Community composition and habitat characterization of a rock sponge aggregation (Porifera, Corallistidae) in the Cantabrian Sea

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SUPLEMENT A

Taxonomic remarks

This supplement adds taxonomical and genetics details to the principal species in this aggregation: *Neoschrammeniella* aff. *bowerbankii* and explained the difficulties to arrive a one correct identification.

Neoschrammeniella aff. *bowerbankii* (Johnson, 1863)

Material examined. DR07060511A; DR7060511B and DR07060511C
Comparative material. *Neoschrammeniella bowerbankii* (HBOM 003:00592), *N. bowerbankii* (HBOM 003:00810) from Madeira (Portugal).
Description. Cup-shape or fan-shape to lamellate masses of 90 x 80 mm in size, with thick walls, 8–25 mm, attached to the substrate by the base; surfaces are smooth with numerous small openings evenly spread. Color is white to light beige when alive and in ethanol.
Skeleton. Ectosome is formed by a layer of dichotriaenes and numerous microscleres; choanosomal skeleton has an intricate net of dicranoclones, some oxeas and several microscleres spread through the skeleton.
Spicules. Dichotriaenes have a smooth cladome, sometimes with the tips bending downwards, 141–220–314 µm in diameter, and a long rhabdome, 234–402–637 x 9–13–15 µm (Fig 1 C–D); oxeas are long and fusiform, 776–1117 x 5,2–7,7 µm in size; dicranoclone desmas are smooth, thin, with several tubercles spread through the clones, 292–432–594 x 18–32–58 µm in size (Fig 1A–B); microscleres are of two types, type I are spirasters with thick rays with several spines, 10,1–17,1–30,9 µm (Fig 1E); type II are metasters, very irregular, varying in shape and the number of rays, 19,3–33,2–45,5 µm (Fig. 1F–G).
DNA sequences. Sequence data of mitochondrial and ribosomal gene fragments were generated and deposited in GenBank (http://www.ncbi.nlm.nih.gov/)under accession nos. XXXX-XXXX (mtDNA COI gene) and XXXXX-XXXXX (rDNA 28S gene).
Remarks. The genus *Neoschrammeniella* is currently represented by six species (Van Soest et al., 2019) and have been reported from the South West Pacific Ocean (Lévi & Lévi, 1983; 1988; Schlacher-Hoenlinger et al., 2005), Antarctic Ocean (Kelly, 2007), Mediterranean Sea (Pisera and Vacelet, 2011) and North East Atlantic (Johnson, 1863; Carvalho et al., 2015). In the Northeast Atlantic and Mediterranean Sea, *Neoschrammeniella bowerbankii* (Johnson, 1863) is the only species recorded so far, and is commonly found in deep waters113–669 m (Johnson, 1863; Carter, 1876, Lendenfeld, 1907; Carvalho et al., 2015) or in shallower water, 12–30 m, often in caves (Pouliquen, 1969; 1972 and Pisera &Vacelet, 2011), respectively.
Pisera and Vacelet (2011) established the genus *Neoschrammeniella* to accommodate corallistids that possess smooth dichotriaenes, anisoxeas and two to three types of microscleres (type I with short and blunt rays and type II with long or short pointed rays). After the re-examination and illustration of *Corallistes bowerbankii* previous reported from Madeira by Johnson as *Dactylocalyx bowerbankii*

(1863), the authors have decided to transfer this species to the new genus, due to the possession of two types of microscleres (type I with short and blunt rays and type II with long and pointed arms), being now this species accepted as *N. bowerbankii.*

The specimens examined in this study, show affinities to the Atlantic species *N. bowerbankii*, however some dissimilarities were noted: i) the desmas of the Cantabrian specimens are less tuberculated and thinner, ii) but, the most preeminent feature is the irregularity of shape and size found on the metasters (Fig. 1F–G) that is not observed in the Atlantic or Mediterranean specimens. DNA sequences were obtained for mtDNA COI and they are identical to *N. bowerbankii* from Madeira (sequences obtained from the HBOM vouchers), while the 28S C1–D2 sequences differs in 2bp. However, these specimens do not form a clade with other *Neoschrammeniella* (data not shown here). Since the phylogenetic position of these specimens could not be clarified at the moment of this publication, one could not infer whether these differences are intraspecific variation due to environmental conditions or whether it is in fact a new species/genus. More information regarding this topic will be provided elsewhere.

Material and methods

The identification of specimens was made through the examination of spicules, using both light and scanning electron microscopy (SEM). For the observation of spicules under optical microscopy, we follow the procedures in Cristobo et al., (1993) and for SEM in Carvalho and Pisera (2019). Spicule measurements were also performed for each spicule type (30 times, when possible).

For DNA extraction, the DNeasy blood and tissue kit was used following the instructions provided by the manufacturer. Two gene fragments were amplified: the mtDNA COI using the primers LCO1490 and HCO2198 (Folmer et al., 1994), and the partition C1–D2 of the 28S rDNA using the primers C1'ATR (Cárdenas et al., 2009) and D2 (Lê et al., 1993), following the protocols in Schuster et al. (2015). The molecular work was performed at Biodiversity Laboratories (BDL, DNA Section) at the Department of Biological Sciences (University of Bergen).

Vouchers of the collected lithistid material have been stored at the Spanish Institute of Oceanography in Gijon and will be part of the reference collection of the SponGES project to be deposited in several Natural History Museums (Madrid, Lisbon, Bergen, Uppsala, London and Ottawa).

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Figure 1. *Neoschrammeniella* aff. *bowervankii* Cantabrian area. A. Dicranoclone desma skeleton. B. Details of articulation and sculpture of dicranoclone desmas. C–D. Ectosomal dichotriaenes with smooth cladome. E. Spirasters. F–G. Metaster. Scale bar A: 200µm. B: 20µm. C: 100µm. D: 30µm. E–F: 2µm. G: 10µm.