



## SYMPOSIUM

### Phylogenetic Novelties and Geographic Anomalies among Tropical Verongida

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**Synopsis** Exploring marine sponges from shallow tropical reefs of the Caribbean and western Central Pacific, as part of large biodiversity (Moorea Biocode Project) and evolutionary (Porifera Tree of Life) research projects, we encountered 13 skeleton-less specimens, initially divided in two morphological groups, which had patterns of coloration and oxidation typical of taxa of the order Verongida (Demospongiae). The first group of samples inhabited open and cryptic habitats of shallow (15–20 m) Caribbean reefs at Bocas del Toro Archipelago, Panama. The second group inhabited schiophilous (e.g., inner coral framework and crevices) habitats on shallow reefs (0.5–20 m deep) in Moorea Island, French Polynesia. We applied an integrative approach by combining analyses of external morphology, histological observations, 18S rDNA, and mtCOI to determine the identity and the relationships of these unknown taxa within the order Verongida. Molecular analyses revealed that none of the species studied belonged to *Hexadella* (Ianthellidae, Verongida), the only fibreless genus of the Order Verongida currently recognized. The species from the Caribbean locality of Bocas del Toro (Panama) belong to the family Ianthellidae and is closely related to the Pacific genera *Ianthella* and *Anomoianthella*, both with well-developed fiber reticulations. We suggest the erection of a new generic denomination to include this novel eurypylous, fibreless ianthellid. The species collected in Moorea were all diplodal verongid taxa, with high affinities to a clade containing *Pseudoceratina*, *Verongula*, and *Aiolochoiria*, a Pacific and two Caribbean genera, respectively. These unknown species represented at least three different taxa distinguished by DNA sequence analysis and morphological characteristics. Two new genera and a new species of *Pseudoceratina* are here proposed to accommodate these novel biological discoveries. The evolutionary and ecological meaning of having or lacking a fiber skeleton within Verongida is challenged under the evidence of the existence of fibreless genera within various verongid clades. Furthermore, the discovery of a fibreless *Pseudoceratina* suggests that the possession of a spongin-chitin fiber reticulation is an “ecological” plastic trait that might be lost under certain conditions, such as growing within another organism’s skeletal framework. These results raise new questions about the ecological and evolutionary significance of the development of a fiber skeleton and of sponges’ adaptability to various environmental conditions.

#### Introduction

The order Verongida Bergquist, 1978 comprises tropical to temperate marine sponges that, mostly, possess a characteristic fiber skeleton comprised of chitin and spongin, organized either in dendritic or

anastomosing patterns (Bergquist and Cook 2002a; Ehrlich et al. 2007). Verongid fibers are amber in color and are composed of an internal dark organic pith and a concentrically layered translucent bark. These two fibrous components may present

variations in their dimensions and structure, the combination of which may characterize a particular taxon, such as a species or genus. *Hexadella* Topsent, 1896 is the only genus to lack any form of a fibrous skeleton and for centuries species from this genus were assigned to other more distantly related skeleton-less taxa, e.g., *Oscarella* Vosmaer, 1884 and *Halisarca* Johnston, 1842. Other evidence from cellular (spherulous cells), chemical (Tyrosine-derived brominated compounds), and genetic traits have allowed for their inclusion within the order Verongida (Bergquist and Cook 2002a).

Recently, studies of two verongid genera demonstrated the presence of chitin and a silica-chitin-aragonite composite on the spongin fibers, making this a unique biochemical characteristic among all the aspiculate sponge taxa that possess a fibrous skeleton (Ehrlich et al. 2007, 2010). Phylogenetic molecular studies, including data from ribosomal, mitochondrial, and nuclear housekeeping genes, confirm that the order Verongida belongs to a clade of aspiculate demosponges, together with the orders Halisarcida and Chondrosida (see Wörheide et al. 2012 for a review; Hill et al. 2013). This clade named “Myxospongiae” by Borchini et al. (2004) was formally provided with a phylogenetic definition by Cárdenas et al. (2012). Erpenbeck et al. (2012), while further characterizing (COI and 28S) fiber-bearing demospongiae taxa, proposed the term “Verongimorpha” for this clade of aspiculate sponges.

Verongid species constitute conspicuous components of the world’s tropical marine faunas. Some verongids grow as large fans, tubes, or repent or erect branches (Fig. 1A) and are abundant on shallow and mesophotic coral reefs, reef walls, and rocky substrates (van Soest 1978; Alvarez et al. 1991; Slattery et al. 2011). In contrast, species of the fiberless genus *Hexadella* grow as crust-shaped individuals, mostly a few millimeter in thickness, on both exposed and cryptic hard substrates in Atlantic, Mediterranean, and Pacific waters (Reveillaud et al. 2012) (Fig. 1B).

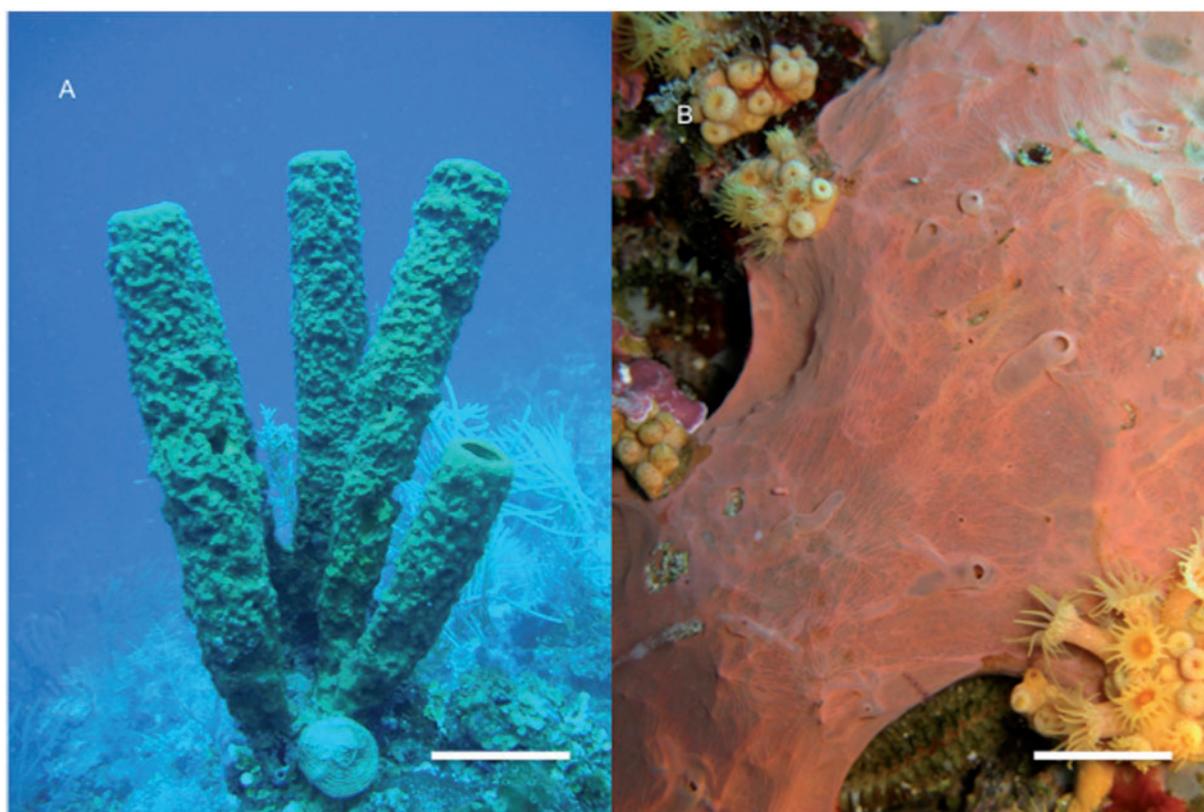
Approximately 89 verongid species have been described (Cárdenas et al. 2012; van Soest et al. 2013) and are currently classified within 10 genera and four families (Aplysinidae Carter, 1875; Aplysinellidae Bergquist, 1980; Ianthellidae Hyatt, 1875; Pseudoceratinidae Carter, 1885) based on morphological criteria (Bergquist and Cook 2002a, 2002b, 2002c, 2002d, 2002e). Two very distinct arrangements of choanocyte chambers allow the separation of the families into two major groups: (1) the family Ianthellidae with sac-shaped to ovate-elongate choanocyte chambers of relatively large size

(>20–50  $\mu\text{m}$  in diameter), which simply open into excurrent canals (eurypylous chambers) (Fig. 2A), and (2) the families Aplysinidae, Aplysinellidae, and Pseudoceratinidae with very small (<20  $\mu\text{m}$ ) round diplodal choanocyte chambers (Bergquist and Cook 2002b, 2002c, 2002e; Reveillaud et al. 2012) (Fig. 2B).

A summary of the major morphologic criteria used to separate the currently described genera of Verongida (*sensu* Bergquist and Cook 2002a) is presented in Table 1. Ianthellidae, with three genera, contains taxa with sac-shaped eurypylous choanocyte chambers. Two ianthellid genera possess well-developed anastomosing fiber reticulations (*Ianthella* and *Anomoianthella*), and one genus (*Hexadella*) lacks any fibrous skeleton. The seven genera of the three other families all possess mostly smaller (<20  $\mu\text{m}$ ), densely packed, diplodal choanocyte chambers. They have been distinguished by two contrasting patterns of reticulation: anastomosing (i.e., Aplysinidae) (see Fig. 3A) or dendritic, and the nature of their fiber structure (bark and pith presence or absence and ratio of each fiber component).

Even though the monophyly of Verongida is nearly certain (Cárdenas et al. 2012; Erpenbeck et al. 2012; Wörheide et al. 2012; Redmond et al. 2013, this issue; Thacker et al. 2013, this issue), the current assignment of genera within families remains equivocal. Recent molecular evaluations of Keratosa (Dictyoceratida and Dendroceratida) and the related Verongida have shown that the development of either an anastomosing or a dendritic fiber reticle does not provide sufficient phylogenetic signal to establish suprageneric relationships (Erwin and Thacker 2007; Erpenbeck et al. 2012). 28S and COI data showed that the family Aplysinellidae (diplodal, dendritic fibrous skeleton, with fibers possessing both bark and pith) is polyphyletic, containing genera that belong to the ianthellid clade (*Aplysinella*) or to the aplysinid clade (*Suberea* and *Porphyria*). 28S and COI data show that the Caribbean genus *Verongula* (Aplysinidae *sensu* Bergquist and Cook 2002) lies within Pseudoceratinidae, together with *Pseudoceratina* (Pseudoceratinidae, Demospongia), an exclusively western Pacific genus. Erpenbeck et al. (2012) suggested that the molecular information and the morphology of choanocyte chambers are the datasets that are most robust for re-evaluating suprageneric verongid taxa and their phylogenetic relationships.

During expeditions by the “Porifera Tree of Life” project to Bocas de Toro Archipelago (Republic of Panama) and to Moorea, French Polynesia (in conjunction with the Moorea-BIICODE project), we



**Fig. 1** Two extreme morphologies among Verongida. (A) Large clump of tubes of *Aplysina fistularis*, Carrie Bow Cay, Belize (photograph by M. C. Diaz); scale = 30 cm. (B) Thin crusts of *Hexadella topsenti* form Marseille (photograph by T. Perez); scale = 5 cm.

encountered various species of sponges that lacked any apparent skeleton under routine microscopic analyses. These species had sulfur yellow to orange colorations that oxidized to darker or even to black coloration when exposed to air or placed in alcohol, which led to hypothesizing that they were verongids, and perhaps species of the genus *Hexadella*, given their lack of fibers or spicules.

The present work evaluates the taxonomic identity and phylogenetic relationships of fibreless verongids found in distant geographic localities, by using an integrative approach, first determining the nature of their choanocyte chambers (large eurypylous versus smaller diplodal) and then by analyzing their molecular affinities using 18S rDNA and, when possible, mitochondrial COI sequences.

## Methods and materials

### Collections from Bocas del Toro

Specimens were collected by scuba diving at three sites within the Bocas del Toro Archipelago (Panama) on shallow reefs (15–20 m deep) (Table 2). Specimens were obtained by collecting fragments of rubble on which the sponges were growing. Samples were preserved in 96% ethanol. During the 2012 expedition,

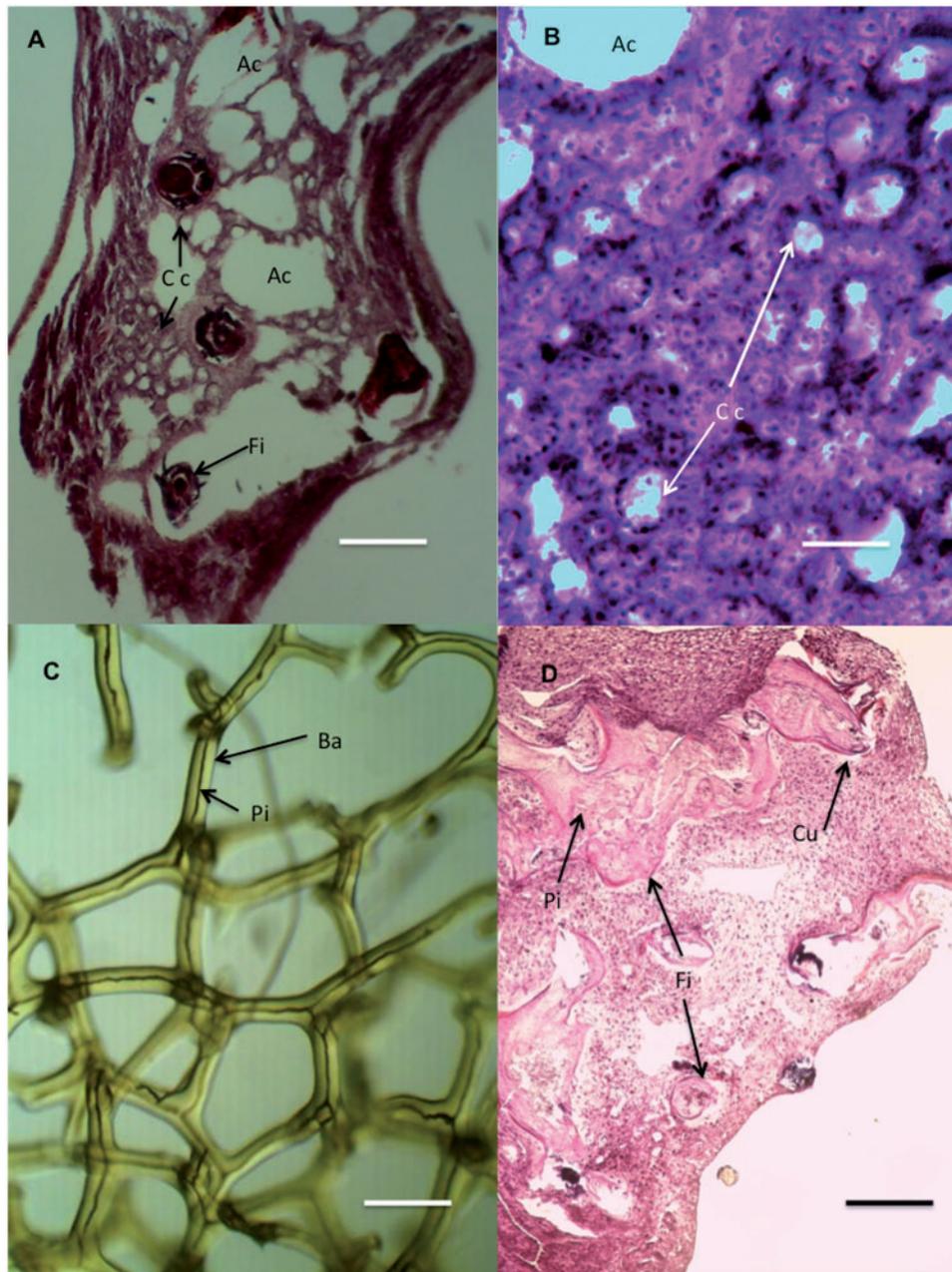
samples were preserved in paraformaldehyde (PF) 4% for histological analysis.

### Collections from Moorea

Collection was carried out on various coral reefs along the northeastern coast of Moorea (0.5–15 m deep) during expeditions in 2010 and 2011 (Table 2). Specimens were growing within a framework of dead coral, in crevices or under the rubble, at depths ranging between 0.5 and 15 m. Fragments were collected from standing coral rubble by breaking small pieces with a hammer and chisel or by using a knife to peel the sponge from crevices or from loose coral rubble. During the 2011 expedition, samples were also preserved in 96% ethanol for DNA analyses and in PF 4% for histologic analysis.

### Morphology

Observations of the shape, surface features, oscules, size, color, and consistency of live animals as well as photographic records (underwater and/or in the laboratory) were used to describe the external morphology of the material collected, and notes on the habitat were made. Samples were preserved routinely in 96% ethanol for DNA studies and in PF 4% for



**Fig. 2** Contrasting morphologies of the aquiferous system and the fibers within Verongida taxa. **(A)** Cross section of *Lanthella basta* shows abundant large (30–50  $\mu\text{m}$ ) eurypylous sac-shaped choanocyte chambers (Cc) distinguished from the larger aquiferous canals (Ac) and fibers (Fi) in cross section showing dark dense pith; scale = 200  $\mu\text{m}$ . **(B)** Cross section of unidentified fibreless verongid from Moorea (CPM1572) showing round small, diplodal choanocyte chambers (darker internal borders) that only can be distinguished at high magnifications; scale = 20  $\mu\text{m}$ . **(C)** Well developed anastomosing polygonal reticulation in *Aplysina* sp. from Bocas del Toro, formed by clear amber-colored fibers with a dark thin pith (Pi) and translucent bark (Ba). **(D)** Longitudinal section of *Pseudoceratina* cf. *purpurea* from Moorea (BMOO-16218, CPM 1550); wide deformed fibers in which the pith dominates and the bark is only represented by a thin cuticle (cu); scale = 100  $\mu\text{m}$ .

histological studies. In the laboratory, samples were dehydrated with a battery of successive ethanol washes (70%, 75%, 80%, 90%, 97%, and 100%), and a final clarification was accomplished using three xylol (100%) washes of 1 min each. An automated microtome was used to obtain the fine

sections (5–8  $\mu\text{m}$ ) from fixed samples embedded in paraffin. Tissue was stained according to a hematoxylin-eosin protocol (Luna 1968).

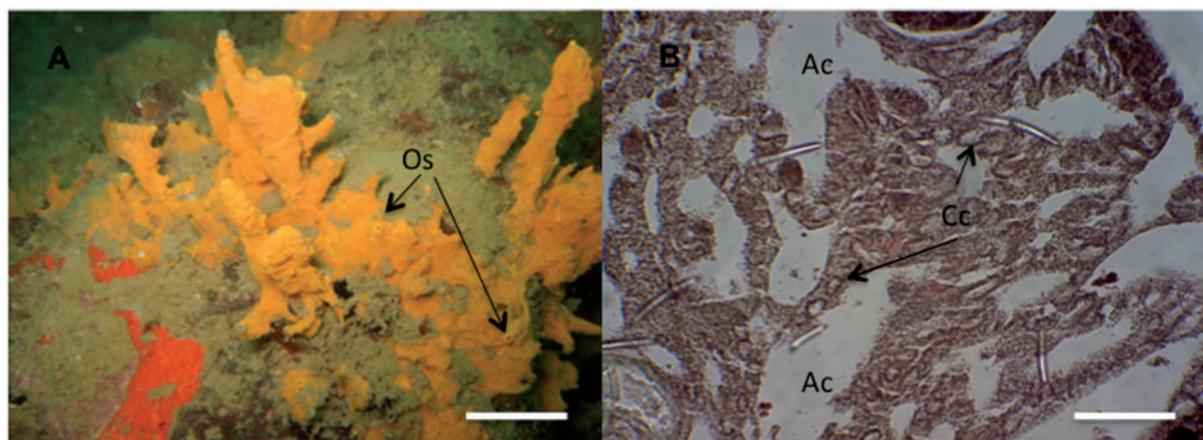
#### 18S analyses

Refer to Redmond et al. (2013, this issue).

**Table 1** Choanocyte chambers, fiber and reticle characterization, species distribution, and diversity of verongid genera

Taxa	Cch	Bark/Pith	Reticle	spp.	Distribution
lanthellidae Hyatt, 1875					
<i>lanthella</i> Gray, 1869	Eury	Y/Y	Anasto.	10	W. Pac
<i>Anomoianthella</i> Bergquist, 1980	Eury	Y/Y	Anasto.	3	W. Pac
<i>Hexadella</i> Topsent, 1896	Eury	Fibreless	None	8	W. Pac, Atl., Med.
Aplysinellidae Bergquist, 1980					
<i>Aplysinella</i> Bergquist, 1980	Dipl	Y/Y	Dendr.	2	W. Pac
<i>Porphyria</i> Bergquist, 1980	Dipl	Y/Y	Dendr.	1	W. Pac
<i>Suberea</i> Bergquist, 1995	Dipl	Y/Y	Dendr.	12	W. Pac
Aplysinidae Carter, 1875					
<i>Aplysina</i> Nardo, 1834	Dipl	Y/Y	Anasto.	45	Tro, Atl., Med.
<i>Verongula</i> Verrill, 1907	Dipl	Y/Y	Anasto.	3	Car.
<i>Aiolochroia</i> Wiedenmayer, 1977	Dipl	Y/Y	Anasto.	3	Car.
Pseudoceratinidae Carter, 1885					
<i>Pseudoceratina</i> Carter, 1885	Dipl	N/Y	Dendr.	4	W. Pac, Res

Note: Choanocyte chambers (Cch) are either eurypylous, sac-shaped (Eury) or diplodal (Dipl); types of reticulation are anastomosing (Anasto.) or dendritic (Dendr). Distribution data include Atlantic Ocean (Atl.), Caribbean Sea (Car.), Mediterranean Sea (Med.), Pan-tropical (Tro.), Red Sea (Res), and Western Pacific (W. Pac).



**Fig. 3** Morphological characteristics of the fibreless lanthellidae from Bocas del Toro. (A) Thin (2–3 mm) fibreless lanthellidae (P67) from Punta Caracol, Bocas del Toro, Panama, overgrowing a specimen of *Oceanapia* sp. Notice the canals and membranous oscula over the whole surface; scale = 10 cm. (B) Cross section of P67 (Puerta Caracol, 15 m) showing unsupported body, with large aquiferous canals, and sac-shaped large choanocyte chambers; broken oxea are from the overgrown *Oceanapia*; scale = 200  $\mu$ m.

### COI analyses

Sequences of the mitochondrial COI barcode region were generated as part of the workflow of the Moorea BIOCODE project (<http://www.mooreabiocode.org/>) for six specimens of Verongida. These sequences were aligned in Geneious with all available COI sequences from GenBank ( $n=125$ ) using its implementation of MAFFT with default settings. Ends of this initial alignment were trimmed, resulting in a 585-bp alignment used for phylogenetic analysis. RaxML was used to conduct maximum

likelihood searches for optimal trees with 500 bootstrap replicates to assess node support. Outgroups from Chondrosida were initially included, but their position was unstable, so the resulting topology is rooted arbitrarily between members of Lanthellidae and the remaining verongids.

### Results

The characterization of external morphological and histological features and subsequent 18S ribosomal and mitochondrial COI analyses (Figs. 2–8)

**Table 2** Samples collected during expeditions to Bocas del Toro, Panama, and Moorea, French Polynesia

PorToL no.	Museum no.	Coordinates	Depth (m)	Expedition
SI06-189	NA	9.30183–82.29433	15–20	Bocas 2006
P67	NA	9.30183–82.29433	15–20	Bocas 2010
P76	NA	9.30183–82.29433	15–20	Bocas 2010
P12 × 342	NA	9.37766–82.30316	15–20	Bocss 2012
CPM1356	UF:Porifera:1604	–17.50988–149.76102	6	Moorea 2010
CPM1400	UF:Porifera:1670	–17.45747–149.83277	10–20	Moorea 2010
CPM1429	UF:Porifera:1683	–17.55681–149.87384	1–2	Moorea 2010
CPM 1434	UF:Porifera:1688	–17.49520–149.86238	6–14	Moorea 2010
CPM1460	UF:Porifera:1711	–17.48395–149.91638	19–20	Moorea 2010
CPM1543	NA	–17.57000–149.90000	15–20	Moorea 2011
CPM1547	NA	–17.57000–149.90000	15–20	Moorea 2011
CPM1550	NA	–17.57000–149.90000	0.5	Moorea 2011
CPM1572	NA	–17.52230–149.76218	15–20	Moorea 2011

Note: Museum numbers not available yet are indicated as NA.

UF, University of Florida Museum of Natural History.

corroborated the verongid nature of all the studied specimens. Distinct clades or branches (well supported) allow the distinction of four taxa distributed among two verongid families.

#### Ianthellidae (*sensu* Bergquist and Cook 2002d) from the Caribbean

The specimens of a thin crustose (1–2 mm thick), yellow-orange (Fig. 3) fibreless sponge presented sac-shaped, large, eurypylous choanocyte chambers, corroborating their histological affinities to the ianthellid genera (Fig. 2A). 18S ribosomal gene analyses depict a solid clade (100% BS) for this Caribbean ianthellid, which pairs with a clade of Pacific *Ianthella* and *Anomoianthella* species (Fig. 5). 18S sequences of fibreless *Hexadella* species do not “pair” with our Caribbean material. This constitutes the first report of the family Ianthellidae for the Caribbean basin and the first report of a non-*Hexadella* fibreless Verongida. Unfortunately, we were not able to generate COI for this sample.

#### Pseudoceratinidae (*sensu* Erpenbeck et al. 2012) from the Central Pacific

The presumed skeleton-less specimens from schiophilous habitats in Moorea all presented very small (10–15 μm), densely packed choanocyte chambers with diplodal organization, suggesting affinities with non-ianthellid verongid families, Aplysinidae or Pseudoceratinidae (*sensu* Erpenbeck et al. 2012).

Histological sections revealed that two of the seven presumed skeleton-less samples studied actually had sparse fibers. The first, BMOO-16218 (Fig. 4A), was

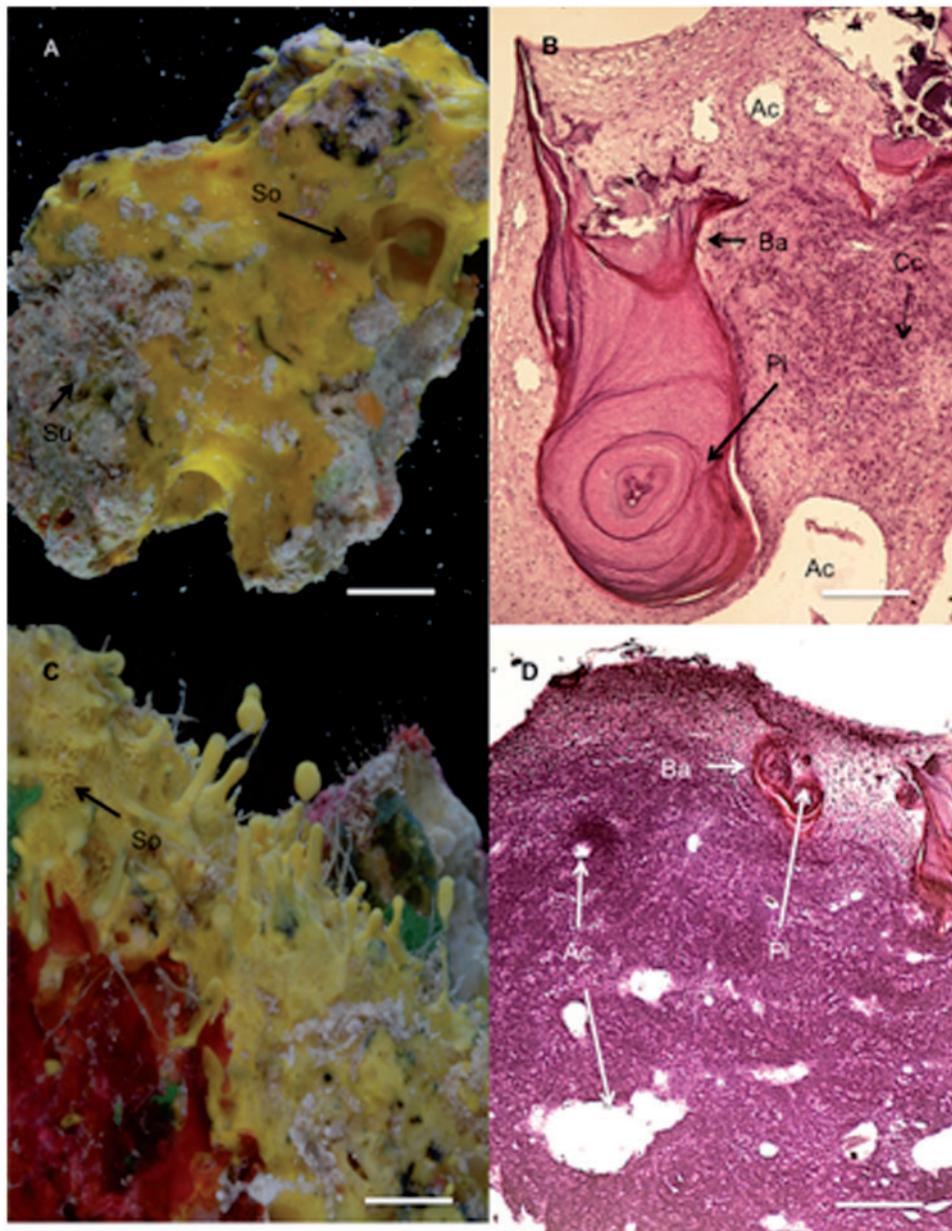
identified as *Pseudoceratina* cf. *purpurea* (Pseudoceratinidae) possessing a dendritic arrangement of deformed fibers dominated by an amorphous pith (Figs. 2D and 4B). The second, BMOO-16211 (Fig. 4C), was identified as *Suberea* cf. *creba* (Aplysinidae *sensu* Erpenbeck et al. 2012) presenting a dendritic reticle formed by fibers with a clear presence of the concentrically layered bark and dark organic pith characteristic of the genus (Fig. 4D). The rest of the taxa from Moorea truly lacked any fibrous elements and consequently are interpreted as fibreless verongid taxa.

A combination of morphological characters evidenced in the histological sections and/or a combination of molecular data was used to establish identity and affinities. COI genetic data, gathered from the BIOCOTE project, was used to further clarify the identity and relationships of these taxa within the Verongida (Figs. 5 and 6). 18S ribosomal and COI gene analyses of those fibreless diplodal verongids reveals diverse taxa, with specimens grouped in three separate branches within the Pseudoceratinidae (*sensu* Erpenbeck et al. 2012).

Below are brief characterizations of the external, histological, and genetic information and the apparent phylogenetic affinities of these three taxa.

#### Pseudoceratinidae 1

This group forms a distinct lineage in both 18S and COI gene analyses and is represented by samples number BMOO 81068 (CPM1460), BMOO 06409 (CPM1400), BMOO 16215 (CPM 1547), and BMOO 04391 (CPM 1356). The group is represented



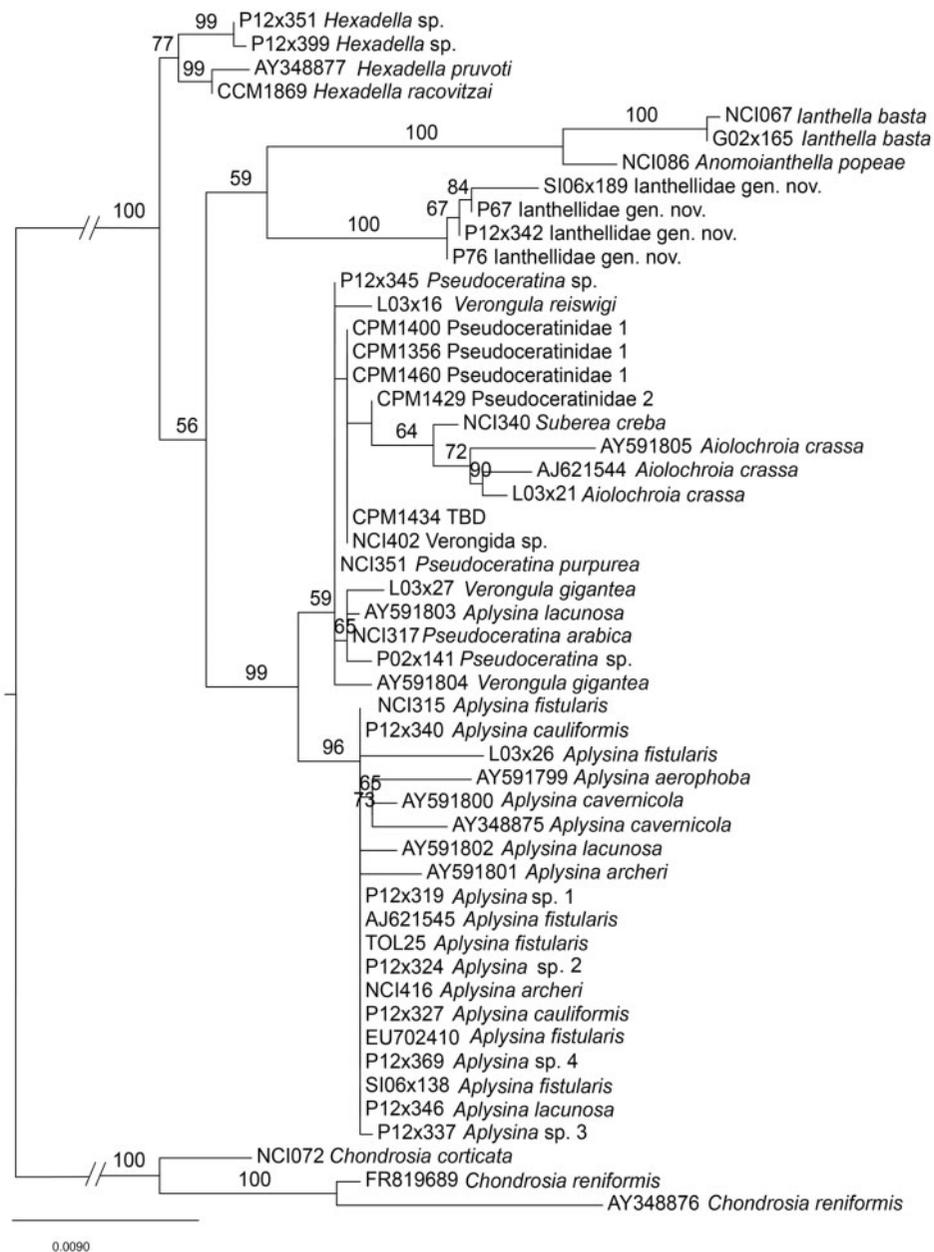
**Fig. 4** Fiber-bearing taxa encountered among the presumed skeleton-less verongids from Moorea. **(A)** Live specimen of *Pseudoceratina cf. purpurea* (BMOO16218, CPM 1550) growing over dead coral conglomerate substrate (Su); sieve of ostia (So) shown with arrow; scale = 0.9 cm. **(B)** Cross section of *Pseudoceratina cf. purpurea* (BMOO16218) showing a transversal section of pith (Pi) dominated fibers, with its thinner dark bark (Ba); few very small choanocyte chambers (Cc) distinguished in the very dense inner body; scale = 140  $\mu$ m. **(C)** Live specimen of *Suberea cf. creba* (BMOO16211, CPM 1543) with abundant sieve of ostia (So) giving off small buds (Bu); scale = 0.5 cm. **(D)** Cross section of *Suberea cf. creba* (BMOO16211) showing a very dense inner body, with large aquiferous canals (Ac), and fibers with pith (Pi) and bark (Ba); scale = 100  $\mu$ m.

by bright yellow crustose to massive specimens (Fig. 7A) that grew embedded in dead coral. When the standing dead coral was broken off, this species was found within the coral structure. The sponge body contains large amounts of coral fragments and detritus, making it hard to get a tissue section from most samples (Fig. 7B). Dark, dense granules (Fig. 7C) and clear diplodal choanocyte chambers

(Fig. 7D) were seen for one of the specimens (BMOO-81068, CPM1460). Specimens darken to light brown in ethanol.

#### Pseudoceratinidae sp. 2

This taxon is represented by sample BMOO-07061 (CPM 1429) and has a light-yellow thick crust habit (3–5 mm thick), overgrown by a cobalt blue



**Fig. 5** Reconstruction of phylogenetic tree resulting from RAxML analysis of near-complete 18S rRNA from 34 verongid taxa. One thousand bootstrap replicates were employed and all bootstrap values >50 are shown on the relevant branches.

spiculose sponge (Fig. 8A). It is represented by a distinct branch both in the 18S and in the COI trees (Figs. 5 and 6). The histological sections show that the sponge does not incorporate detritus on its body. However, due to the poor preservation of this sample, it is only possible to be certain of the lack of fibers and the absence of large sac-shaped choanocyte chambers, based on the sections obtained (Fig. 8B).

#### Pseudoceratinidae sp. 3

This taxon is represented by sample BMOO-16291 (CPM1572) (Fig. 8C) and its identity became evident during the COI analyses (Fig. 6) (amplification of

the 18S rRNA was unsuccessful). Its histology demonstrated a fibreless nature and a particular abundance of collagen strands both within the mesohyl and toward the surface of the specimen (Fig. 8D). COI data demonstrate that this specimen is within a clade (COI tree) containing three recognized *Pseudoceratina* species and one undescribed *Pseudoceratina* from Moorea (BMOO-16218) (Fig. 4A and B).

#### Relationships of the order Verongida

The present study has added important taxa to the genetic database of the order Verongida. 18S ribosomal gene analyses support the existence of three

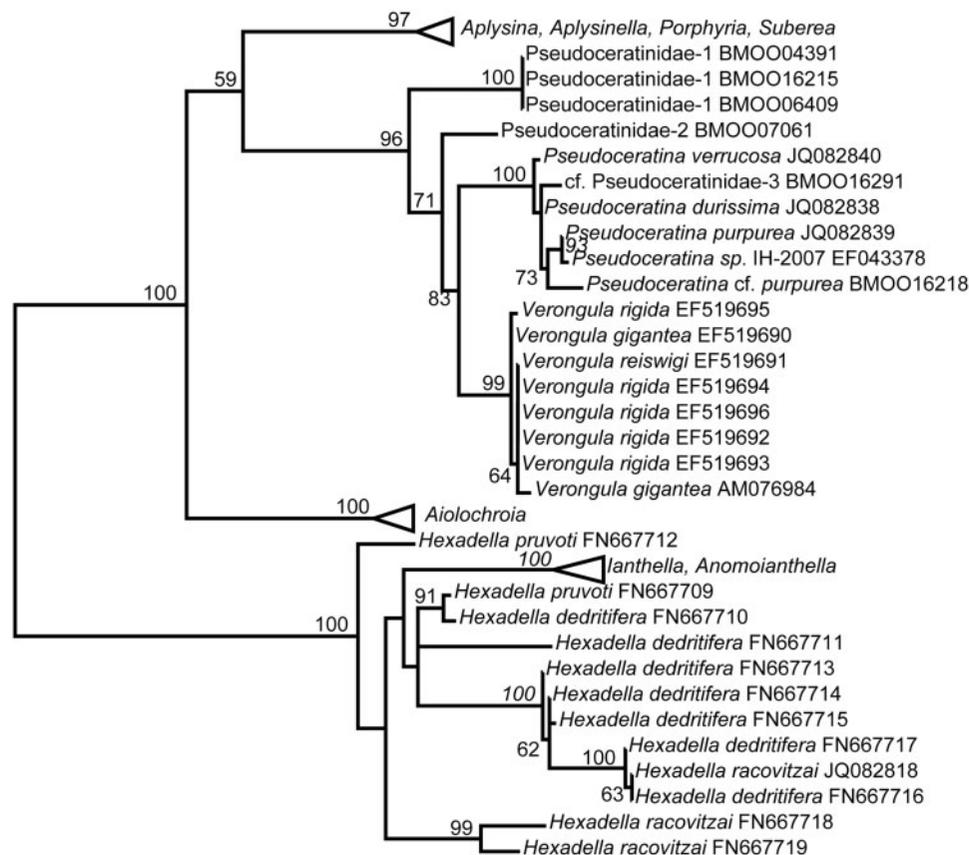


Fig. 6 Reconstruction of phylogenetic tree resulting from RAxML analysis of 585bp COI alignments; 500 bootstrap replicates were used to assess node support. Outgroups from Chondrosida were initially included, but their position was unstable, so the resulting topology is rooted arbitrarily between members of Ianthellidae and the remaining verongids.

clades within this order (Fig. 5): an *Hexadella* clade (77% BS), an *Ianthella/Anomoianthella*/skeleton-less verongid from the Caribbean clade (59% BS), and a Pseudoceratinidae/Aplysinidae clade (99% BS). The lower clades within these three major divisions obtain very high (99–100% support), except the pseudoceratinid clade containing *Pseudoceratina*, *Verongula*, *Suberea*, and *Aiolochoia*. A relatively similar picture is revealed by analysis of COI; there is a division between the species of Ianthellidae and the remaining verongids (Fig. 6). This node in the unrooted topology was chosen as the arbitrary root for purposes of display. Within non-ianthellid verongids, there are three well-supported groups of species: (1) *Aiolochoia*, (2) *Aplysina*, *Aplysinella*, *Porphyria*, and *Suberea*, and (3) *Verongula*, *Pseudoceratina*, and two new skeleton-less taxa.

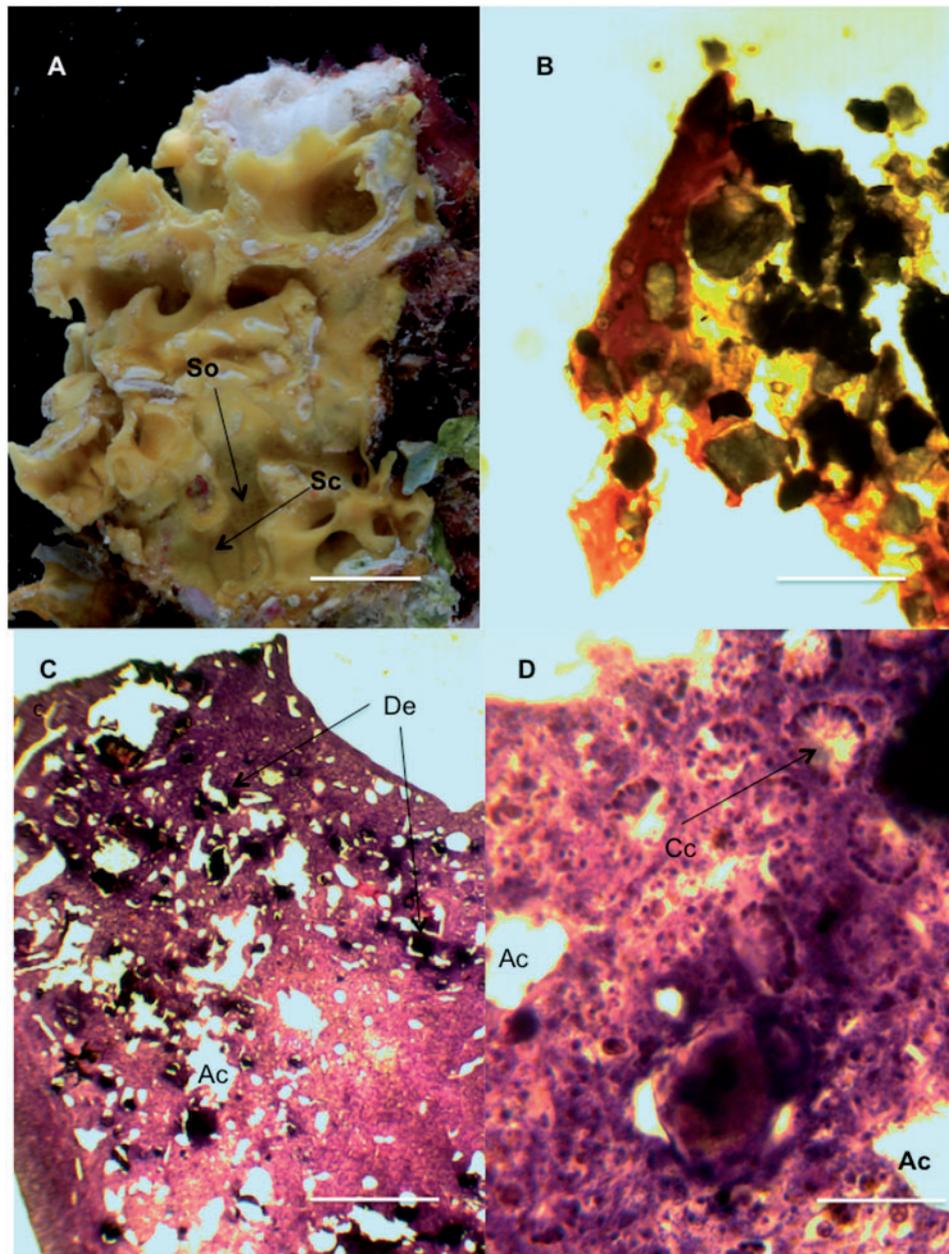
## Discussion

The integrative approach used to study skeleton-less Verongida encountered during expeditions to the Caribbean and the West-Central Pacific has shown

an unsuspected richness of biological diversity within Verongida, with such a unique molecular signature that it is producing three major changes to our vision of Verongida classification.

### Ianthellidae—new skeletal-less genus

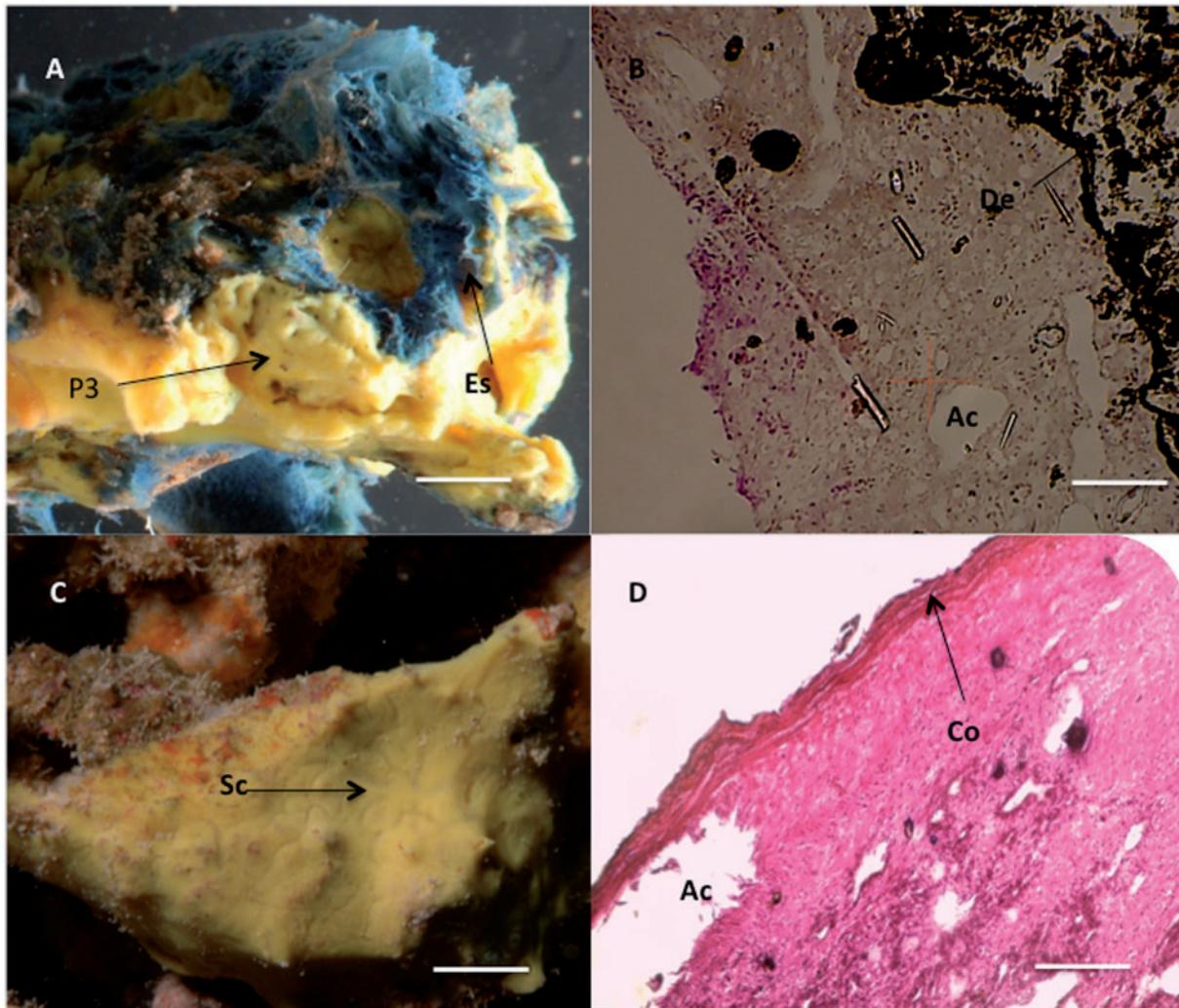
*Hexadella* is the only, currently recognized genus of Verongida lacking any skeleton. Any eurypylous, fibreless verongid otherwise would be classified as *Hexadella*. The encounter of a completely separate ianthellid clade, lacking any fibrous skeleton, brings important changes in the classification of the family by requiring the erection of a new genus and opens up questions with respect to the evolutionary significance of a fibrous skeleton, particularly as it shows that within this family the possession of a fibrous skeleton might be a derived condition and not an ancestral one (Figs. 5 and 6). We propose the erection of a new genus of Ianthellidae to accommodate this unique Caribbean taxon. This new genus represents the first known occurrence of an ianthellid from the Caribbean basin. We are currently preparing its description and nomination of its type



**Fig. 7** Representative morphology of the group Pseudoceratiidae 1. (A) Laboratory live photograph of BMOO 16215 (CPM 1547) of a massive sciophilous sponge embedded on dead coral; thin sub-dermal canals (Sc) and sieve of ostia (So) are visible; scale = 4 mm. (B) Thick section of BMOO 16215 (CPM 1547); scale = 500  $\mu\text{m}$ , showing large amounts of foreign material obscuring inner morphological features. (C) Histological sections of BMOO 81068 (CPM1460) showing dark, dense detritus within the sponge body (De); scale = 200  $\mu\text{m}$ . (D) Histological sections of BMOO 81068 (CPM1460), showing the small round diplodal choanocyte chambers; scale = 20  $\mu\text{m}$ .

species. This genus has bright yellow to orange thin crusts (1–2 mm thick), and it spreads over dead coral or over other sponges (i.e., *Oceanapia* sp.). Its surface is riddled with pores (<1 mm in diameter) and dermal canals (0.5–1 cm wide) and it possesses large sac-shaped (50–60  $\mu\text{m}$  id), eurypylos choanocyte chambers and represents its unique genetic clade (100% support) within ianthellid genera. These

sponges oxidized slowly, reaching dark brown to purple colors. A comparative study to discard a possible relationship of this species to *Halisarca bajalus* (Lendefeld 1889), a species found in the Red Sea and Southeastern Australia, as suggested by van Soest (personal communication), will be carried out to discard the a potential congeneric nature with this very rare Caribbean species.



**Fig. 8** External Morphology and histologic sections of Pseudoceratiniidae 2 (A, B) and Pseudoceratiniidae 3 (C, D). (A) Laboratory live photograph of BMOO-07061 (CPM1429) in bright yellow (P3), with a cobalt blue sponge overgrowing it (Es); scale = 1.5 mm. (B) Cross section of BMOO-07061 (CPM1429); scale = 75  $\mu$ m, showing an organic wall, 5–10  $\mu$ m in diameter, separating the soft body from detritus material (De), aciferous canals (Ac), and no fibers. (C) Underwater photograph of Pseudoceratiniidae 3, BMOO-16291 (CPM1572); scale = 6 mm. (D) Cross section at low magnification showing the absence of fibers and the presence of collagen bundles (Co) particularly at the surface and the aciferous canals (Ac); scale = 120  $\mu$ m.

### Pseudoceratiniidae

Bergquist (1995) revised the definition of the genus *Pseudoceratina* and later revived the order Pseudoceratinida (Bergquist and Cook 2002e) to accommodate four species with a unique fibrous structure, dominated by an amorphous pith (Fig. 2D). COI data support the existence of at least three main groups within the Pseudoceratiniidae: (1) a well-supported *Verongula* clade (with three species presently described in the genus; previously assigned to the family Aplysinidae), (2) a *Pseudoceratina* clade containing all the species of *Pseudoceratina* currently described and one fibreless taxon described herein (Pseudoceratiniidae 3) which must formally

be defined, and (3) two distinct clades with fibreless taxa, Pseudoceratiniidae 1 and Pseudoceratiniidae 2.

The integrative study of five fibreless, sciophilous verongids from shallow reefs in Moorea led to the discovery of three unique phylogenetic branches within the family. We propose that two new genera and one new species must be erected to accommodate their unique morphologic and genetic characteristics. The three new taxa would represent the following synapomorphies.

#### Pseudoceratiniidae 1

Diplodal fibreless verongid massively engrained on coral rubble, heavily incorporating detritus on its body obscuring the mesohyl (Fig. 7).

### Pseudoceratinidae 2

Diplodal fibreless verongid grows under coral rubble, detritus, and/or crevices, with an apparent cuticle separating the sponge body from the external detritus and not incorporating foreign material on its body (Fig. 8A and B).

### Pseudoceratinidae 3 (*Pseudoceratina* sp.)

Diplodal fibreless verongid grows in crevices, and within the coral framework, with a strong development of collagenous matrix both at the surface and within the mesohyl (Fig. 8C and D). COI data shows that this specimen lies within the *Pseudoceratina* clade. The high content of collagen fibrills is a nice synapomorphy with the genus *Pseudoceratina* and the genetic affinity of Pseudoceratinidae 3 (COI tree) suggests that this is a *Pseudoceratina* that lacks a fibrous skeleton.

## Conclusions

The present study has demonstrated the occurrence of Caribbean representatives of the family Ianthellidae among the common shallow-water fauna of Bocas del Toro, Panama.

The generic diversity of the family Ianthellidae is greater than expected, and we have shown that lacking a skeleton is not restricted to *Hexadella* species.

A new genus must be erected to accommodate the existence of a eurypylous, non-*Hexadella* type of fibreless ianthellid.

The exploration of the sponges of the order Verongida in sciophilous habitats from Moorea has shown an unsuspected and previously undescribed biological diversity.

The genetic identities of sciophilous Verongida from Moorea have revealed the existence of several unique phylogenetic branches within the monogeneric family Pseudoceratiniidae, suggesting the existence of at least two more genera within the family, with genetic and morphologic synapomorphies that allow their distinction.

The lack of a fibrous skeleton has been shown to occur in at least two clades of the family Ianthellidae and in various clades within the family Pseudoceratinidae. This leads to a re-evaluation of the evolutionary significance of the acquisition/loss of a fibrous skeleton.

The finding of a fibreless *Pseudoceratina*, living within the coral framework, proposes a possible ecological plasticity in the capacity for building a fibrous skeleton.

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