A proposal for the family level classification of Calcinea (Porifera, Calcarea)

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Integrative approaches have more recently advanced the understanding of the taxonomy and phylogenetic relationships of calcareous sponges of the subclass Calcinea. With the help of DNA phylogenetic analyses and morphological re-interpretation of characters several genera were revised. It became obvious that several classically recognized genera such as example *Clathrina* and *Guancha* comprised species that sometimes were not particularly closely related to each other. As a result, several new genera were proposed, for example *Arthuria* Klautau, Azevedo, Cóndor-Luján, Rapp, Collins & Russo, 2013, *Borojevia* Klautau, Azevedo, Cóndor-Luján, Rapp, Collins & Russo, 2013, *Brattegardia* Klautau, Azevedo, Cóndor-Luján, Rapp, Collins & Russo, 2013 and *Nicola* Cóndor-Luján & Klautau, 2016. These revisions also led to the recognition of new synapomorphic morphological characters for the genera. Consequently, however, uncertainties about the family-level taxonomy of Calcinea exist. We analysed two nuclear DNA markers, the internal transcribed spacer region and the partial 28 ribosomal RNA gene from 18 genera of Calcinea. Based on the results of our phylogenetic analyses, we propose a revised family-level taxonomy for the subclass.

Cinachyrella australiensis (Carter, 1886) In The Indo- West Pacific: An Integrative Approach To Understanding A Complex Species Complex

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Species of *Cinachyrella* Wilson, 1925 (Demospongiae, Tetractinellida, Tetillidae), with their distinctive ball shapes and complements of delicate triaenes protruding beyond their surfaces, are charismatic and conspicuous components of benthic marine sponge fauna. Within the large collection of sponges at Queensland Museum, several hundred specimens are identified provisionally as *Cinachyrella australiensis* (Carter, 1886), largely due to their yellow colouration and spherical habitus. Much of this material was collected by benthic trawls of locations spanning the entire length of the inter-reef seabed of the Great Barrier Reef and Torres Strait. Additional material from subtropical and temperate eastern Australia, Papua New Guinea and other western Pacific and eastern Indian Ocean localities supplements the GBR collection. The physical forces associated with the collection method, and subsequent handling of large volumes of material from the trawls, caused many sponges to be broken and fragmented, making identification using light microscopy alone extremely demanding.

Given the inadequacy of light microscopy for resolving the identities of the specimens within this large and problematic collection, it seemed the perfect candidate for an integrative taxonomy study, using DNA barcoding and detailed electron microscopy. Examinations using SEM demonstrated variation in the morphology of the specimens, suggesting that we did not have a homogenous set of samples, despite their macroscopic similarity. Although biological variation was evident, the partial COI mtDNA barcode sequences we obtained were insufficient to resolve satisfactorily any species limits within the sample. Consequently, we adopted a combined approach to the molecular examination, employing four additional markers: partial COII mtDNA, two mitochondrial intergenic spacers, and partial 28S rDNA.

Our results to date support previous studies (1), which suggest that, for tetillid sponges at least, COII and the intergenic spacers have faster rates of evolution than the COI barcoding region, and are useful for taxonomic studies. Given the very large collection housed at Queensland Museum, we are amassing a substantial data-

base of sequences for these regions. Preliminary phylogenetic analysis indicates broad agreement among the mitochondrial and ribosomal gene trees, and further, that these trees are consistent with observed morphological variation. Given that these four additional markers are quicker and easier to amplify compared to COI (50–70% success rate, compared to 25% for COI), and have a higher information content because they are more rapidly evolving, we advocate strongly for a multilocus approach to the "barcoding" of tetillid sponges. Although the study is still in infancy, already our data show that at least 10 MOTUs hide within the *C. australiensis* species complex.

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Taxonomy of deep-sea sponges living on polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCFZ), East Pacific

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Up-to-date knowledge on the deep-sea sponge fauna (Porifera) of the Clarion- Clipperton Fracture Zone (CCFZ) in the eastern Pacific is relatively scarce and needs to be extended, because the CCFZ is one of the worldwide biggest potential deep-sea mining areas for industrial mining of polymetallic nodules. The framework of this project is the Joint Programming Initiative Oceans (JPIO), which is focused on the ecological aspects of deep-sea mining. The project-related research expedition SO239 EcoResponse by RV *Sonne* was focused on the study of benthic deep-sea communities living in polymetallic nodule field systems. During this expedition, 68 deep-sea sponges of 18 morphotypes and 35 species were collected in depths from 1700 to 5000 m by deployment of a Remotely Operated Vehicle (Figure 1). Main objective of this project is the establishment of a picture-based species catalogue of the deep-sea sponge fauna in the CCFZ. Further main objectives are DNA-Barcoding with four genetic markers (16S, 18S, 28S and COI) and software-based annotation of video material from ROV transect dives. The presentation during this workshop includes preliminary results of the deep- sea sponge taxonomy with additional project-specific information, e.g. on species distribution within the CCFZ or potential occurrence of new deep-sea species.

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Calcarean sponge fauna of the eastern Mediterranean Sea

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Sponge research in the eastern Mediterranean Sea has mainly focused on demosponges. Calcareans have been scarcely studied in this area, especially when compared with the north-western Mediterranean basin and the Adriatic Sea. Recent sponge sampling in various habitat types of the Aegean Sea (e.g. shallow rocky beds, semi- and entirely submerged caves, artificial substrates), in the framework of different research projects, yielded several specimens of calcareous sponges. The examination of this material revealed 11 taxa, of which 6 species are new to science: *Amphoriscus* sp. nov., *Sycantha* sp. nov., *Sycon* sp. nov. and *Vosmaeropsis* sp. nov. 1-3. Furthermore, a detailed overview of the relevant scientific literature was performed and an updated checklist of the calcarean fauna of the eastern Mediterranean Sea was compiled. According to our results, the

new records included, the up-to-date calcarean fauna of the eastern Mediterranean consists of 32 species (40% of the Mediterranean Calcarea) belonging to 16 genera, 13 families, and 4 orders. Calcaronea is the richest subclass, with 23 species, while Calcinea is represented with 9 species. The most diverse genus was Sycon (8). Most species were recorded in the Levantine Basin (17), North Aegean (12), and South Aegean Sea (6). Our review showed that most calcarean records from the eastern Mediterranean Sea were included in old publications and that the calcarean species found to date exclusively in the eastern Mediterranean make up 25% of its total calcarean fauna. This and the fact that recent research effort in the Aegean Sea yielded 6 new species indicate that further research could increase our knowledge on the calcarean diversity of the Mediterranean.

Three new and four poorly known species of *Plakina* (Porifera, Homoscleromorpha)

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Plakina is among the most representative genera of the class Homoscleromorpha, with 29 valid species occurring in subtidal hard substrates, mostly ceilings and walls of dark or semi-dark habitats. The genus is widely distributed around the world, although the regions of the Mediterranean Sea (8 spp.) and Caribbean Sea (5 spp.) record the highest species richness. Two Mediterranean species, *Plakina monolopha* and *P. trilopha,* are allegedly cosmopolitan but most records from outside the Mediterranean need revision, including the Brazilian record of *P. trilopha* (Muricy *et al*., 1998, Domingos *et al.*, 2016). In the present study, we describe three new species of *Plakina* and redescribe four others from different regions (Central Pacific, Aegean Sea, Antarctic, and Southwestern Atlantic). *Plakina* sp. nov. 1 from the Marquises Island, Central Pacific, is distinguished by its lophose calthrops exclusively trilophose and with all actines terminally spined. *Plakina* sp. nov. 2 from Greece is massive, orange to red-pink with whitish borders *in vivo*, and has trilophose and tetralophose calthrops with very irregular shapes. *Plakina monolopha var. antarctica* Topsent, 1917 from Petermann Island, Antarctica, has monolophose calthrops with the basal actines bifurcated and the lophose actine ramified in a complicated 1m, 2d, ts pattern. Furthermore, its spicules are larger than those of Mediterranean *P. monolopha* (cf. Muricy *et al*., 1998). We thus propose to rename it as a new species, provisionally called *Plakina* sp. nov. 3. We also revised other four poorly known species of *Plakina* and added the following new characters to their descriptions: to *P. crypta*, the irregular shape and rare trilophose calthrops; to *P. weinbergi* details of spicule shape (regular and irregular, with blunt and microspined tips); to *P. bowerbanki* the presence of a marginal canal, circular oscules and basal cavities, and SEM analysis of spicules; and to *P. coerulea* the presence of a marginal canal, basal cavities and rare mono-, di- and trilophose calthrops. The geographic distribution of *P. crypta* from SW France is extended to the Aegean Sea, with its first record for Greece. The distribution of the Tropical Southwestern Atlantic species *P. coerulea* is extended from Maceió to Fernando de Noronha oceanic archipelago and to Cabo Frio, in SE Brazil. These preliminary results indicate that the genus *Plakina* is more diverse and that its species are more widely distributed than previously thought.

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Calcareous Sponges From Palau And The Great Barrier Reef, Australia

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Calcareous sponges are composed of calcium carbonate spicules that can be diactines, triactines, tetractines, and/or polyactines. Their aquiferous system is very diverse, being asconoid, syconoid, syllebid, leuconoid or solenoid. Currently there is a dearth of taxonomists and knowledge in calcarea taxonomy in Australia. As part of acquiring taxonomic knowledge and updating the calcareous sponge collections from Queensland Museum, a set of 21 vouchered samples from Palau and the Great Barrier Reef were examined at the first calcareous workshop conducted at the South Australian Research Development Institute (SARDI) Aquatic Sciences, Adelaide, South Australia. Four specimens were from Palau while the remaining specimens were from the Queensland coast with 12 specimens exclusively from the Great Barrier Reef. Eighteen species were identified as calcareous sponges, of which, 12 species were from the subclass Calcinea and five from Calcaronea. Order Clathrinida (Calcinea) was represented by the genera: *Arthuria*, *Ascandra, Ascoleucetta*, *Clathrina*, *Leucaltis*, *Leucetta* and *Levinella.* Whilst the subclass Calcaronea was represented by the genera *Grantia*, *Leucandra*, *Sycettusa* and *Sycon.* Of these, the widespread dominant genera were *Leucetta*, followed by *Ascoleucetta.* New species are expected from this collection for Australia and Palau.

Divergence between molecular and morphological data in Brazilian *Arenosclera* sponges (Haplosclerida, Demospongiae)

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Arenosclera has six accepted species in the Indo-Pacific (5): Australian (3), New Caledonia (1), Red Sea (1) and West Atlantic: Brazil (1). Recent expeditions to the Amazon reefs yielded three specimens assignable to *Arenosclera*, but seemingly quite distinct from the sole species this far known from Brazil, namely *A. brasiliensis*. The rich chemistry known from *A. brasiliensis* caught our attention about relationships among these species and inspired us to perform a phylogenetic analysis to verify if *Arenosclera* is monophyletic. We integrated morphological and metagenome derived molecular analyses (28S). Morphology was studied as usual, and metagenomes were extracted according to (1), and then sequenced with Illumina MySeq technology. A 28S Genbank database was compiled with sequences ≥80% similar to the complete 28S of *A. heroni* (type species of *Arenosclera*). Metagenomes were compared with this database using BLASTN and only 28S-like sequences were saved. Following, we used CAP3 and SPADES to assemble sequences in contigs, and selected the largest contig for the phylogenetic analysis. The latter also used additional haplosclerid 28S sequences collected from Genbank. Sequences were aligned with MAFFT 7 and the Maximum likelihood phylogeny obtained with RAxML. The Amazon species feature delicate oxeas and sand in the fibers (carbonatic in one, siliciclastic in the other), with soft consistency and beige color. Additional differences between both species are the structure of their callyspongiid skeleton and morphology of the oxeas. Aside their arborescent habit, these species are very similar to other *Arenosclera* spp. However, the phylogeny retrieved shows that not only Amazon reef species, as well as *A. brasiliensis* do not form a monophyletic group with *A. heroni*. Brazilian species appear in a distinct clade, suggesting that these species represent a new genus. The confused systematics of the Haplosclerida hinders the objective classification of this new clade for now. Additional studies using other Haplosclerida and molecular markers are necessary to better define this group.

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