). In some parts, spongin becomes scarce and spicules loose, creating a confused reticulation. Sand and foreign debris in moderate quantity dispersed throughout skeleton, but mainly close to surface (Fig. 1C).

3.1.7.3. Spicules. Oxeas, slightly curved, fusiform, acerate, 57–101 x 1.5–4.4 μm. Foreign spicules commonly found.

3.1.7.4. Chemistry. The species is extremely rich in 3-APA with 17 features annotated later in the MN using Sirius and Marinlit mainly as arenosclerins, xestoproxamines, haliclonacyclamines and their derivatives (see Supplementary Material 5).

3.1.8. Ecology and distribution

Found on rocky shores, mainly on horizontal surfaces at depths of 2–12 m (Muricy and Ribeiro, 1999; present study), sometimes used as

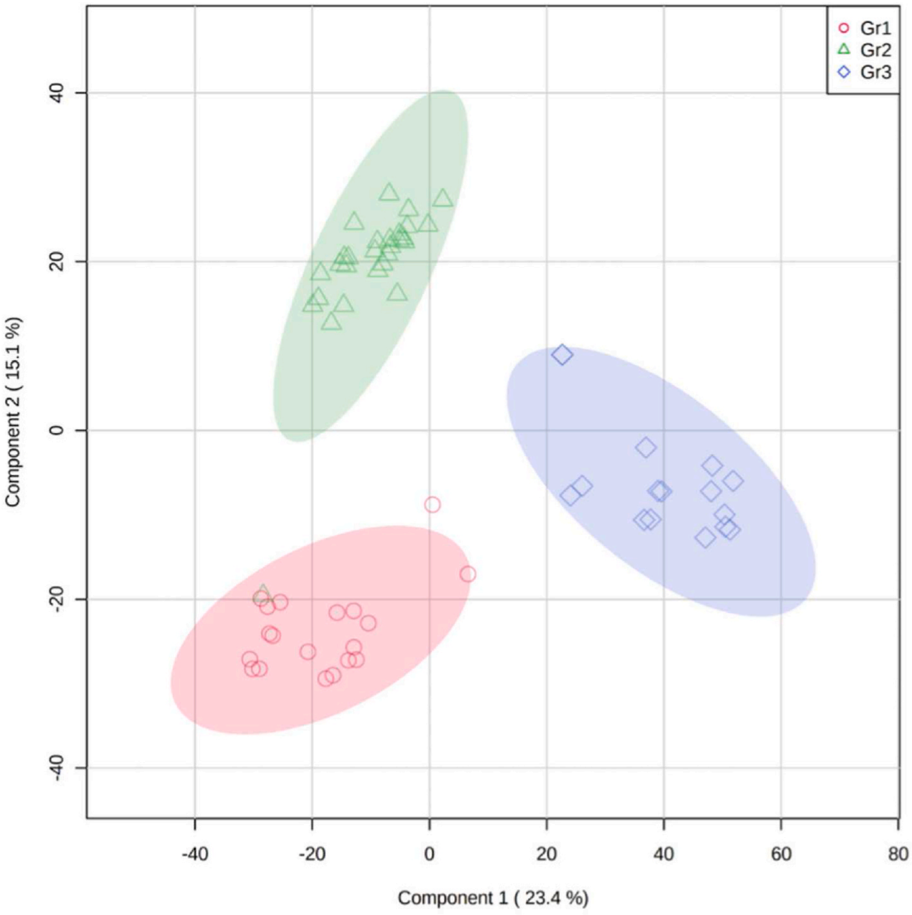

Fig. 6. PLS-DA for the metabolomes of Haplosclerida species. G1 (red), G2 (green) and G3 (blue) = Groups 1, 2 and 3, respectively.

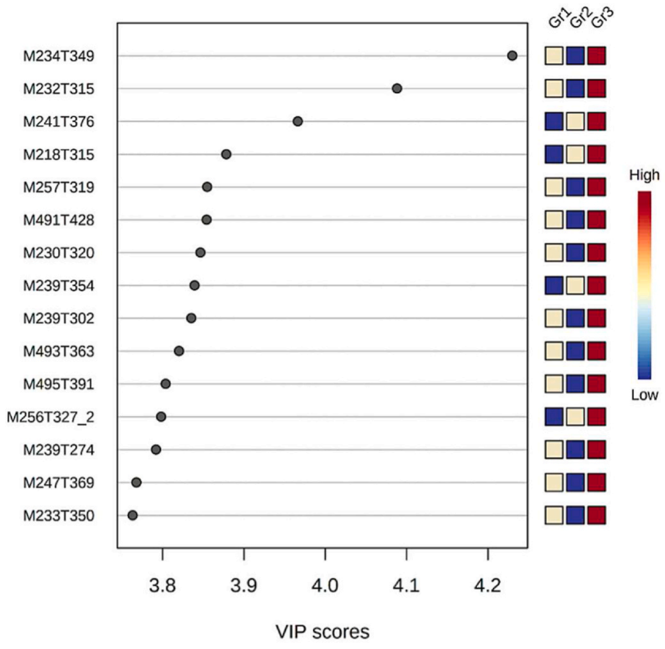

Fig. 7. Variable Influence on Projection (VIP) scores from PLS-DA analysis of Haplosclerida species metabolomes between Gr1, Gr2 and Gr3. Codes in y-axis indicated the molecular mass and the retention time of the UPLC-HRMS peaks.

substrate by sea spiders and the exotic ophiuroid *Ophiothela mirabilis* (Verrill, 1867). The species has its distribution range extended northward to ES state (Eastern Brazil Ecoregion). Previously, it was only known to occur at RJ state (Southeastern Brazil Ecoregion).

3.1.9. Arenospicula amazonensis (Leal et al., 2017) comb. nov

Arenosclera sp. Moura et al. (2016) *in part* (specimens MNRJ, 18798 and MNRJ, 18778, not MNRJ, 18757).

Arenosclera amazonensis Leal et al., 2017(18): 5, Fig. 3.

3.1.10. Material examined

MNRJ 18798 (Holotype) – Station #8, off Marajó Bay, Amazon River mouth, Pará state (00°45.359′N–46°38.49′W), 51 m depth, coll. F. Moraes & R. Moura/NHo Cruzeiro do Sul, 28–IX–2014; MNRJ 18778 (Paratype) – Station #6, off Marajó Bay, Amazon River mouth, Pará state (01°17.989′N–46°46.732′W), 55 m depth, coll. F. Moraes & R. Moura/NHo Cruzeiro do Sul, 27–IX–2014.

3.1.11. Diagnosis

Adapted from Leal et al. (2017).

Erect, solid funnel to lamellate, stalked habit with digitiform projections. Oscula clustered in slightly concave circular regions, randomly arranged. Surface regular, slightly rough to the touch, heavily reinforced by siliceous sand grains. Consistency soft and elastic. Color light brown to beige *in vivo* and after fixation. Ectosomal skeleton with spongin fibres cored by uni-to multispicular tracts, forming irregular to rounded meshes. Choanosomal, a subisotropic (plumo)reticulate architecture of spongin fibres also forming irregular to rounded meshes. Spongin fibres may be absent. Abundant sand grains, among and in the fibres, more so in the ectosome. Oxeas (44–130 μm), straight so slightly curved,

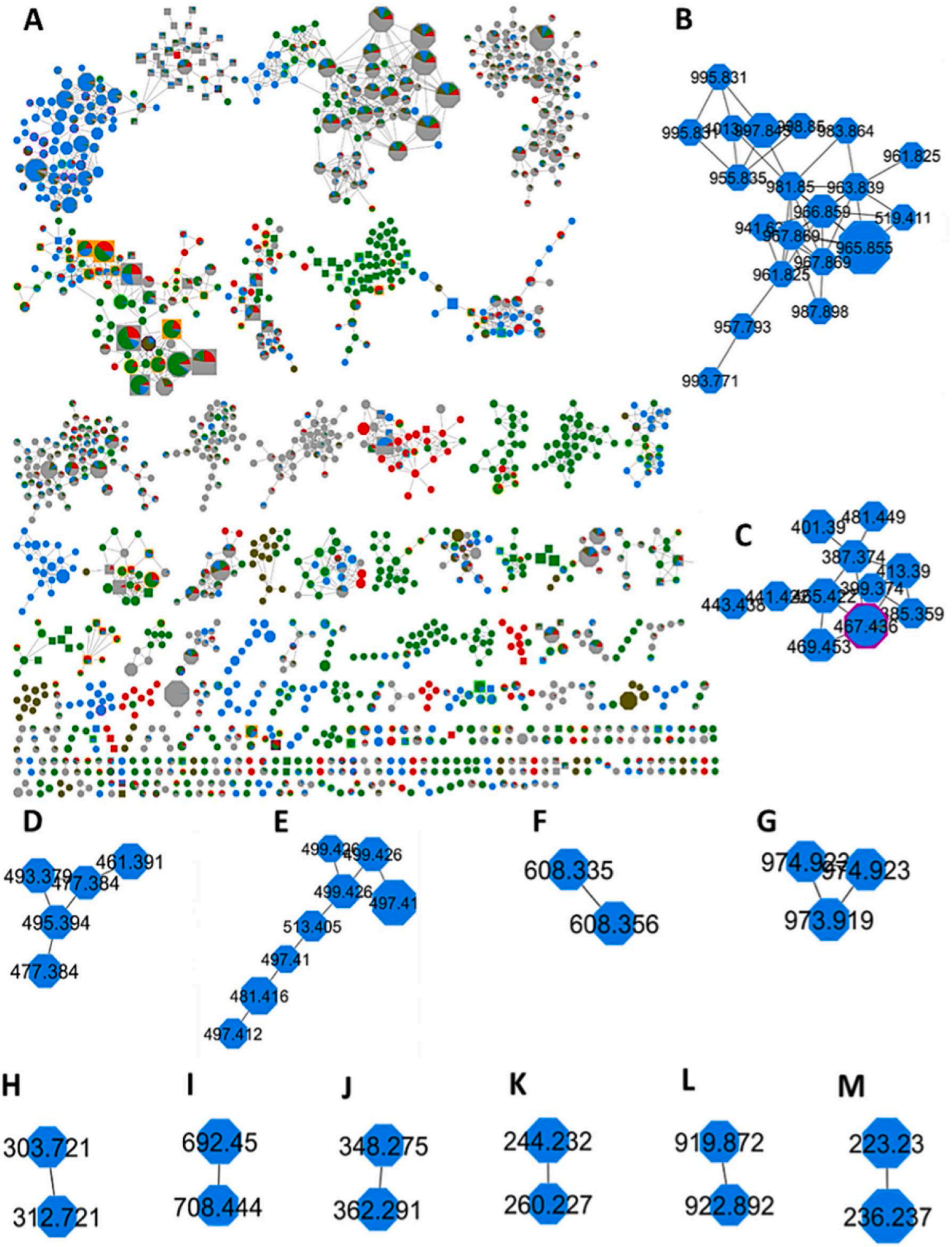

Fig. 8. GNPS molecular network (MN) of the studied Haplosclerida metabolomes. Node colours as follows: red for MNG1; dark green for MNG2; blue for MNG3; olive green for MNG4. MNG1+MNG2 = yellow; MNG1+MNG3 = cyan; MNG2+MNG3 = light green; MNG1+MNG2+MNG3 = orange; MNG3+MNG4 = pink. All signals shared with analytical blanks are in grey. The standard signals shared exclusively with the MNG3 are in lilac. Standard signal shared with samples from other group are in purple, and the exclusive signal of the standards are in moss green. (A) Molecular networking; (B–M) exclusive G3 signal clusters.

fusiform, acerate. In the 3-APA-region described above, this species contains mainly features at *m/z* 487, 445, 469, 471 annotated in the MN as new $C_{32}H_{58}N_2O$, new $C_{30}H_{56}N_2$, haliclonacyclamine A or B, and haliclonacyclamine C or D, respectively using Sirius 5 and Marinlit.

3.1.12. *Description*

3.1.12.1. External morphology. Erect, solid funnel to lamellate, stalked habit (25 cm high), with digitiform projections (9–12 cm long). Oscula (1–2 mm in diameter) clustered in slightly concave circular regions,

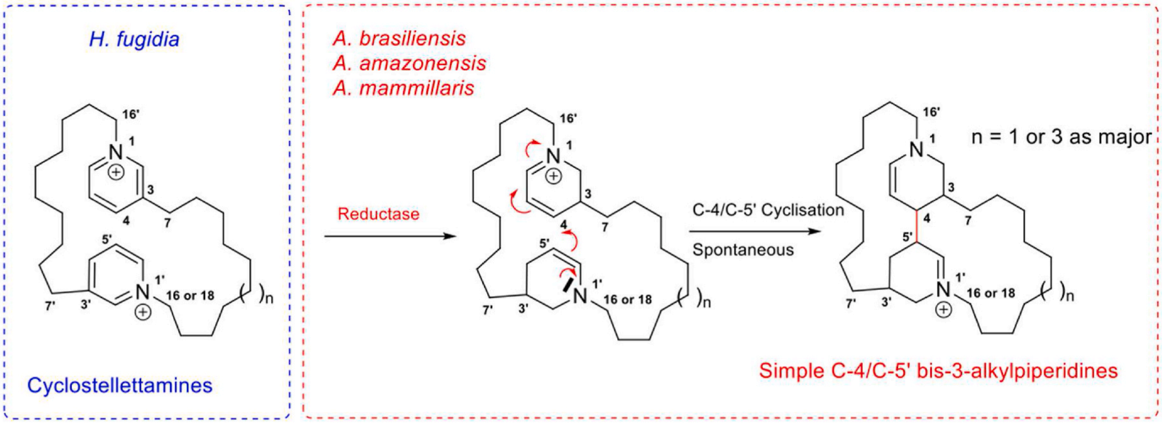

Fig. 9. 3-APA present in species of the genus *Arenospicula.*

which are randomly arranged. Ectosome not detachable. Surface regular, slightly rough to the touch, heavily reinforced by siliceous sand grains. Consistency soft and elastic. Color light brown to beige *in vivo* and after fixation.

3.1.12.2. Skeleton. Ectosomal skeleton with spongin fibres cored by uni-to multispicular tracts (9.6–46 μm in diameter), forming irregular to rounded meshes (97–493 μm in diameter). Choanosomal, a subisotropic reticulation of spongin fibres forming irregular to rounded meshes (116–1189 μm in diameter). Spongin fibres can occasionally be absent, with spicules forming a (plumo)reticulate architecture. Abundant sand grains, among and in the fibres, more so in the ectosome.

3.1.12.3. Spicules. Oxeas, straight to slightly curved, fusiform, acerate, 44–130 x 1–5 μm μm.

3.1.12.4. Chemistry. The species is rich in 3-APA with 16 features annotated later in the MN mainly as new 3-APA in C_{30} and $C_{32}H_xN_2O_y$, xestoproxamine B, haliclonacyclamines and their derivatives (see Supplementary Material 5). We can notice an absence of arenosclerins in this species but the presence of xestoproxamine B and haliclonacyclamines A or B in common with *A. brasiliensis*.

3.1.13. Ecology and distribution

Arenospicula amazonensis is a mesophotic species, occurring at 51–55 m depth. No other associated organisms were recorded in this species. It is currently known only in the northern Brazilian continental shelf, on rhodolith beds at the central sector off the Amazon River mouth (Pará, Brazil).

3.1.14. Arenospicula klausi (Leal et al., 2017) comb. nov

Arenosclera sp. Moura et al. (2016) [65] *in part.* (specimen MNRJ, 18757, not MNRJ, 18798 and MNRJ, 18778).

Arenosclera klausi Leal et al. (2017), p. 6, Fig. 4.

3.1.15. Material examined

MNRJ 18757 (Holotype) – Station #10, off Mutuoca Bay, Maranhão state (00°14.742′S– 44°54.089′W), 23 m depth, coll. F. Moraes & R. Moura/NHo Cruzeiro do Sul, 29–IX–2014.

3.1.16. Diagnosis

Adapted from Leal et al. (2017).

Erect, somewhat lamellate, stalked. Oscula flat on the surface, concentrated on one edge of the sponge. Ectosomal architecture with spongin fibres cored by pauci-to multispicular tracts, forming irregular to rounded meshes. Choanosomal, a subisotropic reticulation of spongin fibres cored by pauci-to multispicular tracts, with irregular meshes, becoming aspicular deeper in the choanosome, and ending in bouquets of oxea near the surface. Carbonate sand and foreign debris dispersed in large quantities among fibres all over the sponge. Oxeas (142–182 μm), straight to slightly curved, fusiform, acerate.

3.1.17. Description

Adapted from Leal et al. (2017).

3.1.17.1. External morphology. Erect (7 cm high), somewhat lamellate with a cylindrical stalk. Oscula (1–3 mm in diameter) flat on the surface, and concentrated on one edge of the sponge. Ectosome not detachable.

3.1.17.2. Skeleton. Ectosomal architecture with spongin fibres cored by pauci-to multispicular tracts (7–22 μm in diameter), forming irregular to rounded meshes (116–793 μm in diameter). Choanosomal, a subisotropic reticulation of spongin fibres cored by pauci-to multispicular tracts (8–28 μm in diameter), with irregular meshes (77–599 μm in diameter), which become aspicular deeper in the choanosome, and ending in bouquets of oxea near the surface. Carbonate sand and foreign debris dispersed in large quantities among fibres all over the sponge.

3.1.17.3. Spicules. Oxeas, straight to slightly curved, fusiform, acerate, 142–182 x 3.1–7.2 μm.

3.1.17.4. Chemistry. Due the rarity of this species we were not able to collect and fixed specimens for chemistry and therefore this species was not included in our metabolomics analysis.

3.1.17.5. Arenospicula mammillaris (Mothes and Lerner, 1994) comb. nov. Fig. 1B–E,F; 2C,D; 3A–E

Haliclona mammillaris Mothes and Lerner (1994): 54, Figs. 26, 27, 35; Lerner (1996): 115, Figs. 22, 23, 32., Mothes et al. (2003): 55; Mothes et al. (2006): 95.

Haliclona (*Haliclona*) *mammillaris* Muricy et al. (2011): 101.

Pachychalina alcaloidifera Pinheiro et al., 2005(25): 274, Fig. 3a–c; Muricy et al., 2011: 106.

3.1.18. Material examined

MNRJ 15568 – Alcatrazes Archipelago, São Sebastião, São Paulo state (24°05.743′S–045°41.548′W), 7.6 m depth, coll. R. Gamba, 29–VI–2012; MNRJ 17226, 17227 – Vermelha Beach, Angra dos Reis, RJ state (23°01.452′S–044°29.682′W), 3–5 m depth, coll. E. Hajdu, 19–VII–2013; MNRJ 21133, 21135, 21137, 21148, 21152, 21170 – Ilha do Pai, off Itaipu Beach, Niterói, RJ state (22°59.120′S–043°4.952′W), 9.1–12.5 m depth, coll. E. Hajdu, 04–IV–2017; MNRJ 21166, 21171, 21172, 21173, 21176 – Ilha Comprida, Cagarras Archipelago, RJ state (23°2.223′S–43°12.176′W), subtidal, depth not recorded, coll. E. Hajdu,

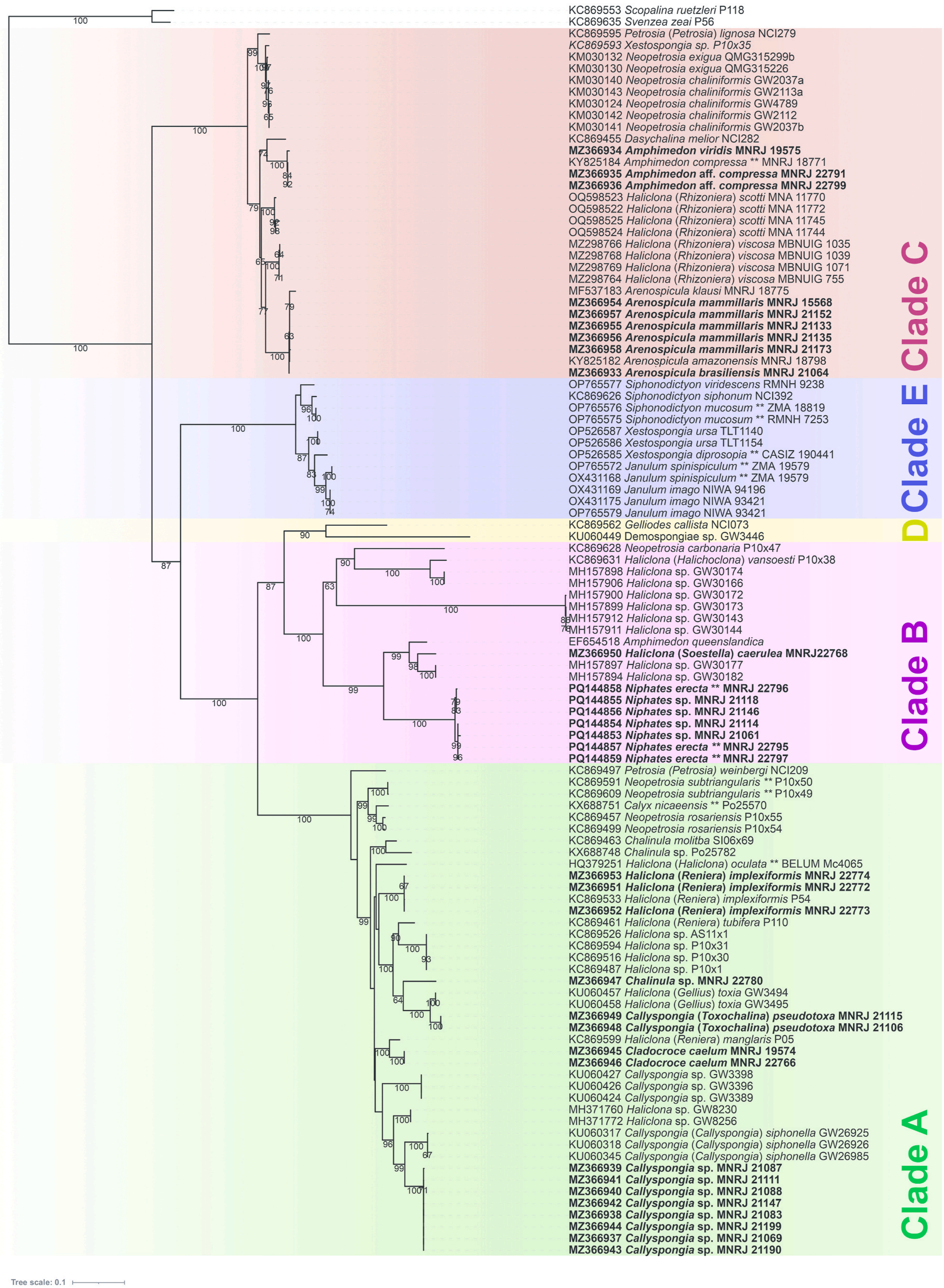

Fig. 10. Maximum likelihood phylogeny of Haplosclerida based on sequences of the 28S rRNA. New sequences of 28S C2–D2 region from specimens used in the metabolomics analysis are highlighted in bold.

04–IV–2017. MNRJ 22808 – Site 1, Ilha das Aranhas, Florianópolis, Santa Catarina state (27°29.300′S–48°21.617′W), 10–14 m depth, coll. J. Carraro, 06–IV–2011. MNRJ 22809 – Site 2, Ilha das Aranhas, Florianópolis, Santa Catarina state (27°29.200′S–48°21.679″W), 12 m depth, coll. J. Carraro, 17–XI–2011.

3.1.19. *Comparative material*

MCNPOR 1482 (Holotype of *H. mammillaris*) – Bombinhas Beach, Santa Catarina state (27°8.800′S–48°29.000′W), intertidal, coll. M.C.M. Tavares, 07–XI–1987. MNRJ 552 (Holotype of *P. alcaloidifera*) – cliff at Ponta do Boi, southern São Sebastião Island, Ilhabela, São Paulo state (23°57.957′S–45°16.120′W), 25 m depth, coll. E. Hajdu, 22–IV–1997; MNRJ 1697 (Paratype of *P. alcaloidifera*) – Itaçusse Islet, off Barequeçaba Beach, São Sebastião, São Paulo state (23°49.806′S–45°25.657′W), 6 m depth, coll. E. Hajdu, 23–IV–1998; MNRJ 1755 (Paratype of *P. alcaloidifera*) – Farol do Moleque, São Sebastião, São Paulo state (23°49.631′S–45°24.754′W), 7 m depth, coll. E. Hajdu, 07–IX–1998. MNRJ 2012 (Paratype of *P. alcaloidifera*) – Pedra Montada, Barequeçaba Beach, São Sebastião, São Paulo state (23°49.746′S–45°26.478′W), 4 m depth, coll. E. Hajdu, 01–II–1999; MNRJ 2024 (Paratype of *P. alcaloidifera*) – western Island of Búzios, off eastern São Sebastião Island, Ilhabela, São Paulo state (23°49.746′S–45°26.478′W), 10–15 m depth, coll. E. Hajdu and M.C. Guerrazzi, 08–II–1999; MNRJ 3099 (Paratype of *P. alcaloidifera*) – do Pai Island, off Itaipu Beach, Niterói, Rio de Janeiro state (22°59.205′S–43°05.252′W), ca. 15 m depth, coll. E. Hajdu, U.S. Pinheiro and S. Ribeiro, 09–V–2000.

3.1.20. *Diagnosis*

Adapted from Pinheiro et al. (2005).

Encrusting to massive, with or without lobate, digitiform or volcaniform projections. Colour varies from white, beige to grey in life. Apical oscula. Ectosomal skeleton an irregular, mostly unispicular paratangential reticulation. Choanosomal a subisotropic (plumo)reticulation, with primary ascending tracts rarely anastomosing, and secondary transverse tracts. Scarce spongin found, never forming fibres. Oxeas (82–165 μm), slightly curved. This species has a very similar chemical profile to *A. brasiliensis* with major metabolites at *m/z* 483, 465, and 467 corresponding to arenosclerins, tetradehydrohaliclonacyclamine A, and haliclonacyclamine E or F respectively after a search in Sirius 5.

3.1.21. *Redescription of the holotype of A. mammillaris*

3.1.21.1. External morphology. Encrusting, ca. 3 mm thick. Oscula circular, small, ca. 2 mm wide, surrounded by a translucent membrane, with few, short, subsuperficial canals visible close to some oscula. Surface smooth, slightly irregular, covered with sediment in many areas. Colour alive white, turning beige in ethanol. (Fig. 3A).

3.1.21.2. Skeleton. Ectosomal specialization absent. Choanosomal skeleton mainly anisotropic, with sinuous, pauci-to multispicular primary lines (3–7 spics across), irregularly connected by unispicular secondary lines. Pattern clearer near the surface, increasingly obscured deeper in the choanosome due to the presence of greater numbers of spicules in confusion. Spongin scarce. (Fig. 3B–D).

3.1.21.3. Spicules. Oxeas, hastate, usually slightly curved, occasionally straight, 110–121.8–133 x 4–4.9–6 μm. (Fig. 3E).

3.1.22. *Description of material examined*

3.1.22.1. External morphology. Encrusting to massive (up to 10 mm thick), usually occupying areas up to 400 cm^2, but sometimes reaching over 1 m in diameter (Fig. 2C and D). Oscula (0.5–1.3 mm) circular, common, usually with a transparent rim, flush with the surface or at the top of small mounds (Fig. 2C and D). Surface slightly hispid to the touch, microconulose, irregular, punctate (Fig. 2C and D). Consistency friable. Colour alive is beige, usually becoming lighter closer to the surface.

3.1.22.2. Skeleton. Ectosome not detachable, discontinuous, mostly unispicular but also with pauci-to multispicular fibres, creating irregular to rounded meshes (Fig. 1B). Choanosome a subisotropic reticulation of pauci-to multispicular tracts (8.0–46.4 μm in diameter); denser and more confuse towards the interior, with many loose spicules, more organized towards the surface, sometimes allowing distinction of tracts in primary and secondary (Fig. 1D and E). Choanosomal spaces (102–1091 μm in diameter) present. Debris are sometimes observed in small quantity, both in the ectosome and choanosome. Spongin scarce.

3.1.22.3. Spicules. Oxeas, straight so slightly curved, fusiform, acerate, 82.6–160.1 x 2.2–6.4 μm.

3.1.22.4. Chemistry. The species is also rich in 3-APA with 11 features annotated later in the MN mainly as arenosclerins, haliclonacyclamines and their derivatives, nearly all of them being shared with *A. brasiliensis* (see Supplementary Material 5).

3.1.22.5. Ecology and distribution. Arenospicula mammillaris comb. nov. is a subtidal species, occurring at 3–25 m depth at rocky shores, found on horizontal surfaces and commonly covered with sand, debris and epi- and endobiontic organisms, including polychaetes, ophiuroids and gastropods. The species is found only in Rio de Janeiro (down from Cabo Frio), São Paulo, and Santa Catarina states.

3.1.22.6. Remarks. We observed that the morphology and anatomy of *H.* (*Rh.*) *mammillaris* is within the known morphological variability of *P. alcaloidifera*, the latter is better known and comprise a wider range of morphological plasticity. Still, they share a similar encrusting habit, apical oscula on projections, fragile consistency, mostly hispid surface, white-beige colour, debris over the surface and in the skeleton, pauci-to multispicular tracts with a great number of spicules in confusion, and oxeas that are similar in dimensions, 99–133 x 3–6 in *H.* (*Rh.*) *mammillaris* vs. 87–172 x 2.4–12 μm in *P. alcaloidifera* (Pinheiro et al., 2005).

Although the holotype (MNRJ 552) and a paratype (MNRJ 569) of *P. alcaloidifera* have a stouter skeleton that deviates slightly from that observed in *H.* (*Rh.*) *mammillaris*, this appearance is granted by the much thicker oxeas in these type specimens of *P. alcaloidifera* (ca. 10 μm vs. ca. 6 μm in other paratypes), which are clear outliers. However, in general both species are indistinguishable and must be synonymized. As the name *mammillaris* has priority over *alcaloidifera*, the species should be called *Arenospicula mammillaris* (Mothes and Lerner, 1994) comb. nov.

3.2. *Metabolomics of Brazilian Haplosclerida*

The matrix resulting from the Galaxy Platform using LC-MS on 14 species of Brazilian haplosclerids was filtered based on the coefficient of variation, resulting in 6217 features among which 3-APA were tentativily annotated (See Supplementary Material 5). The overlaid chromatogram shown in Fig. 4 clearly indicates similar chemical profiles with the three species of the same genus *Arenospicula*. Compounds of similar polarity were also present in *Haliclona (Rhizoniera) fugidia* as a member of the Haplosclerida Clade C, but the family of 3-APA is different. Hierarchical Cluster Analysis (HCA) showed that haplosclerid sponges were divided into three main groups (Fig. 5). Group 1 (G1) comprising *Callyspongia* sp., *Callyspongia pseudotoxa* and *Niphates* sp. Group 2 (G2) comprising *Cladocroce caelum, Chalinula molitba, Chalinula* sp., *Haliclona (Soestella) caerulea*, *H. (Reniera) implexiformis*, *Amphimedon viridis* and *Niphates erecta*. Group 3 (G3) comprising *Haliclona (Rhizoniera) fugidia*, *A. mammillaris* comb. nov., *A. brasiliensis* comb. nov. and *A. amazonensis* comb. nov. In the HCA, one specimen of *Callyspongia* sp. (MNRJ 21071) and another of *Niphates* sp. (MNRJ 21061) nested in G3 while the remaining specimens of these two species stood within G1. However, the unsupervised PCA (Supplementary Material 3) and supervised PLS-DA (Fig. 6) included both specimens in G1.

Brazilian *Arenospicula* gen. nov. spp. formed a chemically similar group, while *Cladocroce* showed a closer relationship with *Chalinula* spp.*, Haliclona caerulea, H. implexiformis, Amphimedon* and *Niphates*. PCA score plots of the second and the first principal components (Supplementary Material 3) showed that the sponge samples were again divided into three main groups. The Partial Least Squares Discriminant Analysis (PLS-DA, Fig. 6) score plot showed that each group obtained from the HCA were significantly separated with $R2 = 0.4$; $Q2 = 0.65$ for two components. The Variable Importance in the Projection (VIP, Fig. 7) obtained from the PLS-DA enabled the determination of chemical diagnostic characters. Considering VIP values greater than 3.8 (Fig. 7), 15 features were discriminants for the three groups obtained in HCA analysis, and these were especially highly concentrated in group G3. The features are mostly cyclostellettamines identified with the general formula $C_nH_{2n-8-2x}N_2O_y$ with $n \geq 30$ $x = 0,1,2,3$ $y = 0,1,2,3$.

Classical Molecular networking analysis using MS/MS data enabled the visualization of clusters within the different sponge species. A total of 1459 nodes were observed in the molecular network: 605 corresponded to analytical blanks, 45 to standards, and 809 to sponge metabolites (Fig. 8; main clusters datailed in the Supplementary Material 4). Thirteen features annotated by GNPS as mainly expressed in G3 were sponge metabolites: arenosclerin A (Torres et al., 2000), deoxy-arenosclerin A; deoxy, 23,24-dihydro-arenosclerin A, arenosclerin E (de Oliveira et al., 2007), 14,15-dihydrohaliclamine B, 13*E*,14, 23*Z*,24-tetradehydrohaliclonacyclamine A, 22ξ-hydroxyhalicyclamine A, halicyclamine A (Arai et al., 2008), madangamines A-E (Matzanke et al., 1997; Amat et al., 2015), and additional 3-APAs using Sirius and *in silico* fragmentation (see Supplementary Material 5).

Some nodes are only present in *Arenospicula* gen. nov. and may represent taxonomic markers for this clade especially the simple C-4/C-5' 3-alkylpiperidines (Fig. 8B–M). The Molecular Network indicated that these VIP features were present exclusively in samples belonging to group MNG3 (Fig. 8). The VIP detected only in G3 enabled us to conclude that *A. amazonensis* comb. nov.*, A. brasiliensis* comb. nov. and *A. mammillaris* comb. nov. share unique 3-APA with major compounds in $C_{32}H_{(56\text{-}2x)}N_2O_y$ that may be used as chemical markers for pinpointing a likely monophyletic group, one among many necessary steps towards a much-needed reshuffling of the classification of Haplosclerida.

The Supplementary Material 5 lists the 3-APA annotated in the molecular network. The VIP analysis was sufficient to indicate the potential of the compounds in G3 to represent chemical markers.

All species assigned below to this new genus were analyzed chemically and all specimens produce 3-APA such as all members of Haplosclerida Clade C should do. All species analyzed in the genus as shown in the SI, displayed major metabolites that eluted between 4 and 8 min and were all annotated as 3-APA using MS and MS/MS data. We therefore targeted this region of the chromatograms for further analyses. As shown in the table SI, all major metabolites belong to 3-APA as described in Tribalat et al., (2016) with the molecular formulae: $C_{30}H_xN_2O_y$ and/or $C_{32}H_xN_2O_y$ as major metabolites. We consider that the presence of these 3-APAs in all studied species of *Arenospicula* place them in the Haplosclerida Clade C (Fig. 9). The reporting of this motif in species other than those contained in Clade C remains questionable, as misidentification of haplosclerid sponges based on morphology is rampant.

3.3. *Phylogeny and molecular taxonomy*

The phylogeny of the 28S C2–D2 marker (Fig. 10) retrieved three of the currently recognized main haplosclerid Clades (Redmond et al., 2013): A (100 BST), B (100 BST), C (100 BST), D (90 BST) and E (100 BST). Our samples were spread in three of these main Clades: *Callyspongia* sp., *Callyspongia pseudotoxa*, *Cladocroce caelum* and *H. implexiformis* are members of Clade A. Two sequences of *Cladocroce caelum* grouped with high support (100 BST) and closely related (100 BST) to a sample of *Haliclona manglaris* Alcolado (1984) collected from the Caribbean Sea (KC869599), both diverging genetically by about 12%. Likewise, our samples of *Haliclona implexiformis* clustered (100 BST) with a sequence (KC969533) from conspecific material from the Caribbean, their sequence divergence ranged up to 0.2% only. In turn, *Ca. pseudotoxa* samples were identical and recovered in a small monophyletic group (100 BST) including samples of *H. toxia* (Topsent, 1897), *Chalinula* sp., *H. tubifera* (George and Wilson, 1919) and *Haliclona* sp., with genetic distances of 6.7%, 21.2%, 21.8% and 21.4%, respectively. Lastly, *Callyspongia* sp. samples were identical and also clustered in a monophyletic group (99 BST) as a sister of *Ca. siphonella* (Lévi, 1962). Both diverge by 16.2–16.7% in genetic distance.

Brazilian samples of *Niphates erecta* and *Niphates* sp. clustered in Clade B in a highly supported Clade (100 BST) sister to another Clade containing *Neopetrosia carbonaria*, *Haliclona vansoesti* and *Haliclona* sp. These are the first taxonomically confirmed sequences of *Niphates erecta*, type species of the genus. Specimens identified as *Niphates* sp. could not be genetically distinguished from *Niphates erecta* and might represent a phenotypical variation.

Arenospicula comb. nov. spp. and *Amphimedon viridis* are members of Clade C. A sample of *Ar. brasiliensis* comb. nov. and all the samples of *Ar. mammillaris* comb. nov. grouped with sequences of *Ar. amazonensis* comb. nov. (KY825182) and *Ar. klausi* comb. nov. (MF537183) with high support (100 BST), motivating our decision to transfer *Ar. mammillaris* comb. nov. to *Arenospicula* gen. nov. On the other hand, the genetic divergence within this “*Arenospicula* clade” was very low, ranging from 0.0 to 1.3%, while divergence of *Arenospicula* comb. nov. spp. to other closely-related taxa is of about 13–16% compared to *Haliclona* (*Rhizoniera*) *viscosa* (Topsent, 1888) and 12–17% to *Haliclona* (*Rhizoniera*) *scotti* (Kirkpatrick, 1907). The samples of *Am. viridis* and *Am.* aff. *compressa* clustered with a sequence of *Am. compressa* Duchassaing de and Michelotti, 1864 from the Amazon mouth available in GenBank (KY825184), forming a well-supported clade (100 BST) with no genetic divergence, except for MNRJ 19575 that differs genetically from the remaining samples by 1.1%. This divergent *Am. viridis* sample is the only sample with a green colour, while the others are brown in colour. Finally, we could not obtain any sequences for samples of *Haliclona (Rhizoniera) fugidia*, and *Chalinula molitba* studied here, despite several different extraction and amplification protocols.

4. Discussion

4.1. *Taxonomic classification of Arenospicula gen. nov*

Two new species of Haplosclerida were previously allocated to *Arenosclera*, even though the 28S molecular phylogeny (Leal et al., 2017) retrieved them in the haplosclerid Clade C, which does not include the type species of *Arenosclera*, namely *Arenosclera heroni*. The latter belongs to Clade A. A new clade in the Phylocode system was named, *Arenospicula*^{P}, including these formerly new *Arenosclera* spp. (*A. amazonensis* and *A. klausi*) and *Arenospicula brasiliensis* comb. nov. (as *Arenosclera brasiliensis*), but not *Arenosclera heroni*. The extensive analysis of type species of most *Arenosclera* spp. known to date (except *Arenosclera digitata*), combined with the integrative taxonomic analysis used here (especially supported by metabolomics analyses), lead us to propose and diagnose *Arenospicula* gen. nov. in the Linnean classification.

Arenosclera species possess an ectosome composed of thicker primary fibers cored exclusively by sand, organized in neat meshes, and clearly separated from the choanosome. Occasionally thinner secondary fibers can be seen with conspicuous spongin and cored by solitary oxeas. Species of *Arenospicula* gen. nov (Fig. 1). have solely loose brushes of oxeas, representing the apical ends of choanosomal lines, mixed up with sand and foreign debris. This is apparent in their whole body, without any clear distinction between ectosome and choanosome (Figs. 1 and 3).

We consider the familial assignment of *Arenospicula* sp. as dubious given the very low support for the few clades retrieved this far that include type species of core genera of currently recognized families in the order. Consequently, we propose for the time being to classify *Arenospicula* spp. as Haplosclerida *incertae sedis*. Therefore, *Arenospicula* gen. nov. is assigned to Haplosclerida *incertae sedis* and comprises four species at present: *Arenospicula amazonensis* comb. nov*., A. brasiliensis* comb. nov*., A. klausi* comb. nov., and *A. mammillaris* comb. nov. The new genus appears to be endemic from Brazil, despite taxonomic inventories carried out in the Caribbean Sea (Wiedenmayer, 1977; van Soest, 1978, 1980, 1984, 1988, 2017; Pulitzer-Finali, 1986; Zea, 1987; Collin, 2005; Rützler et al., 2014) and in other South American countries (Zea, 1987; van Soest, 2017).

Skeletal variability in *Arenospicula* gen. nov. spp. includes the pauci- to multispicular reticulation slightly reinforced by spongin in *A. amazonensis* comb. nov., *A. brasiliensis* comb. nov. and *A. klausi* comb. nov., and the denser, pauci-to multispicular skeleton nearly devoid of spongin observed in *A. mammillaris*. All of them possess foreign debris incorporated in their skeletons, in distinct proportions. These divergent architectures in the same genus, chemically and genetically supported, point to the low systematic value of the skeleton as a widespread diagnostic feature for a given level of the hierarchy in the classification of Haplosclerida. However, careful descriptions of skeletal architectures are still a mandatory requirement for the classification of the 1100 sponges this far recognized in this order (Muricy et al., 2015).

The phylogeny of the 28S C2–D2 marker retrieved a similar topology as previously obtained for other genes and regions (Redmond et al., 2011, 2013). The high similarity in 28S rRNA sequences found among species of *Arenospicula* gen. nov. has been discussed (Leal et al., 2017), and *Arenospicula mammillaris* comb. nov. exhibits the same pattern. The phylogenetic marker (28S rRNA) used here and previously (Erpenbeck et al., 2016b) present a good resolution for Haplosclerida OTUs, even for closely related species. But, the Haplosclerida LSU evolutionary patterns are very limited compared with other Demospongiae (Erpenbeck et al., 2004). Therefore, it is not surprising that this marker does not work well for some Haplosclerida spp. The low variability of 28S C2-D2 marker is not exclusive to *Arenospicula* spp., this marker appears to be less variable also for *Amphimedon viridis* x *A. compressa* (0.000–0.005); and, *Callyspongia* sp. x *C. siphonella* (0.000–0.081). Besides the genetic variation discussed in Leal et al. (2017), the morphological variations among species and the presence of exclusive metabolites in *Arenospicula brasiliensis* comb. nov. and *A. amazonensis* comb. nov. support our species delimitation hypothesis. Also, a small tree using the ITS1-5.8S-ITS2 marker shows the differences among *Arenospicula* spp. improving the resolution of the intrageneric relationships in *Arenospicula* gen. nov. spp. (Supplementary Material 6). The low number of genomic data from sponges and the difficulty of getting good DNA extractions from type specimens usually limits comprehensive phylogenetic analyses. An improvement of such genomic studies in sponges is necessary to obtain a phylogeny with greater resolution.

Four of five clades (including Clade C) proposed by Redmond and collaborators (2011) using the 28S marker were recently confirmed by a paper that used 446 loci of ultra conservative elements (UCEs) confirming the high probability that the evolutionary history of Haplosclerida is not reflected by the morphological characters used so far (van der Sprong et al., 2024). Clade C includes genera from four out of five families recognized in the Order: Callyspongiidae, Chalinidae, Niphatidae and Petrosidae. It is possible that a revision of all type material of the species included in Clade C, as well as the inclusion of type specimens in a phylogenomic tree can help clarify the Systematic of this group allowing the formal naming of these clades at last.

4.2. Metabolomics approach

Seeking new characters to untie the Gordian knot of haplosclerid taxonomy could benefit from metabolomics data (Redmond et al., 2011; Tribalat et al., 2016). There are *ca.* 180 3-APA alkaloids known from haplosclerid sponges. For example, the 3-APAs are known from *Arenospicula mammillaris* comb. nov. (de Oliveira et al., 2004, 2006). Polymeric halitoxins and amphitoxins were found in *Amphimedon* spp. (as *Haliclona* spp.), *Callyspongia* spp. and *Haliclona sarai* (Schmitz et al., 1978; Berlinck et al., 1996; Sepčić et al., 1997; Scott et al., 2000; McClelland et al., 2003; Tribalat et al., 2016). Manzamines were reported from the haplosclerids *Haliclona* sp., *Xestospongia* sp., *Amphimedon* sp., *Pachypellina* sp., and *Acanthostrongylophora* spp., also reviewed in Tribalat et al. (2016). Manzamines were also found in *Oceanapia* Norman, 1869, identified as *Pellina* sp. (Nakamura et al., 1987). Ircinols and ircinals were reported from *Amphimedon* sp. (Tsuda et al., 1994) as well as from *Acantostrongylophora* sp. (Rao et al., 2004). Some 3-APA were reported from non haplosclerid sponges such as theonelladins (Kobayashi et al., 1989) in the tetractinellid *Theonella swinhoei* Gray (1868), of cyclostellettamines in the tetractinellid *Stelletta maxima* Thiele (1898); Fusetani et al. (1994), of manzamines, ircinols and ircinals in the dictyoceratid *Ircinia* (Kondo et al., 1992), and of halitoxin in the poecilosclerid *Batzella* sp. (Segraves and Crews, 2005). Several hypotheses were proposed to explain such divergences (Andersen et al., 1996): (1) multiple, instead of single origin of these compounds (Baldwin and Whitehead, 1992); (2) origin from microorganisms (that might be horizontally transferred among sponge hosts); (3) sponge misidentification, and; (4) samples contaminated by other sponges. Hypotheses 3 and 4 seem the most probable because many of the chemistry-based reports did not include a sponge taxonomist as a co-author.

The diversity of 3-APA and the possibility that these metabolites are synapomorphic characters at the clade level suggest a use of these chemical markers in the recovery of the phylogenetic history of the order (Andersen et al., 1996). Besides previous investigations (*e.g.* Braekman et al.,1992) which favour the recognition that Haplosclerida Clade C has niphatid affinities, several members of this clade are not classified in the Niphatidae. Given the widely divergent morphologies of these haplosclerids, it seems improbable to assess a morphologic diagnosis for Clade C. Rampant parallelism in major morphologic features may indeed be the rule for Haplosclerida, and one needs to search for less conspicuous details of the anatomy aiming at understanding the most obscure phylogenetic relationships. This would allow translation of phylogeny into the Linnean classification.

Other *Haliclona* spp. previously reported as sources of 3-APA alkaloids present in *Arenospicula* gen. nov. species, also belong in *Haliclona (Rhizoniera)*: *H. (R.) rosea* (Bowerbank, 1866)*, H. (R.) sarai* (Cimino et al., 1986) and *H. (R.) viscosa* (Braekman et al.,1992; Volk and Köck, 2004; Schmidt, 2010; Timm et al., 2010; Cychon et al., 2012; Köck et al., 2013; Schmidt et al., 2012). Only *H. (R.) sarai* has already been included in a phylogenetic study (18S rRNA) where it clustered with *Amphimedon compressa*, a Clade C species (Riesgo et al., 2013). Another *H. (Rhizoniera)* species already included in a phylogeny is *H. (R.) curacaoensis* (van Soest, 1980; Redmond et al., 2013), which clustered in Clade C as well. Therefore 3-APA alkaloids appear to be specific to species in Clade C (Tribalat et al., 2016). This, in association with careful morphological assessment and screening for further genetic markers, might enable naming well corroborated monophyletic species groups (smaller clades), reducing the polyphyly of sweeping proportions currently observed within Haplosclerida (Bispo, 2019), as undertaken here for *Arenospicula* gen. nov. For the genus *Arenospicula,* we do propose a reductase enzyme is present that allows a C-4/C-5′ cyclisation into simple C-4/C-5’ 3-alkylpiperidines such as arenosclerins while more advanced enzymes leading to more complex 3-APA (such as saraines) are absent.

Although fresh samples of *Arenosclera heroni* were not available for the metabolomics analysis, we included other species from Clade A, such as *Chalinula molitba* and *Callyspongia* spp., the latter genus being polyphyletic. All species of this latter genus included in the phylogenetic analyses nested in Clade A (Redmond et al., 2011). *Haliclona caerulea* belongs in Clade B according to its phylogenetic analysis, but in HCA this species appears clustering in Group 1, together with species from Clade A. This may be a consequence of no additional species from Clade B being present in the metabolomics analysis, or because Clade C presents more specific and phylogenetically informative metabolites. Metabolomics Groups 1 and 2 are mostly composed of Clade A species. These two groups are more closely related to each other than to Group 3, essentially composed of *Arenospicula* gen. nov. spp. Metabolomics data reinforced the phylogeny and morphology data, corroborating the proposition that *Arenospicula* gen. nov. is distantly related to *Arenosclera*. We include three *Arenosclera* species in the Molecular Network analysis: one specimen of *A. amazonensis,* two specimens of *A. brasiliensis* and two specimens of *A. mammillaris.* Based on these results we were able to propose chemical taxonomic markers for *A. brasiliensis,* and *A. mammillaris.* Arenosclerins are chemical markers common to these two species (and certainly *A. klausi*). In *A. amazonensis,* arenosclerins are absent but haliclonacylamines seem to be in common to these three species. The species *H. (R.) fugidia* was only analyzed in MS but the annotation was possible using literature data and the presence of cyclostelletamines was confirmed which placed this species a bit more distant to the three species currently included in the genus *Arenospicula* as the gene coding for the C-4/C-5 of the bis-3APA is clearly absent. Adding to this the *Haliclona*-like skeleton of *H. (R.) fugidia* we decide to keep this species out of *Arenospicula* for now but the similarity of these species should be investigated in more detail as well as the relationship among the other species in the Clade C.

4.3. Targeted metabolomics as a valuable tool in the integrative taxonomy of marine sponges

The sponge order Haplosclerida is no exception within Metazoa in general, when it comes to a sizeable mismatch between the growing phylogeny of the group and its accepted classification. For this reason, taxonomists must resort to new tools, through which they can gradually recover the phylogeny in parallel with the construction of a more solid classification. Use of integrative taxonomy refines our systematic knowledge and may result in the description of new, well diagnosed taxa (Erpenbeck and van Soest, 2005; Ivanišević et al., 2011b; Boury-Esnault et al., 2013; Morrow and Cárdenas, 2015; Leal et al., 2017). In cases where morphology provides limited evidence to diagnose taxa recognized in well supported molecular phylogenies, metabolomics is a welcome source of additional descriptors, as proposed above for the diagnosis of *Arenospicula* gen. nov. Chemical markers have been proposed in the past to reinforce the diagnosis of some taxa within Porifera, notably Verongiida Bergquist, 1978 (tyrosine derived brominated compounds – Bergquist and Cook, 1978) and Latrunculiidae Topsent, 1922 (pyrroloiminoquinone-type alkaloids – Li et al., 2021). Our metabolomics analyses of some species of the order Haplosclerida enabled us to refine the taxonomy of the genus *Arenosclera* (Callyspongiidae), transferring some of its species to *Arenospicula* gen. nov. (Haplosclerida *incertae sedis*). Our experimental approach followed a recent overview on the metabolomics of marine organisms (Bayona et al., 2022). By applying Molecular Networking-based metabolomics having 3-APA as standards, several members of this family were identified in the analyzed samples. Some previous integrative taxonomy approaches applied to sponges and using metabolomics failed to unambiguously annotate the discriminant metabolites (Ivanišević et al., 2011b; Ruiz et al., 2015; Reverter et al., 2018). However, a successful metabolomics approach was applied to other marine invertebrates such as the zoantharian *Parazoanthus axinellae* (Schmidt, 1862) where standards were available but molecular networking was not used (Cachet et al., 2015). We demonstrate here that Molecular Networking can be of great value to guide the annotation of the chemical markers and therefore in tackling taxonomic challenges based on phylogeny misinterpretations. As far as we are aware, the metabolomics-based integrative approach reported here is the first to propose a new classification for a new genus of marine sponge.

5. Conclusion

An integrative approach highlighted chemical similarities among species of *Arenospicula* gen. nov. and pointed out that species of *Arenosclera* do not occur in Brazil, nor in the Atlantic based on our current knowledge. The PhyloCode *Arenospicula*^{P} can now naturally be included in the Linnean Hierarchy system as *Arenospicula* gen. nov. Brazilian *Arenosclera* spp., and *Pachychalina alcaloidifera/Haliclona mammillaris* (synonymized herein) are all here transferred to *Arenospicula* gen. nov. Metabolomic analyses were key to define the taxonomic markers. Integration of morphology, molecular and metabolomic data is a robust way to resolve the challenge of widespread polyphyly in every non-monotypic higher taxon within the Haplosclerida. Integration of these data could also lead to a more accurate and natural view of genera and family boundaries of currently para- and polyphyletic taxa in marine invertebrates. With several new taxa described every day, one realizes that we are still in the 'Age of Discovery', but at the same time, in the sixth mass extinction. Inclusion of new approaches to taxonomy, making it more integrative, can stimulate the next generation of taxonomy experts to ally investigations with specialists in chemistry, ecology, microbiology and other fields, further enhancing the importance of species description and the understanding of biological evolution.

CRediT authorship contribution statement

Camille V. Leal: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **André Bispo:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Mirelle Takaki:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Vitor F. Freire:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Maggie M. Reddy:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Fabiano L. Thompson:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition. **Eduardo Hajdu:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Olivier P. Thomas:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Roberto G.S. Berlinck:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics

Not applicable.

Data accessibility

All specimens used here have a voucher deposited at MNRJ collection. Supporting material for this article including sponge *in vivo* and skeleton morphology images, metadata, main compounds tables and the complete systematic are included in the electronic supplementary material. All sequences generated in this article have been uploaded to the GenBank database. The Genbank identification numbers for the datasets used in this article are: MZ366933 – MZ366958; PQ144853-PQ144859. The new genus and the new combination of the species were recorded at the Zoobank with the follow code:

urn:lsid:zoobank.org:pub:2D559EFB-B451-4375-B0CB-4B007971C93A. The Molecular Network Data are available in GNPS and can be accessed by the follow link: https://gnps.ucsd.edu/ProteoSAFe

/status.jsp?task=1cedb566ce6b4670b41ef055a9a01150. The results and parameters for *Insilico Peptidic Natural Product Dereplicator* and the *Dereplicator+: Identification of Metabolites Through Database Search of Mass Spectra* GNPS analyses can be accessed by the following links: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=87a29cb30ade43eda54818b48a70a323 and https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=75902a51c97b4b80b4d981d253722105, respectively.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2025.104971.

Data availability

Data in GNPS can be accessed via the link (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=1cedb566ce6b4670b41ef055a9a01150).Sequences are deposited in NCBI GenBank under the accession numbers MZ366933–MZ366958; PQ144853-PQ144859. Other data will be made available on request.

References

Afgan, E., Baker, D., Van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Eberhard, C., Grüning, B., Guerler, A., Hillman-Jackson, J., Von Kuster, G., Rasche, E., Soranzo, N., Turaga, N., Taylor, J., Nekrutenko, A., Goecks, J., 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44, W3–W10.

Afoullouss, S., Balsam, A., Allcock, A.L., Thomas, O.P., 2022. Optimization of LC-MS2 data acquisition parameters for molecular networking applied to marine natural products. Metabolomics 12, 1–17.

Alcolado, P.M., 1984. Nuevas especies de esponjas encontradas en Cuba. POEYANA 271, 1–22.

Almeida, A.M.P., Berlinck, R.G.S., Hajdu, E., 1997. Alcalóides alquilpiridínicos de esponjas marinhas. Quím. Nova 20, 170–185.

Alvarez, B., van Soest, R.W.M., Rützler, K., 1998. A revision of axinellidae (Porifera: Demospongiae) in the central west atlantic region. Smithsonian Contrib. Zool. 598, 1–47.

Amat, M., Pérez, M., Ballette, R., Proto, S., Bosch, J., 2015. The alkaloids of the madangamine group. Alkaloids - Chem. Biol. 74, 159–199.

Andersen, R.J., Van Soest, R.W.M., Kong, F., 1996. Alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida. In: Pelletier, S.W. (Ed.), Alkaloids: Chemical and Biological Perspectives. Springer, New York, USA, pp. 301–355.

Arai, M., Sobou, M., Vilchéze, C., Baughn, A., Hashizume, H., Pruksakorn, P., Ishida, S., Matsumoto, M., Jacobs Jr., W.R., Kobayashi, M., Halicyclamine, A., 2008. A marine spongean alkaloid as a lead for anti-tuberculosis agent. Bioorg. Med. Chem. 16, 6732–6736.

Baldwin, J.E., Whitehead, R.C., 1992. On the biosynthesis of manzamines. Tetrahedron Lett. 33, 2059–2062.

Bayona, L.M., de Voogd, N.J., Choi, Y.H., 2022. 2022 Metabolomics on the study of marine organisms. Metabolomics 18, 1–24.

Bergquist, P.R., Cook, S.D.C., 1978. Order verongida bergquist. In: Hooper, J.N.A., Van Soest, R.W.M. (Eds.), Systema Porifera: a Guide to the Classification of Sponges. Kluwer Academic/Plenum, NY, p. 1081.

Bergquist, P.R., 1965. The sponges of Micronesia, Part I. The Palau Archipelago. Pac. Sci. 19 (2), 123–204.

Bergquist, P.R., 1978. *Sponges.* Hutchinson. University of California Press, London, pp. 1–268.

Bergquist, P.R., Warne, K.P., 1980. The Marine Fauna of New Zealand: Porifera, Demospongiae, Part 3 Haplosclerida and Nepheliospongida. N. Z. Oceanogr. Inst. Mem. 87, 1–77.

Berlinck, R.G.S., Ogawa, C.A., Almeida, A.M.P., Sanchez, M.A.A., Malpezzi, E.L.A., Costa, L.V., Hajdu, E., De Freitas, J.C., 1996. Chemical and pharmacological characterization of halitoxin from Amphimedon viridis (Porifera) from the southeastern Brazilian coast. Comp. Biochem. Physiol., C 115, 155–163.

Bispo, A., 2019. Integrative systematics of Haplosclerida (Callyspongiidae, Chalinidae e Niphatidae) from Brazil. Universidade Federal do Rio de Janeiro. PhD thesis.

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. Mol. Phylogenet. Evol. 32, 823–837.

Boury-Esnault, N., Lavrov, D.V., Ruiz, C.A., Pérez, T., 2013. The integrative taxonomic approach applied to Porifera: a case study of the homoscleromorpha. Integr. Comp. Biol. 53, 416–427.

Bowerbank, J.S., 1866. A Monograph of the British Spongiadae, ii. Ray Society, London.

Braekman, J.C., Daloze, D., Stoller, C., Van Soest, R.W.M., 1992. Chemotaxonomy of *agelas* (Porifera: Demospongiae). Biochem. Systemat. Ecol. 20, 417–431.

Cachet, N., Genta-Jouve, G., Ivanišević, J., Chevaldonné, P., Sinniger, F., Culioli, G., Pérez, T., Thomas, O.P., 2015. Metabolomic profiling reveals deep chemical divergence between two morphotypes of the zoanthid *Parazoanthus axinellae*. Sci. Rep. 5, 1–9.

Cantino, P.D., de Queiroz, K., 2010. International code of phylo-genetic nomenclature [Internet]. Available from: http://www.ohio.edu/phylocode/.

Cárdenas, P., Pérez, T., Boury-Esnault, N., 2012. Systematics facing new challenges. Adv. Mar. Biol. 61, 79–209.

Carter, H.J., 1882. Some sponges from the west indies and acapulco in the liverpool free museum described, with general and classificatory remarks. Ann. Mag. Nat. Hist. 9, 266–301.

Carter, H.J., 1885. Descriptions of sponges from the neighbourhood of port phillip heads, South Australia. Ann. Mag. Nat. Hist. (5), 301–321.

Ceballos, G., García, A., Ehrlich, P., 2010. The sixth extinction crisis loss of animal populations and species. Journal of Cosmology 8, 1821–1831.

Chombard, C., Boury-Esnault, N., Tillier, S., 1998. Reassessment of homology of morphological characters in tetractinellid sponges based on molecular data. Syst. Biol. 47, 351–366.

Cimino, G., Stefano, S de, Scognamiglio, G., Sodano, G., Trivellone, E., 1986. Sarains: a new class of alkaloids from the marine sponge *Reniera sarai*. Bull. Soc. Chim. Belg. 95, 783–800.

Collin, R., 2005. Ecological monitoring and biodiversity surveys at the Smithsonian Tropical Research Institute's Boas del Toro Research Station. Caribb. J. Sci. 41, 367–373.

Criscione, F., Hallan, A., Puillandre, N., Fedosov, A., 2021. Snails in depth: integrative taxonomy of *Famelica, Glaciotomella* and *Rimosodaphnella* (Conoidea: raphitomidae) from the deep sea of temperate Australia. Invertebr. Systemat. 35, 940–962.

Cychon, C., Schmidt, G., Mordhorst, T., Köck, M., 2012. Structure elucidation of submilligram quantities of natural products - application to haliclamines G and H from the Arctic marine sponge *Haliclona viscosa*. Z. Naturforsch. B Chem. Sci. 67, 944–950.

de Laubenfels, M.W., 1936. A Discussion of the Sponge Fauna of the Dry Tortugas in Particular and the West Indies in General, with Material for a Revision of the Families and Orders of the Porifera, vol. 467. Carnegie Institute of, pp. 1–225.

de Laubenfels, M.W., 1949. Sponges of the western Bahamas. Am. Mus. Novit. 1431, 1–25.

de Laubenfels, M.W., 1932. The marine and fresh-water sponges of California. Proc. U. S. Natl. Mus. 81 (2927), 1–140.

de Oliveira, J.H.H.L., Grube, A., Köck, M., Berlinck, R.G., Macedo, M.L., Ferreira, A.G., Hajdu, E., Ingenamine, G., 2004. Cyclostellettamines G-I , K , and L from the new Brazilian species of marine sponge *Pachychalina* sp. J. Nat. Prod. 67, 1685–1689.

de Oliveira, J.H.H.L., Nascimento, A.M., Kossuga, M.H., Cavalcanti, B.C., Pessoa, C.O., Moraes, M.O., Macedo, M.L., Ferreira, A.G., Hajdu, E., Pinheiro, U.S., Berlinck, R.G., 2007. Cytotoxic alkylpiperidine alkaloids from the Brazilian marine sponge *Pachychalina alcaloidifera*. J. Nat. Prod. 70, 538–543.

de Oliveira, J.H.H.L., Seleghim, M.H.R., Timm, C., Grube, A., Köck, M., Nascimento, G.G. F., Martins, A.C.T., Silva, E.G.O., de Souza, A.O., Minarini, P.R.R., Galetti, F.C.S., Silva, C.L., Hajdu, E., Berlinck, R.G.S., 2006. Antimicrobial and antimycobacterial activity of cyclostellettamine alkaloids from sponge *Pachychalina* sp. Mar. Drugs 4, 1–8.

de Voogd, N.J., Alvarez, B., Boury-Esnault, N., Carballo, J.L., Cárdenas, P., Díaz, M.C., Dohrmann, M., Downey, R., Hajdu, E., Hooper, J.N.A., Kelly, M., Klautau, M., Manconi, R., Morrow, C.C., Pisera, A.B., Ríos, P., Rützler, K., Schönberg, C., Vacelet, J., Van Soest, R.W.M., 2024. World Porifera Database. Accessed at.

De Weerdt, W.H., 2002. Family Chalinidae Gray, 1867. Systema Porifera. Guide to the classification of sponges 852–873.

Desqueyroux-Faúndez, R., Valentine, C., 2002a. Family Petrosiidae van soest, 1980. In: Hooper, J.N.A., van Soest, R.W.M. (Eds.), Systema Porifera. Guide to the Classification of Sponges. Kluwer Academic/Plenum Publishers, New York, USA, pp. 906–917.

Desqueyroux-Faúndez, R., Valentine, C., 2002b. Family Phloeodictyidae carter, 1882. In: Hooper, J.N.A., van Soest, R.W.M. (Eds.), Systema Porifera. Guide to the Classification of Sponges. Kluwer Academic/Plenum Publishers, New York, USA, pp. 893–905.

Desqueyroux-Faúndez, R., Valentine, C., 2002c. Family Callyspongiidae de Laubenfels, 1936. In: Hooper, J.N.A., van Soest, R.W.M. (Eds.), Systema Porifera. Guide to the Classification of Sponges. Kluwer Academic/Plenum Publishers, New York, USA, pp. 835–851.

Desqueyroux-Faúndez, R., Valentine, C., 2002d. Family Niphatidae van soest, 1980. In: Hooper, J.N.A., van Soest, R.W.M. (Eds.), Systema Porifera. Guide to the Classification of Sponges. Kluwer Academic/Plenum Publishers, New York, USA, pp. 874–890.

Desqueyroux-Faundez, R., 1984. Description de la Faune des Haplosclerida (Porifera) de la Nouvelle-Calédonie I. Niphatidae-Callyspongiidae. Rev. Suisse Zool. 91, 765–827.

Desqueyroux-Faúndez, R., 1999. Convenient genera or phylogenetic genera? Evidence from Callyspongiidae and Niphatidae (Haplosclerida). Memoir. Queensl. Mus. 44, 131–146.

Duchassaing, F.P., Michelotti, G., 1864. Spongiaires de la mer Caraïbe. Harlem,Les htiers Loosjes. De Erver Loosjes, Haarlem.

Dührkop, K., Fleischauer, M., Ludwig, M., Aksenov, A.A., Melnik, A.V., Meusel, M., Dorrestein, P.C., Rousu, J., Böcker, S., 2019. SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nat. Methods 16 (4), 299–302. https://doi.org/10.1038/s41592-019-0344-8.

Erpenbeck, D., Ekins, M., Enghuber, N., Hooper, J.N.A., Lehnert, H., Poliseno, A., Schuster, A., Setiawan, E., De Voogd, N.J., Wörheide, G., Van Soest, R.W.M., 2016a. Nothing in (sponge) biology makes sense – except when based on holotypes. J. Mar. Biol. Assoc. U. K. 96, 305–311.

Erpenbeck, D., Voigt, O., Al-Aidaroos, O.M., Berumen, M.L., Büttner, G., Catania, D., Guirguis, A.N., Paulay, G., Schätzle, S., Wörheid, G., 2016b. Molecular biodiversity of red sea demosponges. Mar. Pollut. Bull. 105, 507–514.

Erpenbeck, D., van Soest, R.W.M., 2005. A survey for biochemical synapomorphies to reveal phylogenetic relationships of halichondrid demosponges (Metazoa: Porifera). Biochem. Systemat. Ecol. 33, 585–616.

Erpenbeck, D., Knowlton, A.L., Talbot, S.L., Highsmith, R.C., van Soest, R.W.M., 2004. A molecular comparison of alaskan and north east atlantic halichondria panicea (pallas, 1766) (Porifera: Demospongiae) populations. In: Pansini, M., Pronzato, R., Bavestrello, G., Manconi, R., Sarà, M. (Eds.), Sponge Science in the New Millennium, vol. 68. Bollettino dei Musei e degli Istituti Biologici dell'Universitá di Genova, pp. 319–325.

Fusetani, N., Asai, N., Matsunaga, S., Honda, K., Yasumuro, K., 1994. Cyclostellettamines A-F, pyridine alkaloids which inhibit binding of methyl quinuclidinyl benzilate (QNB) to muscarinic acetylcholine receptors, from the marine sponge, *Stelletta maxima*. Tetrahedron Lett. 35, 3967–3970.

Galitz, A., Nakao, Y., Schupp, P.J., Wörheide, G., Erpenbeck, D., 2021. A soft spot for chemistry-current taxonomic and evolutionary implications of sponge secondary metabolite distribution. Mar. Drugs 19, 1–25.

George, W.C., Wilson, H.V., 1919. Sponges of beaufort (N.C.) harbor and vicinity. Bulletin of the Bureau of Fisheries, Washington 36, 129–179.

Grant, R.E., 1836. Animal kingdom. In: Todd, R.B. (Ed.), The Cyclopaedia of Anatomy and Physiology, vol. 1. Sherwood, Gilbert, and Piper.

Grant, R.E. Porifera, 1841. 5-9, 310-313, pls II-IV. In: Bailliere, H. (Ed.), Outlines of Comparative Anatomy, vol. 1, pp. 1–656.

Gray, J.E., 1868. Note on *Theonella*, a new genus of coralloid sponges from Formosa. Proc. Zool. Soc. Lond. 37, 565–566.

Gray, J.E., 1867. Notes on the arrangement of sponges, with the descriptions of some new genera. Proc. Zool. Soc. Lond. (2), 492–558.

Griessinger, J.M., 1971. Etude des Réniérides de Méditerranée (Démosponges Haplosclérides). Bull. Mus. Natl. Hist. Nat. 3, 97–182.

Hajdu, E., Peixinho, S., Fernandez, J.C., Aguiar, M.C.P., 2011. Esponjas marinhas da Bahia: guia de campo e laboratório. Museu Nacional, Rio de Janeiro.

Hechtel, G.J.A., 1965. Systematic Study of the Demospongiae of Port Royal, vol. 20. Jamaica. Peabody Museum of Natural History Yale University Bulletin, pp. 1–94.

Hooper, J., Capon, R., Keenan, C., Parry, D., Smit, N., 1992. Chemotaxonomy of marine sponges: families Microcionidae, Raspailiidae and Axinellidae, and their relationships with other families in the orders Poecilosclerida and Axinellida (Porifera: Demospongiae). Invertebr. Systemat. 6, 261–301.

Ivanišević, J., Pérez, T., Ereskovsky, A.V., Barnathan, G., Thomas, O.P., 2011a. Lysophospholipids in the mediterranean sponge *Oscarella tuberculata*: seasonal variability and putative biological role. J. Chem. Ecol. 37, 537–545.

Ivanišević, J., Thomas, O.P., Lejeusne, C., Chevaldonné, P., Pérez, T., 2011b. Metabolic fingerprinting as an indicator of biodiversity: towards understanding inter-specific relationships among Homoscleromorpha sponges. Metabolomics 7, 289–304.

Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings Bioinf. 20, 1160–1166.

Keller, C., 1889. Die Spongienfauna des rothen Meeres (I. Hälfte). Zeitschrift für wissenschaftliche Zoologie 52, 294–368.

Kirkpatrick, R., 1907. Preliminary report on the monaxonellida of the national antarctic expedition. Ann. Mag. Nat. Hist. 7 (20), 271–291.

Kobayashi, J., Murayama, T., Ohizumi, Y., Sasaki, T., Ohta, T., Nozoe, S., 1989. Theonelladins A ∼ D, novel antineoplastic pyridine alkaloids from the Okinawan marine sponge *Theonella swinhoei*. Tetrahedron Lett. 30, 4833–4836.

Köck, M., Muñoz, J., Cychon, C., Timm, C., Schmidt, G., 2013. The Arctic sponge *Haliclona viscosa* as a source of a wide array of 3-alkyl pyridine alkaloids. Phytochemistry Rev. 12, 391–406.

Komarek, A., Beutel, R.G., 2006. Problems in taxonomy and suggestions for a standardized description of new insect taxa. Entomologicals problems 36, 55–70.

Kondo, K., Shigemori, H., Kikuchi, Y., Ishibashi, M., Sasaki, T., Kobayashi, J., 1992. Ircinals A and B from the okinawan marine sponge *Ircinia* sp.: plausible biogenetic precursors of manzamine alkaloids. J. Org. Chem. 57, 2480–2483.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Leal, C.V., Moraes, F.C., Fróes, A.M., Soares, A.C., de Oliveira, L.S., Moreira, A.P.B., Thompson, F.L., Hajdu, E., 2017. Integrative taxonomy of Amazon reefs' *Arenosclera* spp.: a new clade in the Haplosclerida (Demospongiae). Front. Mar. Sci. 4, 1–14.

Lerner, C.B., 1996. Esponjas da Ilha da Galé, Reserva Marinha Biológica do Arvoredo, Santa Catarina, Brasil (Porifera; Demospongiae). Biociencias 4 (2), 101–129.

Lévi, C., 1962. Spongiaires récoltés par l'expédition israélienne dans le sud de la Mer Rouge en 1962. Israel South Red Sea Expedition 39, 3–27. *Report 13*.

Lévi, C., 1973. Systématique de la classe des Demospongiaria (Démosponges). Pp. 577-631. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Anatomie, Systématique, Biologie. Spongiaires, pp. 1–716, 3(1). Masson et Cie: Paris.

Li, F., Kelly, M., Tasdemir, D. Chemistry, 2021. Chemotaxonomy and biological activity of the latrunculid sponges (order poecilosclerida, family Latrunculiidae). Mar. Drugs 19 (1), 27.

Marchán, D.F., Fernández, R., de Sosa, I., Sánchez, N., Cosín, D.J.D., Novo, M., 2018. Integrative systematic revision of a Mediterranean earthworm family: hormogastridae (Annelida, Oligochaeta). Invertebr. Systemat. 32, 652–671.

Matzanke, N., Gregg, R.J., Weinreb, S.M., Parvez, M., 1997. A concise approach to the tricyclic core of the cytotoxic marine alkaloid madangamine A. J. Org. Chem. 62, 1920–1921.

McClelland, D., Evans, R.M., Abidin, I., Sharma, S., Choudhry, F.Z., Jaspars, M., Sepčić, K., Scott, R.H., 2003. Irreversible and reversible pore formation by polymeric alkylpyridinium salts (poly-APS) from the sponge Reniera sarai. Br. J. Pharmacol. 139, 1399–1408.

Morrow, C., Cárdenas, P., 2015. Proposal for a revised classification of the Demospongiae (Porifera). Front. Zool. 12, 1–27.

Mothes, B., Lerner, C.B., 1994. Esponjas marinhas do infralitoral de Bombinhas (Santa Catarina, Brasil) com descrição de três espécies novas (Porifera: Calcarea e Demospongiae). Biociencias 2 (1), 47–62.

Mothes, B., Lerner, C.B., Silva, C.M.M., 2003. Guia Ilustrado - Esponjas Marinhas - Costa Sul-Brasileira, first ed. USEB, Pelotas, p. 83.

Mothes, B., Lerner, C.B., Silva, C.M.M., 2006. Illustrated guide of the marine sponges from the southern coast of Brazil/Guia ilustrado de esponjas marinhas da costa sul-brasileira, second ed., p. 119 (revised and extended).

Moura, R.L., Amado-Filho, G.M., Moraes, F.C., Brasileiro, P.S., Salomon, P.S., Mahiques, M.M., Bastos, A.C., Almeida, M.G., Silva, J.M., Araujo, B.F., Brito, F.P., Rangel, T.P., Oliveira, B.C.V., Bahia, R.G., Paranhos, R.P., Dias, R.J.S., Siegle, E., Figueiredo, A.G., Pereira, R.C., Leal, C.V., Hajdu, E., Asp, N.E., Gregoracci, G.B., Neumann-Leitão, S., Yager, P.L., Francini-filho, R.B., Fróes, A., Campeão, M., Silva, B.S., Moreira, A.P.B., Oliveira, L., Soares, A.C., Araujo, L., Oliveira, N.L., Teixeira, J.B., Valle, R.A.B., Thompson, C.C., Rezende, C.E., Thompson, F.L., 2016. An extensive reef system at the Amazon River mouth. Sci. Adv. 2, e1501252.

Muricy, G., Lopes, D.A., Hajdu, E., Carvalho, M.S., Moraes, F.C., Klautau, M., Menegola, C., Pinheiro, U., 2011. Catalogue of Brazilian Porifera. Museu Nacional, Série Livros 300.

Muricy, G., Esteves, E.L., Monteiro, L.C., Rodrigues, B.R., Albano, R.M., 2015. A new species of *Haliclona* (Demospongiae: Haplosclerida: Chalinidae) from southeastern Brazil and the first record of *Haliclona vansoesti* from the Brazilian coast. Zootaxa 3925, 536–550.

Muricy, G., Ribeiro, S.M., 1999. Shallow-water Haplosclerida (Porifera, Demospongiae) from Rio de Janeiro state, Brazil (southwestern atlantic). Beaufortia 49, 83–108.

Nakamura, H., Deng, S., Kobayashi, J.I., Ohizumi, Y., Tomotake, Y., Matsuzaki, T., Hirata, Y., 1987. Keramamine-A and -B, novel antimicrobial alkaloids from the Okinawan marine sponge *Pellina* sp. Tetrahedron Lett. 28, 621–624.

Norman, A.M., 1869. Last report on dredging among the shetland isles. Part 2. On the Crustacea, tunicata, polyzoa, echinodermata, actinozoa, Hydrozoa, and Porifera. Report of the thirty-eighth meeting of the 327–335.

Pappalardo, P., Collins, A.G., Lohan, K.M.P., Hanson, K.M., Truskey, S.B., Jaeckle, W., Ames, C.L., Goodheart, J.A., Bush, S.L., Biancani, L.M., Strong, E.E., Vecchione, M., Harasewych, M.G., Reed, K., Lin, C., Hartil, E.C., Whelpley, J., Blumberg, J., Matterson, K., Redmond, N.E., Becker, A., Boyle, M.J., Osborn, K.J., 2021. The role of taxonomic expertise in interpretation of metabarcoding studies. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 78, 3397–3410.

Pinheiro, U.S., Berlinck, R.G.S., Hajdu, E., 2005. Shallow-water Niphatidae (Haplosclerina, Haplosclerida, Demospongiae) from the São Sebastião Channel and its environs (tropical southwestern Atlantic), with the description of a new species. Contrib. Zool. 74, 271–278.

Pons, L.N., Calcinai, B., Gates, R.D., 2017. Who's there? – First morphological and DNA barcoding catalogue of the shallow Hawai'ian sponge fauna. PLoS One 12, 1–59.

Pulitzer-Finali, G., 1986. A collection of West Indian Demospongiae (Porifera). appendix, a list of the Demospongiae hitherto recorded from the West Indies 86, 65–216.

Pulitzer-Finali, G., 1982. Some new or little-known sponges from the great barrier reef of Australia. Boll. dei Musei degli Ist. Biol. dell Univ. Genova 48, 87–142.

Pulitzer-Finali, G., 1993. A collection of marine sponges from East Africa. Annales Museo Civico Storia Naturale "Giacomo Doria 89, 247–350.

Rao, K.V., Kasanah, N., Wahyuono, S., Tekwani, B.L., Schinazi, R.F., Hamann, M.T., 2004. Three new manzamine alkaloids from a common Indonesian sponge and their activity against infectious and tropical parasitic diseases. J. Nat. Prod. 67, 1314–1318.

Reddy, M.M., Jennings, L., Thomas, O.P., 2021. Marine Biodiscovery in a Changing World *Progress In the Chemistry of Organic Natural Products*, vol. 116, pp. 1–36.

Redmond, N.E., Morrow, C.C., Thacker, R.W., Diaz, M.C., Boury-Esnault, N., Cárdenas, P., Hajdu, E., Lôbo-Hajdu, G., Picton, B.E., Pomponi, S.A., Kayal, E., Collins, A.G., 2013. Phylogeny and systematics of demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. Integr. Comp. Biol. 53, 388–415.

Redmond, N.E., Raleigh, J., Van Soest, R.W.M., Kelly, M., Travers, S.A.A., Bradshaw, B., 2011. Phylogenetic relationships of the marine Haplosclerida (Phylum Porifera) employing ribosomal (28S rRNA) and mitochondrial (cox1, nad1) gene sequence data. PLoS One 6, 1–10.

Reverter, M., Tribalat, M.A., Pérez, T., Thomas, O.P., 2018. Metabolome variability for two Mediterranean sponge species of the genus *Haliclona*: specificity, time, and space. Metabolomics 14, 1–12.

Ridley, S.O., Dendy, A., 1886. Preliminary report on the Monaxonida collected by H.M.S. Challenger. Part I. Ann. Mag. Nat. Hist. 18, 325–493.

Riesgo, A., Novo, M., Sharma, P.P., Peterson, M., Maldonado, M., Giribet, G., 2013. Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). Zool. Scripta 43, 101–117.

Ruiz, C., Ivanišević, J., Chevaldonné, P., Ereskovsky, A.V., Boury-Esnault, N., Vacelet, J., Thomas, O.P., Pérez, T., 2015. Integrative taxonomic description of *Plakina kanaky*, a new polychromatic sponge species from New Caledonia (Porifera: homoscleromorpha). Mar. Ecol. 36, 1129–1143.

Rützler, K., Piantoni, C., Van Soest, R.W., Díaz, M.C., 2014. Diversity of sponges (Porifera) from cryptic habitats on the Belize barrier reef near Carrie Bow Cay. Zootaxa 3805, 1–129.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press, New York, USA.

Santos, G.G., da Silva, L.P., Alliz, A., Pinheiro, U.S., 2014. *Cladocroce caelum* sp. nov. from the Brazilian coast; first record of the genus in the South Atlantic. Zootaxa 3847, 297–300.

Schmidt, G., Timm, C., Grube, A., Volk, C.A., Köck, M., 2012. Viscosalines B 1,2 and E 1,2: challenging new 3-alkyl pyridinium alkaloids from the marine sponge Haliclona viscosa. Chem. Eur J. 18, 8180–8189.

Schmidt, G., 2010. Secondary metabolites in the arctic sponge Haliclona viscosa - spatial and temporal variation. PhD thesis.

Schmidt, O., 1870. Grundzüge einer Spongien-Fauna des atlantischen Gebietes. Wilhelm Engelmann: Leipzig. 1–88.

Schmidt, O., 1868. Die Spongien der Küste von Algier. Mit Nachträgen zu den Spongien des Adriatischen Meeres (Drittes Supplement). Wilhelm Engelmann.

Schmidt, O., 1862. Die Spongien des adriatischen Meeres. Wilhelm Engelmann.

Schmitz, F.J., Hollenbeak, K.H., Campbell, D.C., 1978. Marine natural products: balitoxin, toxic complex of several marine sponges of the genus *Haliclona. The Journal of Organic Chemistry*, 43, 3916–3922.

Schönberg, C.H.L., 2021. No taxonomy needed: sponge functional morphologies inform about environmental conditions. Ecol. Indicat. 129, 107806.

Scott, R.H., Whyment, A.D., Foster, A., Gordon, K.H., Milne, B.F., Jaspars, M., 2000. Analysis of the structure and electrophysiological actions of halitoxins: 1,3 alkyl-pyrdinium salts from *Callyspongia ridleyi*. J. Membr. Biol. 176, 119–131.

Segraves, N.L., Crews, P.A., 2005. Madagascar sponge *Batzella* sp . as a source of alkylated iminosugars. J. Nat. Prod. 68, 118–121.

Sepčić, K., Batista, U., Vacelet, J., Maček, P., Turk, T., 1997. Biological activities of aqueous extracts from marine sponges and cytotoxic effects of 3-alkylpyridinium polymers from *Reniera sarai*. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 117 (1), 47–53.

Sollas, W.J., 1885. A classification of the sponges. Ann. Mag. Nat. Hist. 16, 1–395.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

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. Integr. Comp. Biol. 53, 373–387.

Thiele, J., 1898. Studien über pazifische Spongien. I. Japanische Demospongien. Zoologica : Original-Abhandlungen aus dem Gesamtgebiete der Zoologie 24, 1–72.

Timm, C., Mordhorst, T., Köck, M., 2010. Synthesis of 3-alkyl pyridinium alkaloids from the arctic sponge *Haliclona viscosa*. Mar. Drugs 8, 483–497.

Topsent, É., 1888. Contribution à l'étude des Clionides. Arch. Zool. Exp. Gen. 2, 1–165.

Topsent, É., 1928. Spongiaires de l'Atlantique et de la Méditerranée provenant des croisières du Prince Albert ler de Monaco. Résultats des campagnes scientifiques accomplies sur son yacht par Albert Ier, prince souverain de Monaco 74, 1–376.

Topsent, É., 1897. Pictet dans l'Archipel malais). Rev. Suisse Zool. 4, 421–487.

Topsent, E., 1922. Les mégasclères polytylotes des Monaxonides et la parenté des Latrunculiines. Bulletin de l'Institut océanographique Monaco 415, 1–8.

Torres, Y.R., Berlinck, R.G., Magalhães, A., Schefer, A.B., Ferreira, A.G., Hajdu, E., Muricy, G., 2000. Arenosclerins A-C and haliclonacyclamine E, new tetracyclic alkaloids from a Brazilian endemic Haplosclerid sponge *Arenosclera brasiliensis*. J. Nat. Prod. 63, 1098–1105.

Tribalat, M.A., Marra, M.V., McCormack, G.P., Thomas, O.P., 2016. Does the chemical diversity of the order Haplosclerida (Phylum Porifera: class demospongia) fit with current taxonomic classification? Planta Med. 82, 843–856.

Tsuda, M., Kawasaki, N., Kobayashi, J., 1994. Ircinols A and B, first antipodes of manzamine-related alkaloids from an Okinawan marine sponge. Tetrahedron 50, 7957–7960.

van Soest, R.W.M., Braekman, J.C., 1999. Chemosystematics of porifera: a review. Memoir. Queensl. Mus. 44, 569–589.

van Soest, R.W.M., Hooper, J.N.A., 2002. Order Haplosclerida topsent, 1928. In: Hooper, J.N.A., Van Soest, R.W.M. (Eds.), Systema Porifera. Guide to the Classification of Sponges, vol. 2002. Kluwer Academic/Plenum Publishers, New York, USA, pp. 831–832.

van Soest, R.W.M., 1980. Marine sponges from Curaçao and other Caribbean localities. Part II. Haplosclerida. In: Hummelinck, P.W., Van der Steen, L.J. (Eds.), *Uitgaven van de Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen. No. 104.* Studies on the Fauna of Curaçao and other Caribbean Islands, vol. 62, pp. 1–173.

van Soest, R.W.M., 2017. Sponges of the Guyana shelf. Zootaxa 4217, 1–225.

van der Sprong, J., de Voogd, McCormack, G.P., Sandoval, K., Schätzle, S., Voigt, O., Erpenbeck, D., Wörheide, G., Vargas, S., 2024. A novel target-enriched multilocus assay for sponges (Porifera): Red Sea Haplosclerida (Demospongiae) as a test case. Molecul. Ecol. Resour. 24 (2), e13891. https://doi.org/10.1111/1755-0998.13891.

van Soest, R.W.M., 1978. Marine sponges from Curaçao and other Caribbean localities. Part I. Keratosa. In: Hummelinck, P.W., Van der Steen, L.J. (Eds.), *Uitgaven van de Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen.* No. 94. Studies on the Fauna of Curaçao and other Caribbean Islands, vol. 56, pp. 1–94.

van Soest, R.W.M., 1984. Marine sponges from Curaçao and other Caribbean localities. Part III. Poecilosclerida. In: Hummelinck, P.W., Van der Steen, L.J. (Eds.), Uitgaven van de Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen. No. 112. Studies on the Fauna of Curaçao and other Caribbean Islands, vol. 66, pp. 1–167.

van Soest, R.W.M., 1988. *Tetrapocillon atlanticus* n. sp. (Porifera, Poecilosclerida) from the Cape Verde islands. Beaufortia 38 (2), 37–46.

Vargas, S., Schuster, A., Sacher, K., Büttner, G., Schätzle, S., Läuchli, B., Hall, K., Hooper, J.N.A., Erpenbeck, D., Wörheide, G., 2012. Barcoding sponges: an overview based on comprehensive sampling. PLoS One 7, 1–7.

Verrill, A.E., 1867. Notes on Radiata in the museum of Yale College with descriptions of new genera and species. No. 2. Notes on the echinoderms of Panama and the west coast of America, with descriptions of a new genus. Trans. Conn. Acad. Arts Sci. 1 (2), 251–322.

Volk, C.A., Köck, M., 2004. Viscosaline: new 3-alkyI pyridinium alkaloid from the Arctic sponge *Haliclona viscosa*. Org. Biomol. Chem. 2, 1827–1830.

Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y., Nguyen, D.D., Watrous, J., Kapono, C.A., Luzzatto-Knaan, T., Porto, C., Bouslimani, A., Melnik, A. V., Meehan, M.J., Liu, W.T., Crüsemann, M., Boudreau, P.D., Esquenazi, E., Sandoval-Calderón, M., Kersten, R.D., Pace, L.A., Quinn, R.A., Duncan, K.R., Hsu, C. C., Floros, D.J., Gavilan, R.G., Kleigrewe, K., Northen, T., Dutton, R.J., Parrot, D., Carlson, E.E., Aigle, B., Michelsen, C.F., Jelsbak, L., Sohlenkamp, C., Pevzner, P., Edlund, A., McLean, J., Piel, J., Murphy, B.T., Gerwick, L., Liaw, C.C., Yang, Y.L., Humpf, H.U., Maansson, M., Keyzers, R.A., Sims, A.C., Johnson, A.R., Sidebottom, A. M., Sedio, B.E., Klitgaard, A., Larson, C.B., Boya, P.C.A., Torres-Mendoza, D., Gonzalez, D.J., Silva, D.B., Marques, L.M., Demarque, D.P., Pociute, E., O'Neill, E.C., Briand, E., Helfrich, E.J.N., Granatosky, E.A., Glukhov, E., Ryffel, F., Houson, H., Mohimani, H., Kharbush, J.J., Zeng, Y., Vorholt, J.A., Kurita, K.L., Charusanti, P., McPhail, K.L., Nielsen, K.F., Vuong, L., Elfeki, M., Traxler, M.F., Engene, N., Koyama, N., Vining, O.B., Baric, R., Silva, R.R., Mascuch, S.J., Tomasi, S., Jenkins, S., Macherla, V., Hoffman, T., Agarwal, V., Williams, P.G., Dai, J., Neupane, R., Gurr, J., Rodríguez, A.M.C., Lamsa, A., Zhang, C., Dorrestein, K., Duggan, B.M., Almaliti, J., Allard, P.M., Phapale, P., Nothias, L.F., Alexandrov, T., Litaudon, M., Wolfender, J. L., Kyle, J.E., Metz, T.O., Peryea, T., Nguyen, D.T., VanLeer, D., Shinn, P., Jadhav, A., Müller, R., Waters, K.M., Shi, W., Liu, X., Zhang, L., Knight, R., Jensen, P.R., Palsson, B.Ø., Pogliano, K., Linington, R.G., Gutiérrez, M., Lopes, N.P., Gerwick, W. H., Moore, B.S., Dorrestein, P.C., Bandeira, N., 2016. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat. Biotechnol. 34, 828–837.

Wiedenmayer, F., 1977. Shallow-water sponges of the western Bahamas. Experientia Suppl. 28, 1–287.

Xia, J., Wishart, D.S., 2016. Using metaboanalyst 3.0 for comprehensive metabolomics data analysis. Current Protocols in Bioinformatics 55, 14.10.1–14.10.91.

Zea, S., 1987. Esponjas del Caribe Colombiano. Bogotá: Editorial Catálogo Científico.