

New family and genus for Dendrilla-like sponges with characters of Verongiida. Part I redescription of Dendrilla lacunosa Hentschel 1912, diagnosis of the new family Ernstillidae and Ernstilla n. g.

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New family and genus for *Dendrilla*-like sponges with characters of Verongiida. Part I Redescription of *Dendrilla lacunosa* Hentschel 1912, diagnosis of the new family Ernstillidae and *Ernstilla* n. g.

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Abbreviated title: New family and genus of Verongiida

Abstract Morphological and molecular characters have identified a new family and genus of Verongiida in the Porifera: Demospongiae. The taxa are based on a new study of *Dendrilla lacunosa* Hentschel, 1912, a species that, using integrative taxonomy, has been found to belong to Verongimorpha instead of Keratosa. The species suite of characters require the erection of a new genus, *Ernstilla* n. gen. with affinities to Ianthellidae. However, the dendritic skeleton, absence of cells within the fibers, and molecular characters distinguish it from this family and prompts the establishment of a new family, Ernstillidae.

Keywords Porifera, Verongiida, new family, new genus, Australia

Research Highlights We present a new family and genus within the order Verongiida based on distinctive morphological and molecular characters: eurypylous choanocyte chambers, a dendritic skeleton, lack of cells within fibers, and distinct molecular distances.

Graphical Abstract
Type-specimen of *Dendrilla lacunosa* Hentschel, 1912, Zoologisches Museum, Hamburg,

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Introduction

It is now well known that integrative taxonomy, with the addition of molecular and chemical data to classical morphological characters, may result in significant changes in the classification of sponges. Correct classification of sponges is not only pivotal for systematics, molecular biology, development and evolution but has high relevance for all downstream applications of sponges such as in biotechnology and secondary metabolite research. Here we give a new example of a species, which was well described by Hentschel in 1912 under the name *Dendrilla lacunosa* and turns out to belong to a different subclass of Demospongiae, and is the subject of ongoing cancer research (Le, 2017). We show here that this sponge is not a *Dendrilla*, which in current sponge classification following Morrow & Cardenas (2015) is in the subclass Keratosa, order Dendroceratida, but belongs instead to the subclass Verongimorpha, Order Verongiida. Recently, it is recognized that chitinous skeletons are typical for 12 representatives of three families within this order (Ehrlich et al., 2007; 2010; 2013; 2017; Brunner et al., 2009; Cruz-Barraza et al., 2012; Wysokowski et al., 2013; Zoltowska-Aksamitowska et al., 2018) in contrast to proteinaceous spongin-based skeletons, which remain to be characteristic for Dendroceratida and Dictyoceratida (see for review Jesionowski et al., 2018). There are twelve *Dendrilla* (Lendenfeld, 1883) species currently recognized (Van Soest et al., 2018), and four species originally described as *Dendrilla* that have been reinterpreted as belonging to the verongiid genera *Pseudoceratina*, *Ianthella* or *Suberea*. The species in this study presents unique genetic and morphological characters that do not allow its classification under any currently recognized verongiid genus or family. We present here the morphological and molecular characters justifying such a significant systematic transfer, and the affinities of this unique taxa within the Verongiida. The chemical analysis of the skeleton is presented in the Part II.

Material and methods

Observation and collection sites

Specimens were collected on SCUBA or by benthic sled or trawl in the Kimberley and Pilbara regions of Western Australia in a depth range from 6-63 m. They were preserved in 70 or 100% ethanol or wet frozen. Specimens studied in this project are stored at the Western Australian Museum, which currently holds 28 specimens of this species.

Skeleton and tissue preparation

The skeleton was isolated from the surrounding tissue either manually by forceps or by 1 hour digestion of the tissue in 2.5 M NaOH. The skeleton and the tissue were observed by

light microscopy of sections made from specimens, either parts of the whole sponge or isolated fibers, embedded in Araldite and cut with a low-speed saw using a diamond wafering blade. These thick sections were then thinned by polishing on wet-ground polishing discs.

Molecular data

For molecular taxonomic support we amplified and sequenced two gene regions frequently used for molecular taxonomy (see e.g., Erpenbeck et al., 2016), the C-Region of the nuclear large ribosomal subunit (in the following referred to as 28S) and the 5' region of the mitochondrial cytochrome oxidase subunit 1 (in the following referred to as CO1). DNA was extracted with the NucleoSpin (Machery-Nagel) DNA extraction Kit. The CO1 fragment was amplified using the primers dgLCO1490 and dgHCO2198; 28S with the primers 28S-C2-fwd and 28S-D2-rev under conditions as described in Erpenbeck et al., (2016). PCR products were purified out of the agarose gels via freeze squeeze (Tautz and Renz, 1983) before cycle sequencing with BigDye-Terminator Mix v3.1 (Applied Biosystems) following the manufacturer's protocol. Forward and reverse strand of the template were sequenced on an ABI 3730 automated sequencer. Raw sequences were basecalled, trimmed and assembled in CodonCode Aligner v 3.7.1.1 (www.codoncode.com). Sequences are deposited in NCBI Genbank under accession numbers MK532285 (28S) and MK522799 - MK522802 (CO1), and with location data, specimen photos and additional morphological information in the Sponge Barcoding Database (SBD, www.spongebarcoding.org, Wörheide & Erpenbeck, 2007), accession numbers SBD#2109-2112~~Sequences are deposited in the European Nucleotide Archive (www.ebi.ac.uk/enaENA) under accession numbers XXXXXXX, and with location data, specimen photos and additional morphological information in the Sponge Barcoding Database (SBD, www.spongebarcoding.org, Wörheide & Erpenbeck, 2007), accession numbers xxxxxxxx.~~

Before the reconstructions, the ordinal classification of *Ernstilla* were assessed in a CO1 and 28S reconstruction of all currently published demosponge sequences by aligning to the sequences of the Sponge Genetree Server (www.spongegenetrees.org, (Erpenbeck et al. 2008)). For detailed molecular phylogenetic reconstructions we created a dataset of CO1 and 28S sequences published in NCBI Genbank (www.ncbi.nlm.nih.gov) including relevant representatives of Keratosa and Verongimorpha, particularly Verongiida. The sequences were aligned with MAFFT (Katoh and Standley, 2013) as implemented in Geneious 8.1.9 (Biomatters, http://www.geneious.com Kearse et al., 2012) under default settings and optimized by eye. Maximum-likelihood reconstructions were generated with RAxML 7.2.8 (Stamatakis, 2014) under the GTR+GAMMA model and 1,000 rapid bootstrap replicates, as implemented in Geneious 8.1.9.

Description

Demospongiae
Verongimorpha, Verongiida
Family Ernstillidae FAM. NOV.
Type species: *Ernstilla* n. g. *lacunosa* (Hentschel, 1912)
Diagnosis: Verongiida with eurypylous choanocyte chambers, and a well-developed dendritic skeleton of cell-less fibers differentiated into thick axial fibers that extend from a thickened chitinose base, with thinner wispy fibers that echinate them.
Etymology: Named after Ernst Hentschel who described many new species of sponges in the Indo-Pacific.
Origin and location of specimens
Maret Islands, Kimberley (14°21′52.97″S, 125°20′48.47″E to 14°21′51.76″S, 125°20′51.21″E), 25 m, O. Gomez and J. Ritchie, sled, 13/12/2015, RV *Solander*, WAMSI Kimberley survey

station SOL11, (WAM Z94223); Eclipse Islands, Kimberley (13°29′37.615″S, 125°51′05.878″E to 13°29′35.828″S, 125°51′08.352″E), 41.7-42.5 m, O. Gomez and J. Ritchie, sled, 2/3/2016, RV *Solander*, WAMSI Kimberley survey station ECLR15, (WAM Z94785); Eclipse Islands, Kimberley (13°49′49.861″S, 126°08′18.881″E to 13°49′48.743″S, 126°08′21.132″E), 60.3-60.6 m, O. Gomez and J. Ritchie, sled, 5/3/2016, RV *Solander*, WAMSI Kimberley survey station ECL24, (WAM Z94910); Eclipse Islands, Kimberley (13°37′48.803″S, 125°51′04.248″E to 13°37′45.681″S, 125°51′03.298″E), 55.5-55.6 m, O. Gomez and J. Ritchie, sled, 11/3/2016, RV *Solander*, WAMSI Kimberley survey station ECLR13, (WAM Z95153); Camden Sound, Kimberley (15°44'51.53″S, 124°08′47.41″E to 15°44′50.23″S, 124°08′46.82″E), 43.0-43.2 m, J. Fromont and L. Kirkendale, sled, 26/3/2015, RV *Solander*, WAMSI Kimberley survey station SOL69, (WAM Z87079); off Onslow, Pilbara (21°35′46″S, 115°03′38″E), 12.3 m, J. Fromont and M. A. Wahab, SCUBA, 7/7/2015, RV *Solander*, WAMSI Dredging survey station 10, (WAM Z88302); Exmouth Gulf, Ningaloo (21°51′29″S, 114°12′04″E to 21°51′88″S, 114°11′92″E), 22.3-22.5 m, S. Morrison and P. Unsworth, trawl, 6/11/2004, RV *Naturaliste*, Exmouth Gulf survey III station 10/200/P, (WAM Z29204).

Morphology.

All the specimens are erect, arborescent, composed of a single or up to seven branches of various heights, arising from a short common stem (Fig. 1). This can be a large sponge, with branches are up to 120 cm in length with a diameter up to 5 cm, sometimes with multiple branches (Figure 1 A, B, D). Most specimens are attached by an enlarged flattened basal stalk or stalks (Fig. 1 A, E, F) adhering to rocky substrates, such as broken pavement usually with a thin sediment veneer (Fig. 1 F). In some specimens the basal stalk is divided in rhizoid-like fibers (Fig. 1 F), 7-8 cm long, 8 mm in diameter near their base where they are often coalescent, and 1 mm at their extremity. This composite stalk is encrusted by sediment. All specimens display a large number of holes, 4-10 mm in diameter, giving a lacunose aspect, with relatively thin surface tissue. Numerous large conules, often with protruding terminal fiber ends, are present (Fig. 1). The surface is covered by an easily detachable, smooth dermal membrane with a fine network structure, made by thickened ectoderm of variable thickness from 20 to 120 µm in diameter, forming meshes 50 x 80 µm to 120 x 130 µm (Fig. 2 E). The ectodermal thickening is produced by very elongate cells, arranged in parallel (Fig. 2 G). In the living tissue, beneath the dermal membrane, the choanosome has many choanocyte chambers. They are clearly eurypylous, round or more or less elongate, measuring 30 x 120 µm, up to 70 x 120 µm (Fig. 2 F). The aquiferous canals are bordered by a layer of elongate contractile cells. Spherulous cells, 15 µm diameter, with spherules 2 µm diameter, are very rare and difficult to see with an optical microscope on specimens fixed in ethanol. Small holes, 15-20 µm in diameter, are present in the dermal membrane, possibly pores. Oscules are not visible in the preserved specimens. In underwater images, they appear as small holes, approximately 2-3 mm in diameter with a thin, slightly translucent margin. Consistency of the living tissue is soft, fleshy, that of the skeleton is very hard, difficult to bend. When curved for fixation and preservation in a jar, the new shape of the sponge becomes permanent. The color is yellow to light blue-green, sometimes bluish, in vivo, it rapidly oxidises when exposed to air, becoming deep violet to purplish black in ethanol.

The skeleton is dendritic, without any anastomoses (Fig. 2 A). It is composed of strong fibers, very hard, even the thinnest are difficult to bend. These fibers are made of chitin, as reported in part II of this publication. At the base of the stem, the axis is a very thick fiber (4-5 mm thick), single although probably composite in a first growth stage as indicated by divided pith in cross section (Fig. 2 D). The lowest part of the thick basal stem (5 to 8 cm from the base) is echinated by a few fibers, approximately 5-6 mm long, inserted

perpendicularly by a conical basis 500-1300 µm in diameter (Fig. 2 B, D). From this stem diverge obliquely (angle approximately 50-70 °) ramified lateral branches of decreasing diameter, the thinner, 200-300 µm, ending at the surface conules as obtuse terminal ends. In the specimen with basal rhizoid-like skeleton, it is also made by diverging fibers, but less regular, and sometimes not cylindrical in diameter, forming irregular plates with reduced pith and sometimes including a few foreign bodies (Fig. 2 C). The color of the fibers varies, from dark brown for the thick fibers of the main axis, becoming clearer on the diverging fibers to being clear brown at their extremity. In cross section (Fig. 2 C, D), the fibers show a variable proportion of pith and bark. At the base of the stem, in a section 3.5 mm in mean diameter, the pith is 600 µm in diameter for a bark 1500 µm thick. In 500 µm diameter fibers in the branches, the bark is approximately 50-80 µm thick and the pith 200-350 µm in diameter. In the extremity, in fibers 200-300 µm in diameter, the bark is 50 µm thick and the pith 120-130 µm in diameter. The pith is finely fibrillar, with fibrils 0.4-0.8 µm in diameter, roughly parallel along the axis of the fiber, sometimes forming domes. The transition between bark and pith is more or less clear, in places very distinct, more progressive in others. In the centre of the pith, a small hole, 30-130 µm, which seems to be empty, is always present. The bark shows conspicuous strata, 25-30 µm thick with clearer interstrata 20-60 µm thick in the base of the stem. The strata are very dense, dark in cross section, especially in the outer part of the fiber. In the thinnest sections, they appear to be constructed of the same fibrils as the pith, but considerably denser.

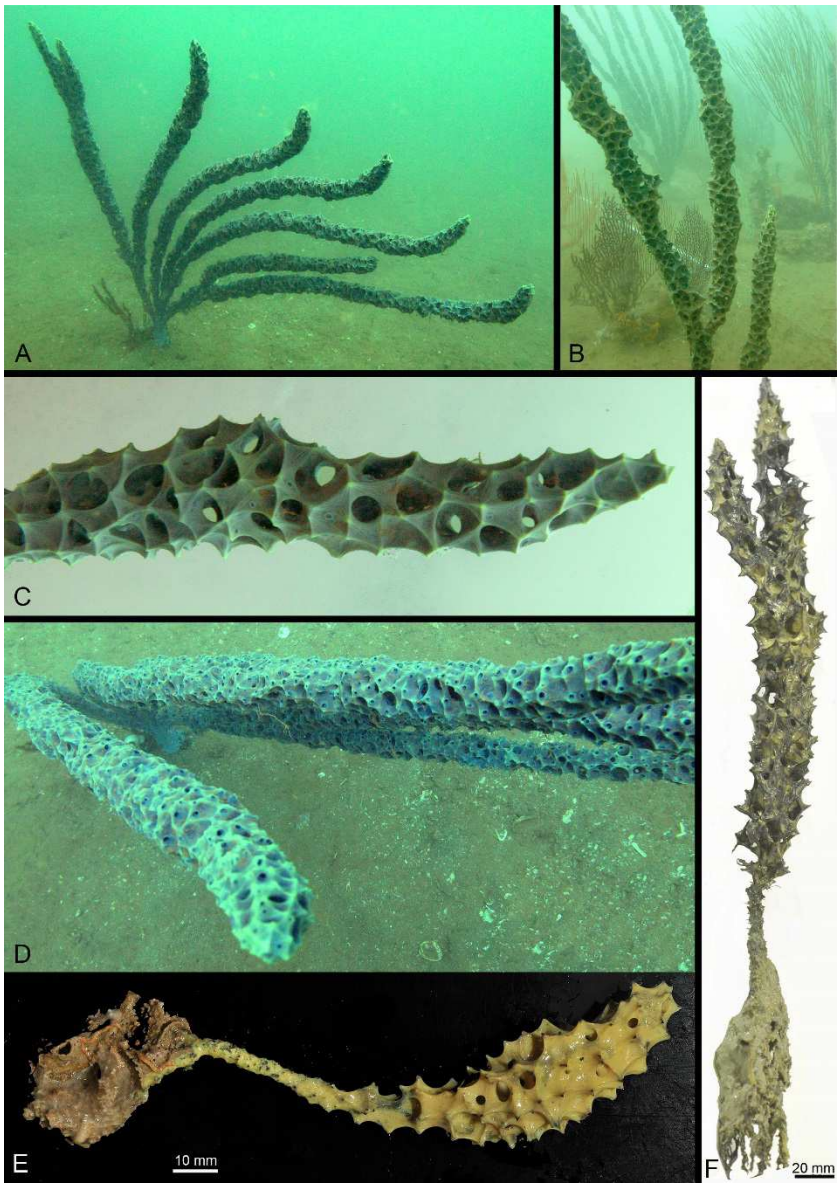


Fig.1: A-D: In situ pictures, 26/03/2013, location off Onslow, Pilbara. C, freshly collected. E: specimen WAM Z94785 Eclipse Islands, Kimberley, freshly collected. F: Specimen WAM Z94223, Maret Islands, Kimberley, with a divided stalk, freshly collected (photographers C. Schönberg A, D; E. Büttner B, C; J. Keesing E, F).



Fig. 2. A: Part of the dendritic skeleton, cleaned digestion of the tissue in 2.5 M NaOH. B: Skeleton of the basal stem, showing echinating short fibers. C: Cross section through the divided stalk of specimen WAM Z94223, showing a composite fiber and sediment. D: Cross section through the fiber of the basal stem, showing pith, bark and the conical base of an echinating fiber. E: Dermal membrane. F: Section through the living tissue near the surface, showing dermal membrane, choanocyte chambers and canals. G: Section through dermal membrane. C-D are from specimen WAM Z94223.

Molecular analysis

The initial phylogenetic analyses with the alignment of the Sponge Genetree Server in CO1 (2471 sequences) and 28S (1095 sequences) clearly indicated a verongiid origin of *Ernstilla* (not shown). As a consequence a detailed analysis with *Ernstilla* and all Verongiida as published in NCBI Genbank was undertaken, with Chondrillida as outgroup. The resulting phylogenetic trees from the CO1 and 28S reconstructions are given in Fig 3. The CO1 data set resulted in 148 taxa and 508 characters; the 28S rDNA data set was considerably smaller due to the fewer sequences available from Genbank at the moment, and consists of 18 taxa with 348 characters. The phylogenetic signal of both trees is congruent. Ianthellidae (*Ianthella* + *Hexadella* + *Anomoianthella*) form a supported monophyletic group. The deeper splits with

relationships of the remaining taxa is lacking support in 28S rDNA due to the high variability of this barcoding fragment. *Ernstilla* constitutes the sister group to Ianthellidae, well supported in 28S, unsupported in CO1. Remarkable is the large branch of *Ernstilla* in comparison to the confamilial taxa, and the outgroup.

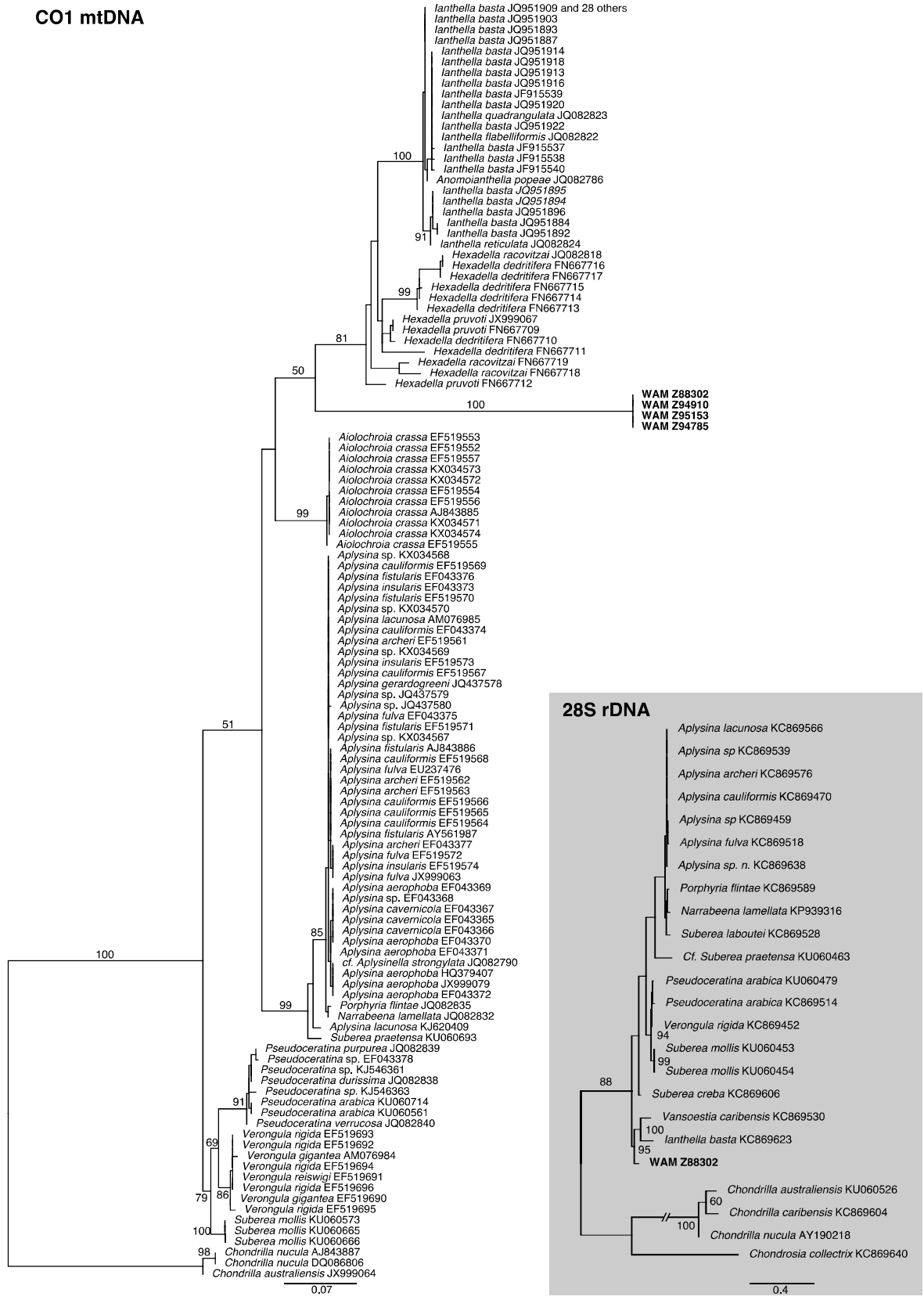


Fig.3: Maximum-likelihood phylogram on the position of *E. lacunosa* (boldface taxa) in the Verongiida based on CO1 and 28S C-Region (gray inset). Numbers following the taxon names are NCBI Genbank accession numbers. Numbers on branches depict selected bootstrap support (only on relevant branches for readability). The scale bar depicts substitutions per site.

Distribution

Ernstilla lacunosa (Hentschel, 1912) is common, but has a patchy distribution throughout tropical Australasia. Additional specimens are known from the Gulf of Carpentaria (QM G320824, G320828), and areas throughout the Northern Territory such as Darwin and Bynoe Harbours, Wessel, Melville and Bathurst Islands, and the Bonaparte Basin in the Timor Sea (NTM Z005145, Z005146, Z006301, Z00608, Z006514, Z006899, Z007083, Z007285, Z007491) with a large depth range from 4 to 109 m. Thus, the species distribution is currently known to be from the Gulf of Carpentaria in the north east of Australia, across the tropical north to the Aru Islands, and to Exmouth Gulf in Western Australia. It can be abundant in filter feeder habitats. Nine specimens were recorded along a 5x1 m transect off Onslow in the Pilbara, and an average of 0.6 specimens per square meter were recorded in Camden Sound in the Kimberley, Western Australia.

Discussion.

The morphology and the skeletal characters of this sponge correspond to the description by Hentschel (1912) of his new species *Dendrilla lacunosa*, (holotype ZMH S 2130, Zoological Museum Hamburg) based on two specimens from the Aru Islands, 4 m depth.

These characters are in good accordance with the genus *Dendrilla* as redefined by Bergquist (1996) and Bergquist (2002): erect shape, skeleton strictly dendritic with a thick basal stem and fibers tapering in diameter towards the surface, fibers with a stratified bark and a central pith without foreign bodies, choanocyte chambers eurypylous. Thus Hentschel's allocation of the species *lacunosa* to *Dendrilla* was fully justified with a taxonomy based on morphological characters. Our present results both for molecular analysis in 28S and CO1 and for the chitinous nature and fine structure of the fibers (Part II of this work) clearly indicate that the sponge does not belong to the order Dendroceratida in the subclass Keratosa, but to the subclass Verongimorpha, order Verongiida, as defined in Systema Porifera (Bergquist, 2002) and recognized by Morrow & Cardenas (2015). However, the position of such a sponge with eurypylous chambers and dendritic skeleton in the order Verongiida is somewhat puzzling. Two families of Verongiida have a dendritic skeleton, but they differ by their diplodal choanocyte chambers and by other important characters. Pseudoceratinidae, with a single genus, has a reduced skeleton of fibers composed only of pith, without bark. Aplysinellidae has three genera, differing respectively by presence of fibrous spicules and weak fiber development (*Aplysinella*), by a reduced fiber skeleton with pith predominant in the fibers (*Suberea*), or by thin fibers and goblet shape (*Porphyria*). In accordance with the suggestion of Erpenbeck et al., (2012) that the morphology of choanocyte chambers are, besides the molecular data, the most robust data to evaluate suprageneric taxa in Verongiida, the eurypylous choanocyte chambers suggest the classification of this sponge in Ianthellidae. However, several characteristics of the fibers and their arrangement are not shared with Ianthellidae fibers. First in all Ianthellidae the fiber skeleton is reticulate while it is clearly dendritic in this species. Furthermore, in *Ianthella* and *Anomoianthella*, the only genera of the Ianthellidae with a fiber skeleton (absent in *Hexadella* and *Vansoestia)*, there are cells inside the fibers, absent in this species. The morphology of the fiber skeleton at the base, axial with echinating fibers, is also unique among other dendritic fibers occurring in Verongiida. These differences suggest a new genus, characterized mainly by a dendritic skeleton and the absence of cells inside the fibers. The distinction between dendritic versus reticulate skeletons is not significant at a high level of classification, but remains highly indicative at a lower level among verongiids growing over open substrates (Erpenbeck et al, 2012; Diaz et al., 2013). As presented in the Results and discussed below, erection of a new genus and family is in line with the

molecular results. The distinction from other Verongiida is corroborated by the molecular data as the mitochondrial branch lengths by far exceed all other inter-familial distances. We thus propose the erection of a new genus, *Ernstilla*, which does not fit within any existing Verongiida family, thus the erection of a new family, Ernstillidae. ~~As presented in the Results and discussed below, erection of a new genus is in line with the molecular results. The distinction from other Verongiida is corroborated by the molecular data as the mitochondrial branch lengths by far exceed all other inter-familial distances. We thus propose the erection of a new family, Ernstillidae, for the genus *Ernstilla*.~~

Given the morphological similarities between the new genus and other *Dendrilla* species, it is possible that some sponges described as *Dendrilla* are in fact members of this new genus and family. Several *Dendrilla* species have already been transferred to Verongiida. According to the World Porifera Database, this is the case for several species described by Lendenfeld: *D. aerophoba* Lendenfeld 1883, accepted as *Ianthella aerophoba*, *D. elegans* Lendenfeld 1888 accepted as *Suberea*, *D. ianthelliformis* Lendenfeld 1888, accepted as *Suberea ianthelliformis*. Similarly, *D. verongiformis* de Laubenfels 1954, accepted as *Pseudoceratina purpurea* (Carter, 1880). However, none of these species have a dendritic skeleton and thus were originally incorrectly described as *Dendrilla*. It appears very difficult to decide from historic morphological descriptions alone if species with branching shape and a dendritic skeleton are true *Dendrilla* or could belong to the new genus and family. A morphological and molecular assessment is required for these species. An example of this could be *Dendrilla mertoni* Hentschel, 1912 described in the same publication as *E. lacunosa*, and also from the Aru Islands. It differs mostly by a more massive form, thinner fibers at the base, and fibers occasionally connected in the branches. This last character is sometimes observed in dendritic skeletons in which the tree-like fibers have a decreasing diameter from the base to the extremity of the branches, and it does not imply a reticulate skeleton. We refrain from reallocating this species until thorough examination of the type specimen is undertaken. At first sight, another case could be *Dendrilla antarctica* Topsent, 1905, which is described as being yellow in life and turning deep blue in alcohol, a character frequently found in Verongiida, but this is not confirmed by molecular data (Ana Riesgo, personal communication und unpublished data).

Legends of Figures

Fig.1: A-D: In situ pictures, 26/03/2013, location off Onslow, Pilbara. C, freshly collected. E: specimen WAM Z94785 Eclipse Islands, Kimberley, freshly collected. F: Specimen WAM Z94223, Maret Islands, Kimberley, with a divided stalk, freshly collected (photographers C. Schönberg A, D; E. Büttner B, C; J. Keesing E, F).

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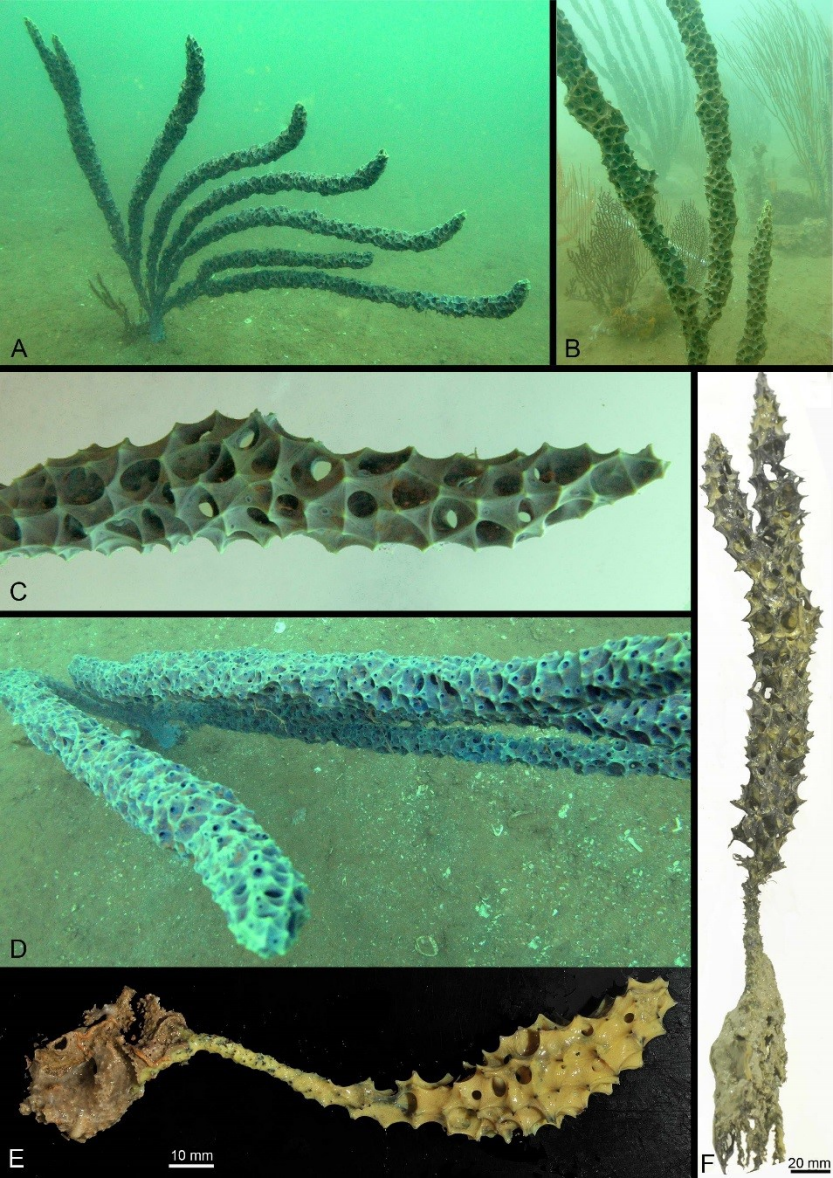
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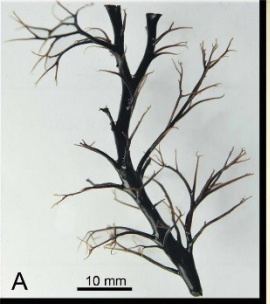
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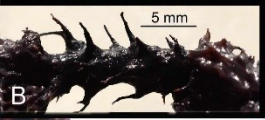
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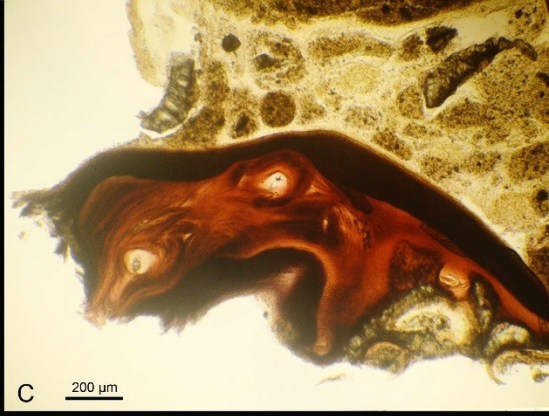
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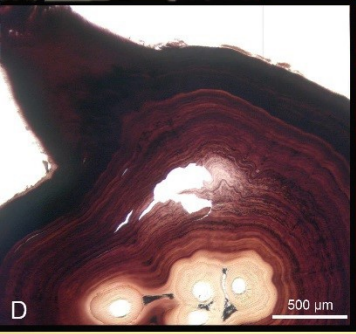
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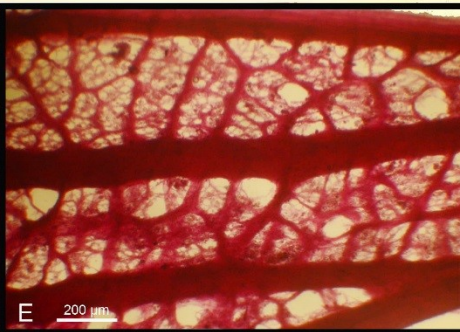

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B
C
D
E
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F
20 mm

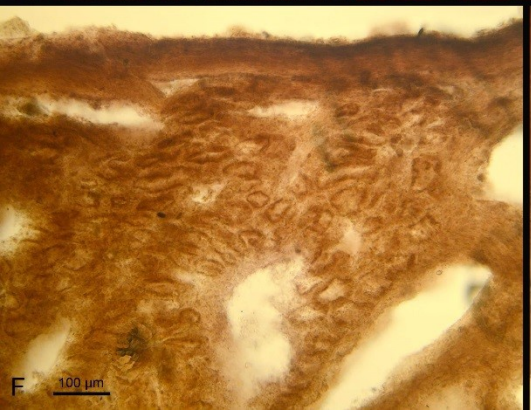

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5 mm
B

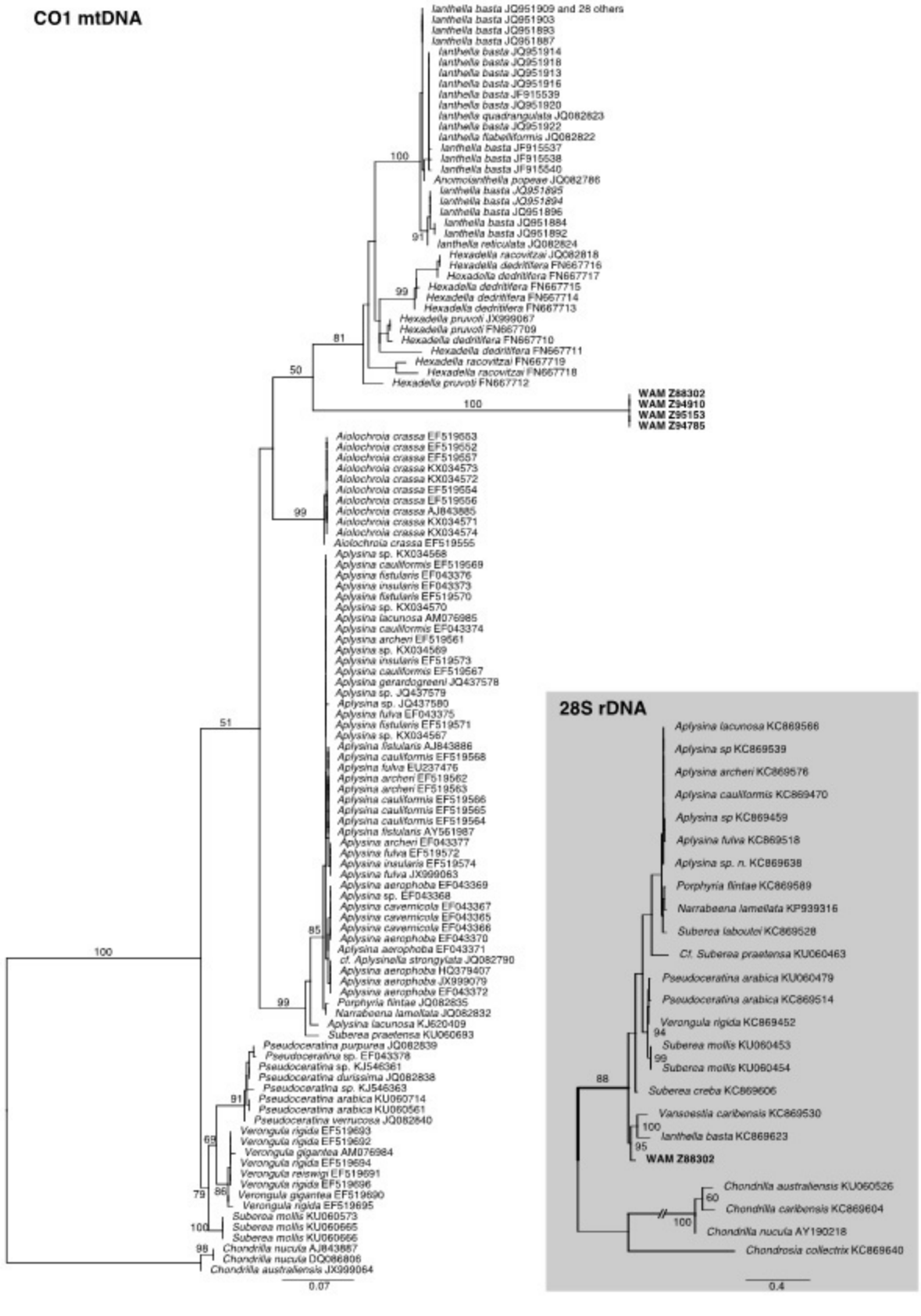

C
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D
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E
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F
100 µm


G
15 µm


CO1 mtDNA
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Ianthella basta JQ951893
Ianthella basta JQ951887
Ianthella basta JQ951914
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