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Redescription and resurrection of *Pachymatisma normani* (Demospongiae: Geodiidae), with remarks on the genus *Pachymatisma*

Paco Cárdenas*§, Joana Xavier†, Ole Secher Tendal‡, Christoffer Schander*ʃ and Hans Tore Rapp*

*Department of Biology, University of Bergen, Bergen High-Technology Center, PO Box 7800, N-5020 Bergen, Norway. †Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, PO Box 94766, 1090 GT Amsterdam, The Netherlands. ‡Zoological Museum, SNM, University of Copenhagen, Universitetsparken 15, DK - 2100 Copenhagen Ø, Denmark. ʃCentre for Geobiology, University of Bergen, Allégaten 41, N-5007 Bergen, Norway.
§Corresponding author, e-mail: paco.cardenas@bio.uib.no

An increasing number of cryptic species are being discovered within sponges with assumed wide geographical distribution. *Pachymatisma johnstonia* (Bowerbank in Johnston, 1842) (Astrophorida: Geodiidae) is one of the most common and known sponges throughout the north-east Atlantic coasts. *Pachymatisma normani* Sollas, 1888 is a northern species previously synonymized with *P. johnstonia* by Topsent. It is here redescribed on the basis of fresh material from the type locality, the Korsfjord in the Bergen area (Norway). Spicules are shown to be reliable characters to distinguish the species investigated. *Pachymatisma normani* is characterized by larger spicules, most markedly in the sterrasters. Our observations also suggest that habitat and gross morphology distinguish *P. johnstonia* from *P. normani*. Furthermore, using a partial sequence of the cytochrome c oxidase subunit I (COI) and an ITS1-5.8-ITS2 nuclear fragment, we show that consistent genetic differences exist between the two species. A brief overview of the genus and a key to the known species are also given.

INTRODUCTION

Bowerbank (1868) stated that shape was not a reliable character for the specific study of sponges. This has been abundantly confirmed by numerous studies dealing with the effect of the environment on sponge morphology (Bell & Barnes, 2000; McDonald et al., 2002; Meroz-Fine et al., 2005; Carballo et al., 2006). Acknowledging this polymorphism, sponge morphologists have preferred to rely on spicule morphology to discriminate sponge groups and species. Unfortunately, these characters have two major drawbacks. First, they are themselves affected by the environment (Palumbi, 1986; Bavastrello et al., 1996; Maldonado et al., 1999; Bell et al., 2002), which could lead to mis-identifications or phylogenetic mis-placements. Second, highly similar spicule types can hide cryptic species that could be distinguished on the basis of other characters: histology, choanosome and canal ultrastructure, ecology, biochemistry, symbionts and especially genetics. With the advent of molecular biology a growing number of cryptic sponge taxa within geographically widespread species have been discovered (van Soest et al., 1991; Solé Cava et al., 1992; Boury-Esnault et al., 1992, 1999; Klautau et al., 1994, 1999; Lazoski et al., 2001; Wulff, 2006). Hooper & van Soest (2004) stated that although 7000 sponge species are currently known worldwide, there are probably more than twice as many. New species were expected in rare or difficult-to-access species, but even well-known and common species turned out to be species complexes (Rapp, 2006; Wulff, 2006).

Pachymatisma johnstonia (Astrophorida: Geodiidae) is one of the most common and well-known sponges of the southern part of the north-east Atlantic coasts. This species was first mentioned by Bowerbank at a meeting of the Microscopical Society of London in 1841 under the name *Halichondria johnstoniana*. The name was later mis-spelled by Johnston in 'A history of British sponges and litophytes' (1842) as *Halichondria johnstonia*. Johnston also revealed that Bowerbank considered this species as the type of a new genus. Thus, at the end of Johnston's book, *H. johnstonia* appears as *Pachymatisma johnstonia* (p. 244). *Pachymatisma* comes from the greek '*pachy-*' meaning 'thick' or 'dense' and '*matisma*' meaning 'dressed' or 'covered with fur' (Johnston, 1842). This obviously refers to the microrhabds in the ectocortex. As for the presence of sterrasters in the endocortex, it prompted Bowerbank (1864, p. 172) to suggest a close phylogenetic relationship between *Pachymatisma* and *Geodia*. *Pachymatisma* was thus associated to the Geodiidae (Gray, 1867). When Sollas (1882) studied the Norman Collection of sponges from western Norway, he described a sponge collected at 329 m depth in the Korsfjord, south of Bergen, under the name *Pachymatisma johnstonia*. Later, Sollas (1888, p. 243) decided to regard the Norwegian specimens as a seperate species that he named *Pachymatisma normani*. He included also specimens identified by Bowerbank from the Orkney Islands and Wick in Scotland, as well as other samples from the Shetlands. The

Table 1. *Collection accession number, species, locality of collection, depth of collection, Genbank and Morphobank accession numbers for the specimens used in this study. Previously published sequences are indicated in bold.*

Institute/Museum accession number	Species	Collection location	Depth (m)	COI	ITS1-5.8-ITS2	Morphology
ZMBN-77858	*Pachymatisma normani*	Korsfjord (Western Norway)	200–400	EF564322	EF577051	M9622 M9624 M9625 M9626
UiB-PC6			200–400	EF564323	EF577048	M9627
UiB-PC7			200–400	EF564324	EF577049	M9628 M9629
UiB-PC11			200–400	EF564325	EF577050	M9630
UiB-PC62			200–400	EF564326	–	-
UiB-PC105			200–400	EF564327	–	M9631 M9632
UiB-PC184			200–400	EF564328	–	M9633
UiB-PC145		Skagerrak (south of Norway)	149–137	EF564329	EF647867	M9634 M9635
UiB-PC196		Røst Reef (northern Norway)	301	–	–	M10544
UiB-PC185	*Pachymatisma johnstonia*	Mingulay Reef (west of Scotland)	168	EF564330	–	M9717 M9718
MC 3159		Rathlin Island (Northern Ireland)	subtidal	EF564331	EF577052	–
MC 3175			subtidal	EF564332	EF577053	–
MC 3213			subtidal	EF564333	EF577054	–
MC 3216			subtidal	EF564334	EF577055	–
MC 3366*			25	–	–	M9704 M9705
MC 3367*			25	EF564337	EF577056	–
?		Roscoff (France)	?	–	**AF062601**	–
MNHN-DCL4015			Inter-subtidal	EF564335	EF647868	M9706
UiB-PC170		Lagosteira, Berlengas Islands (Portugal)	6	EF564338	–	M9707
UiB-PC172		Rio das Estelas, Berlengas Islands (Portugal)	12	EF564339	–	M9708
UiB-PC174		Punta do Segaño, Galicia (Spain)	12	EF564340	–	M9709
UiB-PC176			12	EF564341	–	M9710

*, MC3366 and MC3367 are parts of the same specimen.

major difference from *P. johnstonia* was, according to him, the thicker endocortex filled with sterrasters. The dimensions of the spicules are also slightly larger than the ones given for *P. johnstonia*. Topsent (1894, p. 326) noted that since the thicker sterrastreal layer must be related to the larger sizes of the sterrasters, *P. normani* is probably just a *P. johnstonia* with a more robust spiculation. Subsequently, Topsent (1928, p. 114) described a *P. johnstonia* from Belle-Île (France) with spicules intermediate of *P. normani* and *P. johnstonia*. He therefore remained doubtful on the validity of *P. normani*. Authors have since then considered *P. normani* as a junior synonym of *P. johnstonia* (Burton, 1930; Vosmaer, 1933; Arndt, 1935; Koltun, 1966; Uriz, 2002). Recently, while dredging in the Korsfjord, we came across the deep-water *Pachymatisma* described by Sollas in 1882. It immediately appeared to us morphologically different from the common *P. johnstonia*. Suspecting another case of sibling species, we decided to examine these specimens more closely, comparing them to *P. johnstonia*.

One nuclear gene and one mitochondrial gene were chosen in order to gain independent molecular data. The mitochondrial cytochrome c oxidase subunit I (COI) gene is, in sponges, a slow-evolving gene and has successfully been used to distinguish sponge species (Erpenbeck et al., 2003; Wulff, 2006). Chombard (1998) suggested that internal-transcribed-spacer 2 (ITS2) might be a good molecular marker to discriminate Geodiidae species. ITS1 and ITS2 rDNA sequences have been shown to be useful in sponge phylogeography and to distinguish closely related sponge species (Lopez et al., 2002; Wörheide et al., 2002a,b; Addis & Peterson, 2005; Nichols & Barnes, 2005; Schmitt et al., 2005). Because of possible intragenomic variation ITS results must nonetheless be treated with care (Duran et al., 2004a; Lôbo-Hajdu et al., 2004; Wörheide et al., 2004). This is especially true for analyses at the population-level (Wörheide et al., 2004; Nichols & Barnes, 2005) which is beyond the scope of this study. Since ITS sequences were suspected to evolve faster than COI, we supposed they would be a suitable marker to confirm the absence of gene flow between the two species.

The main aim of this study is to gain new morphological data on *P. normani*. Molecular data will help us to understand the relationships between *P. normani* and *P. johnstonia*. We also seize this opportunity to give a brief account of the genus *Pachymatisma* around the world, as well as a key to species here considered as valid.

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MATERIALS AND METHODS

Sponge collection

Abbreviations

MC, Ulster Museum, Northern Ireland; MNHN, Museum of Natural History in Paris; UiB-PC, Private Collection of H.T. Rapp, currently housed at the University of Bergen; ZMA, Zoological Museum in Amsterdam; ZMBN, Museum of Bergen.

Sampling

Sampling was mainly done in the Korsfjord (60°10'N 05°10'E) (Bergen area, Norway) using a triangular dredge at depths between 200 and 400 m. Korsfjord is the type locality of *Pachymatisma normani*. One specimen was dredged off the south coast of Norway in the Skagerrak trench (58°13'N 08°35'E). Finally, another specimen was brought back by the 'Jago' manned-submersible during a dive in the Røst Reef (67°30'145"N 09°24'524"E). *Pachymatisma johnstonia* from Portugal and Spain were collected by SCUBA diving at depths between 20 m and 6 m. Samples from the Rathlin Island (Northern Ireland) were photographed and collected by B. Picton and C. Goodwin by SCUBA diving. The samples from Roscoff (DCL 4015) and Mingulay Reef were kindly provided, respectively, by the MNHN and the ZMA. All samples are preserved in 95% ethanol and stored at room temperature. Voucher specimens are available upon request. Digital colour images of most *Pachymatisma* specimens used in this study are available on Morphobank, www.morphobank.org (O'Leary & Kaufman, 2007). Species, collection numbers, collecting localities and depth, Genbank and Morphobank accession numbers are given in Table 1.

Distribution

Known localities were taken from the literature, the Marine Life Information network for Britain and Ireland and our collection localities (Table 1). References concerning *P. normani* will be given in the following redescription. For the distribution of *P. johnstonia* on the British Isles and Ireland, we refer to Neish (2007). We here give references concerning *P. johnstonia* in the rest of the north-east Atlantic and the Mediterranean Sea: Sark, Guernsey Islands and France: Topsent (1894, 1895), Beauchamp (1923), Descatoire (1969), Zidane et al. (1996); Spain: Ferrer-Hernández (1912, 1918), Solórzano (1991); Italy: Russ & Rützler (1959); Adriatic Sea: Maurizio Pansini (personal communication). The distribution map was made with Online Map Creation (www.aquarius.geomar.de/omc). Geographical distributions of *P. normani* and *P. johnstonia* are shown in Figure 1.

Spicule observations

Spicule mounts were made following standard procedure (Rapp, 2006). Thirty spicules per spicule type were measured, except for the triaenes. Being long and fragile, the rhabdomes or cladomes were often broken. For measurements of sterrasters, special care was taken to measure only the fully-grown ones. This means that the rosettes at the tip of the actines were fully developed at the surface of the sterrasters. Three directions can be measured in a sterraster: length, width and thickness. The abundant literature on *P. johnstonia* was used to complement our measurements.

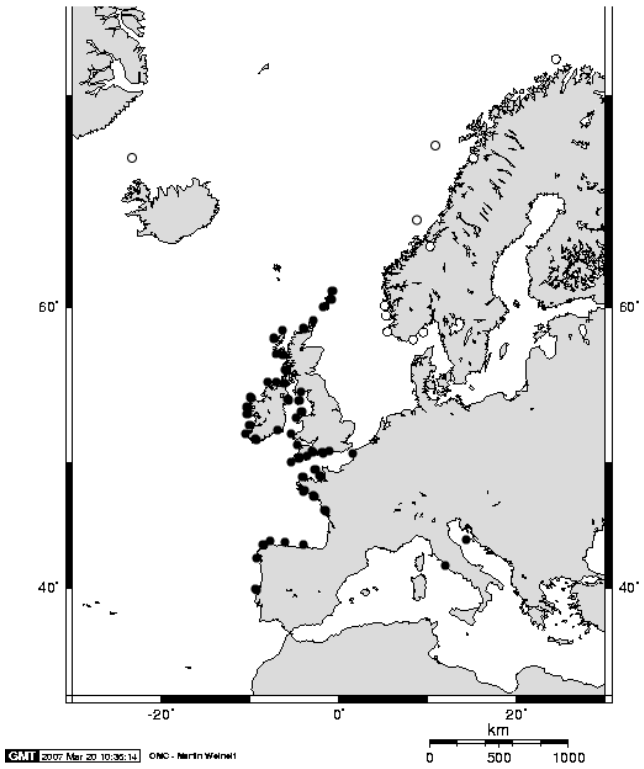

Figure 1. Geographical distribution of *Pachymatisma normani* (❍) and *Pachymatisma johnstonia* (●) in the north-east Atlantic.

DNA extraction, amplification, sequencing and phylogenetic analyses

DNA extraction from samples was performed using the Tissue Genomic DNA extraction kit (Viogene, Sunnyvale, USA) in accordance with the manufacturer's instructions. A single centrifugation step was added just before pipetting the mixture into the columns in order to remove the spicules, the latter would otherwise clog the mini-columns' filters. The primers LCO and HCO (Folmer et al., 1994) were used to amplify a 658 bp region of COI. Polymerase chain reaction (PCR) (50 µl) comprised 4 µl 10×PCR Takara Buffer (TaKaRa Bio Inc.), 4 µl dNTP mix (TaKaRa Bio Inc.), 1 µl of each primer, 0.2 µl of TaKaRa Ex TaqTM Hot Start Version concentrated at 5 units/µl (TaKaRa Bio Inc.), 1–4 µl of template and 36.8 µl of distilled water. The PCR was performed on a Thermo-Cycler PTC-200 (MJ Research) using a two-cycle program. An initial denaturation at 94°C for 5 min followed by five cycles of 94°C for 30 s, 45°C for 1 min and 30 s, 72°C for 1 min. This was followed by 30–35 cycles of 94°C for 30 s, 50°C for 1 min and 30 s, 72°C for 1 min. A final extension step of 72°C for 7 min terminated the program.

The 18SFow and the 28SRev sponge primers (Lôbo-Hajdu et al., 2004) were used to amplify a 794 bp region comprising the 3' end of the 18S, ITS1, 5.8S, ITS2 and the 5' end of 28S. PCR 50 µl contents were the same as for COI. An initial denaturation at 95°C for 5 min, then 35 cycles of 94°C for 30 s, 55–56°C for 45 s, and 72°C for 1 min were used. This was followed by one cycle of 72°C for 7 min, with a hold at 6°C.

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The PCR products were stored at 4°C prior to sequencing. COI sequences were purified using an E.Z.N.A.® Cycle-Pure Kit (Omega Bio-tek, Doraville, USA). ITS1-5.8S-ITS2 sequences were gel extracted using an E.Z.N.A.® Gel Extraction Kit (Omega Bio-tek, Doraville, USA). Cycle sequencing was performed using a dye-labelled dideoxy terminator (Big Dye® Terminator v. 3.1, Applied Biosystems). Products were analysed using an ABI Prism 3700 DNA Analyser (Applied Biosystems). The poriferan origin of the sequences was checked by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/). All sequences were manually aligned in Se-Al v2.0a11 (Rambaut, 1996). A maximum parsimony (MP) analysis of the ITS1-5.8S-ITS2 alignment was conducted using PAUP 4.0b10 (Swofford, 2002). Variable nucleotide positions were treated as unordered discrete characters. Runs were performed by an exhaustive search. Nodal support was estimated with a bootstrap procedure computed after 10,000 replicates of heuristic search with stepwise addition of taxa.

SYSTEMATICS

Class DEMOSPONGIAE Sollas, 1885
Order ASTROPHORIDA Lévi, 1973
Family GEODIIDAE Gray, 1867
Genus *Pachymatisma* Bowerbank in Johnston, 1842

Type species

Halichondria johnstonia Bowerbank in Johnston, 1842 (by subsequent designation).

Diagnosis

Geodiidae with microrhabds tangentially disposed in the ectocortex and along the walls of canals. Often flattened sterrasters, in the endocortex. Cribriporal pores and uniporal oscules.

Pachymatisma normani Sollas, 1888

(Figures 2D–F & 3)
(Morphobank, see Table 1)

Original description

Pachymatisma normani, Sollas, 1888, p. 243.

Synonymy and citations

Pachymatisma normani (?part.), Sollas, 1888, p. 243; Lendenfeld, 1903, p. 91.

Pachymatisma johnstonia, Sollas, 1882, p. 11; Burton, 1930, pp. 490–491; Burton, 1931, p. 2; Alander, 1942, p. 74; Burdon-Jones & Tambs-Lyche, 1960, p. 6; Koltun, 1966, p. 59; Tendal et al., 2001, p. 41; Reitner & Hoffman, 2003, p. 78.

Type material

Holotype: presumably lost (Clare Valentine, personal communication).

Neotype: adult specimen broken in two pieces (9 cm and 7 cm) collected at the type locality: Skorpeodden in the Korsfjord (60°10'N 05°10'E), Norway; water depth: 200–400 m. Bergen Museum collection number: ZMBN 77858. Collected by H.T. Rapp, 21 March 2007.

Additional material examined

Seven specimens from the Korsfjord, one specimen from the Skagerrak, and one specimen from the Røst Reef, UiB-PC (see Table 1).

Comparative material examined

Pachymatisma johnstonia MNHN-DCL4015, Roscoff, France; MC3213, MC 3366, Rathlin Island, Northern Ireland; UiB-PC185, Mingulay Reef, Scotland; UiB-PC174, UiB-PC176, Punta do Segaño, Spain (see Table 2).

Diagnosis

Whitish *Pachymatisma*. Flattened shape with irregular surface due to bumps and outgrowths or regular cone shapped with oscules on a flat top. The spicule set is characterized by large sterrasters (more than 140 μm long), absence of oxyasters II and orthotriaenes. 'G' at position 489 in the Folmer COI sequence. 'C' at position 672 in the ITS1-5.8-ITS2 sequence.

Description

Outer morphology. Most of our specimens have a massive irregularly bumpy flattened shape (Figure 2D–F). Our specimen from the Røst Reef has a more regular shape, like that of a cone (M10544). The biggest specimen found had a diameter of 10 cm and a height of 4 cm. Most of our samples, like *P. johnstonia*, appear to be attached through a large basal area. One whole young specimen (Figure 2D) was stalked. This specimen then flattened out at its upper surface. External colour is usually white to light brownish with whitish patches. One specimen had a peculiar whitish-dark green colour (Figure 2D). Choanosome brownish to whitish. Uniporal oscules (2–3.5 mm) are white-rimmed, slightly raised or flushed with surface. They are gathered in small groups in any area of the sponge (Figure 2F) or restricted to a flat top (M10544). 'Fusion' of two oscules was seldom observed, these could also be interpreted as very simple cribriporal oscules. Pores 0.1–0.01 mm in diameter, cribriporal, similar to those in *P. johnstonia*. Uniporal pores have been observed in four specimens (Figure 3). The cortex is on average 1 mm thick but can reach 2 mm. Surface is rather smooth except in some sheltered hispid areas.

Skeleton

The ectocortex of microrhabds is usually poorly developed, except in pore areas. The endocortex of sterrasters is quite developed. Microrhabds and the flattened sterrasters are tangentially disposed with respect to the surface. Microrhabds can also be found in the wall of large canals. Plagiotriaenes and oxeas are more or less radially disposed near the surface; this arrangement is less obvious in the interior of the sponge. Oxyasters can be found throughout the choanosome. The skeleton is similar to that of *P. johnstonia*, except for the presence of hispid areas in sheltered convolutions where oxeas cross the cortex.

Spicules

Megascleres. All intermediates between strongyles and oxeas exist but the latter are far more abundant. Strongyles and oxeas are straight or bent. Numerous fused oxeas forming

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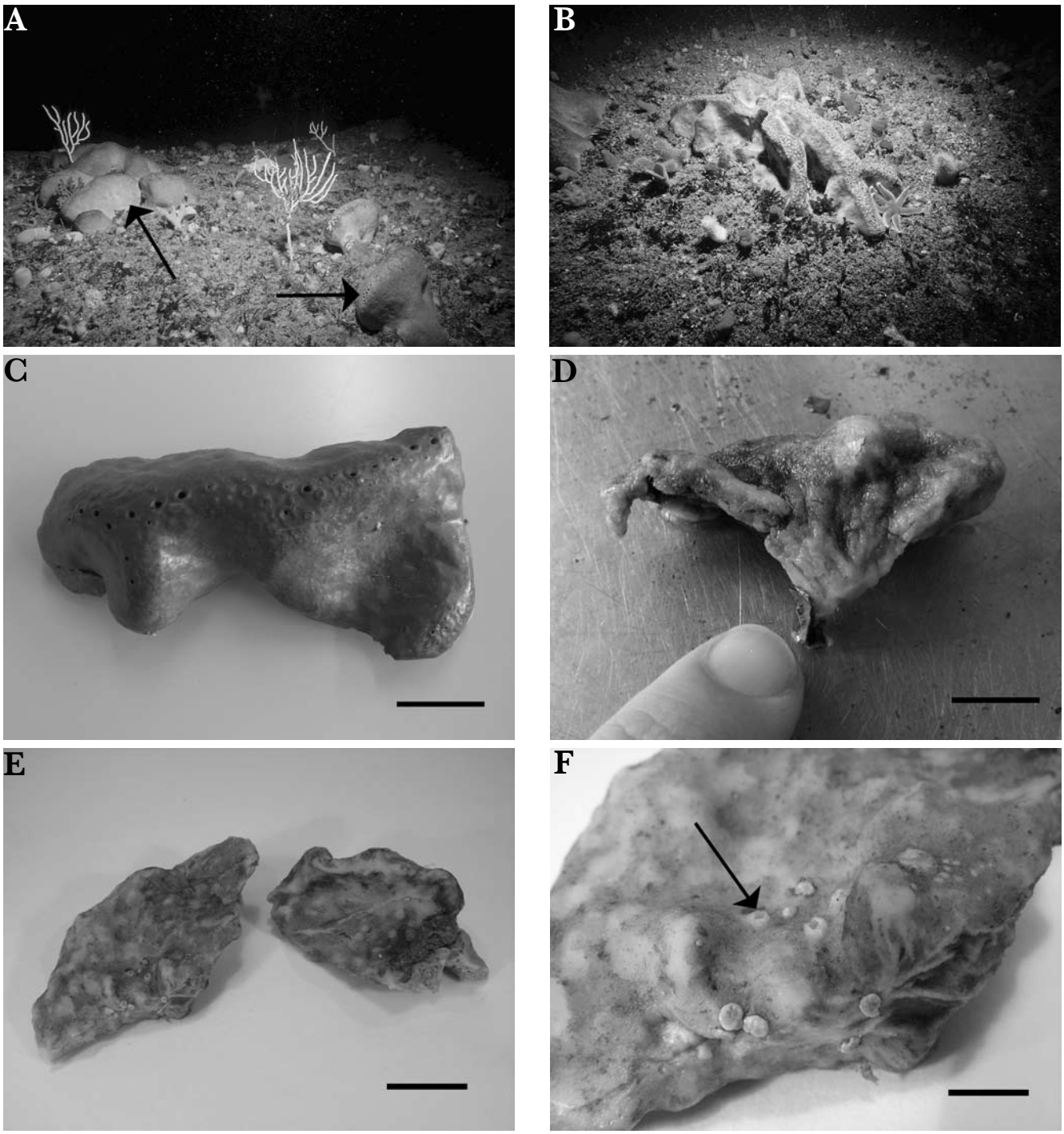

Figure 2. (A) Cushion-shaped *Pachymatisma johnstonia* (arrows), 37 m: west of Bentlevemore, Inishmore, Aran Islands (photograph: B. Picton); (B) interlacing of pillow shapes is characteristic of *P. johnstonia*, sublittoral: north of Brannock Island, Aran Islands (photograph: B. Picton); (C) oscules aligned on the ridges of a *P. johnstonia* collected at 12 m depth, Punta Segaño, Galicia; (D) whitish-greenish young specimen of *Pachymatisma normani* with attaching point (UiB-PC105), Korsfjord; (E) irregularly bumpy flattened shape of *P. normani* (neotype, ZMBN 77858) with few oscules, Korsfjord; (F) closer view of an area defined in E; the arrow points to one of the three white-rimmed oscules present. Parasitic foraminiferans *Hyrrokin sarcophaga* have infested this specimen. Scale bars: C,E, 2 cm; D,F, 1 cm.

'X' or 'Y' shapes (Figure 4C), or even more complicated assemblies, were observed. Large plagiotriaenes are present, with rare dichotriaenes (found only in one specimen).

Microscleres. Large spiny regular oxyasters with 4–7 slender actines are fairly abundant. Three smaller spiny oxyasters with eight actines were found, all with a diameter of 36 μm, and all in the same young specimen. We remain unsure whether these are true oxyasters II or just young oxyasters I. Spiny microrhabds, sometimes centrotylote. Large, slightly flattened sterrasters are present, with all intermediate forms between globular and ellipsoid (Figure 4A). See Table 3 for measurements.

Habitat

Pachymatisma normani lives attached to the steep cliff walls of the fjords (200–400 m) and in Norwegian *Lophelia pertusa*

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Table 2. *Individual spicule dimensions for specimens of* Pachymatisma johnstonia *(in μm) with dimensions of the specimens. Means are in bold; other values are ranges; N=30 unless stated otherwise in parentheses.*

Material	Depth (m)	Height (cm)	Length (cm)	Oscule diameter (mm)	Cortex thickness	Microrhabds (length/width)	Sterrasters (length/width/ thickness)	Oxyasters I (diameter)	Oxyasters II (diameter)	Ortho/Plagiotriaenes (rhabdome: length/ width)	Ortho/ Plagiotriaenes (clads)	Strongyles/Oxeas (length/width)
Plymouth (Sollas, 1888)	–	–	–	–	1	23.6/–	97/83.8/–	63	–	636/16	130	1030/13
Douon Island (Sollas, 1888)	sublittoral	–	–	–	1	31.6/5	90/71/–	60	–	440/19	238	924/13
Puffin Island (Hanitsch, 1890)	infra-sublittoral	largest: 1.5	largest: 10		–	18/3	45–60/60–90/–	48–56	–	405/16	255	570–750/12–24
Roscoff (Topsent, 1894)	inter-subtidal	largest: 8	largest: 15	–	≈1	22–27/–	93–110/72–93/–	34–60	–	550/13	300	up to 1000/20
Belle-Île (Topsent, 1928)	85	5	4		0.42–1.05	16–26/–	140/100/–	45–65	–	700–770/20–26	245–320	800–1200/20–30
Sherkin Island (van Soest et al., 1981)	infra-sublittoral	–	largest: 20	–	–	13–25/4–6	90–130/50–80/–	35–52	–	700/25	–	600–3000/30–45
Torquay, ?Type, (Uriz, 2002)	–			2–3	1	18–32/2.7–4.5	90–120/71–93/–	22–63	–	440–700/13–26	up to 300	600–1100/13–20
Roscoff, MNHN, DCL 4015	inter-subtidal	2.5 (piece)	4 (piece)	n.o.	≤1	10–**20.3**–26/ 2–**3.9**–7	82–**104.7**–119/ 80–**92.1**–107/ 52–**66.1**–76	30–**46.1**–63	22–**25.1**–28 (7)	492–**643.3**–744/ 14–**20.3**–24 (11)	174–**254.7**–328	520–**836.6**–1176/ 10–**17.3**–26
Rathlin Island MC 3366	25	1.5	3 (piece)	n.o.	≤1.5	13–**18.5**–25/ 2–**3.6**–5	91–**102.7**–113/ 70–**82.8**–91/ 57–**62.8**–72	30–**42.3**–57	17–**22.1**–26 (6)	427–**655**–883/ 16–**18**–20 (2)	179–**254.2**–427	575–**938.6**–1272/ 7–**16.6**–23
Mingulay Reef UiB-PC185	168	n.o.	n.o.	n.o.	1	16–**23.7**–43/ 3–**4.9**–10	102–**115.5**–130/ 68–**80.0**–91/ 52–**62.3**–77	41–**50.5**–80	n.f.	570–**849.5**–1104/ 19–**24.5**–29 (8)	197–**363.8**–465	530–**1059.8**–1330/ 4–**18.3**–25

–, not referred; n.f., not found; n.o., not observed.

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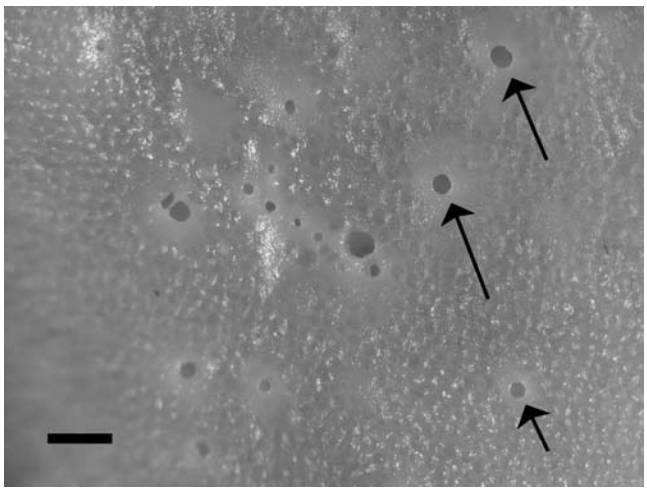

Figure 3. Cribriporal and uniporal pores observed in *Pachymatisma normani* (ZMBN 77858). Lighter surfaces indicate pore areas, rich in microrhabds. Arrows show uniporal pores. Scale bar: 1 mm.

(L., 1758) reefs. In the latter case, it was found living on coral rubble. In the Skagerrak, *P. normani* was found living on a rock and mud bottom (149–137 m). Burton (1931) found two specimens in the Foldafjord at two different stations: 50–100 m and 10–75 m.

Associated fauna

Small ectosymbionts such as sponges (e.g. *Crella* (*Yvesia*) sp.), bivalves (e.g. *Pododesmus squama* Gmelin, 1791), gastropods (e.g. *Iothia fulva* (O.F. Müller, 1776)), brachiopods (e.g. *Terebratulina retusa* Linnaeus, 1758), bryozoans, hydrozoans, polychaetes (e.g. *Spirorbis* sp.), foraminifera (e.g. *Cibicides refulgens* Montfort, 1808; *Hyrrokin sarcophaga* Cedhagen, 1994) were commonly found.

Distribution

(Figure 1.) To date, *P. normani* has been unambiguously identified only in Norway. According to Koltun (1966), it can be found in the south-west of the Barents Sea. *Pachymatisma normani* has also been observed off north-west Iceland (H.T. Rapp, unpublished data).

Remarks

Since the material was collected by dredging, much of our material is fragmented and we have only three complete specimens. With respect to its unusual stalked shape, greenish colour, very thick cortex (2 mm) and dichotriaenes, one cannot help to think that the specimen UiB-PC105 (Figure 2D) is significantly different from the rest of the material. It is tentatively named *P. normani* until further investigations.

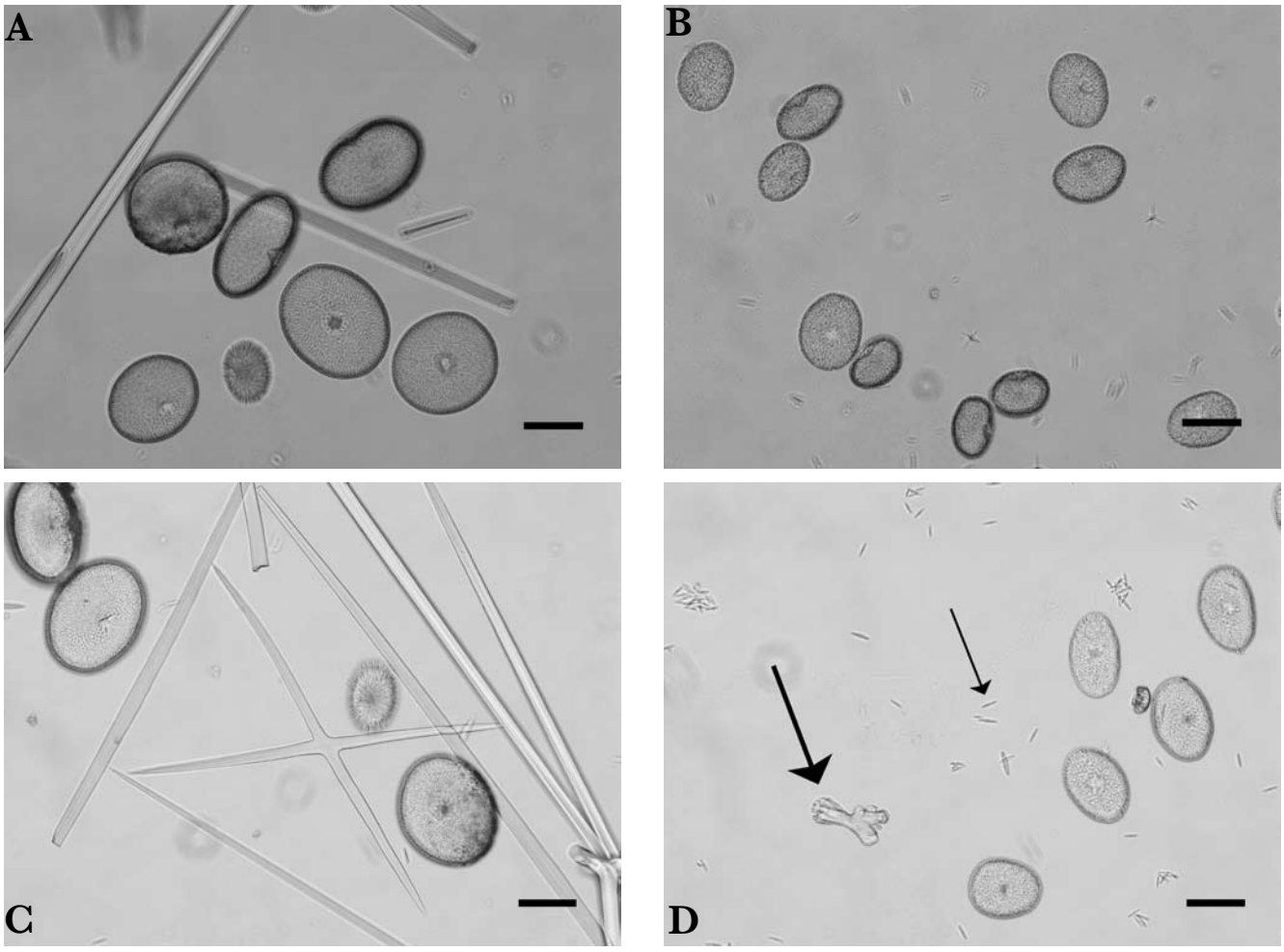

Figure 4. (A) Round and ellipsoid flattened sterrasters in *Pachymatisma normani* (ZMBN 77858); (B) round and ellipsoid flattened sterrasters in *Pachymatisma johnstonia* (MC3366); (C) fused oxeas in *P. normani* resulting in an 'X' shape (ZMBN 77858); (D) normal (small arrow) and gigantic (long arrow) microrhabds in deep *P. johnstonia* specimen from Mingulay Reef. Scale bars: 100 μm.

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Table 3. *Individual spicule dimensions for specimens of* Pachymatisma normani *(in μm) with dimensions of the specimens. Means are in bold; other values are ranges; N=30 unless otherwise stated in parentheses.*

Material	Depth (m)	Height (cm)	Length (cm)	Oscule diameter (mm)	Cortex thickness (mm)	Microrhabds (length/width)	Sterrasters (length/width/ thickness)	Oxyasters I (diameter)	Ortho/Plagiotriaenes (rhabdome: length/ width)	Plagiotriaenes (clads)	Strongyles/Oxeas (length/width)
Type, Korsfjord (Sollas, 1888)	329	–	–	–	–	19–22/–	200/160	80	830/26	446	1680/27.6
Korsfjord UiB-PC6	200–400	1–2	8 (piece)	2	≤1	17–**27.5**–34/3–**4.7**–7	154–**174.9**–190/ 132–**149**–161/ 80–**97.8**–117	49–**59.1**–78	790–**833.2**–1128/ 34–**38.7**–41 (4)	291–**473.7**–650 (26)	640–**1337.2**–1920/ 17–**24.5**–36
Korsfjord UiB-PC105	200–400	3	5	n.f.	≤2	12–**18.5**–26/3–**4.2**–5	162–**195.3**–228/ 137–**152**–182 91–**105.6**–129	36–**56.4**–73	586–**763.2**–930/ 27–**32.8**–38 (9)	325–**453.6**–576 (29)	418–**1241.2**–1992/ 13–**25.3**–36
Skagerrak UiB-PC145	149–137	1–1.5	8	2	≤1	14–**22.5**–32/2–**4.8**–6	128–**158.6**–180/ 118–**129.7**–139/ 77–**94.4**–107	36–**58.1**–73	492–**839.1**–1032/ 11–**24.0**–38	219–**463.8**–558	409–**1200.5**–1776/ 11–**19.7**–29
Neotype, Korsfjord ZMBN 77858	200–400	2.5–3	9+7 (2 pieces)	2	≤1	10–**20**–24/3–**4.1**–5	145–**168.4**–207/ 130–**140.7**–157/ 91–**106.4**–120	41–**67.2**–122	576–**729.7**–837/ 23–**25.2**–28 (4)	339–**433.7**–520 (22)	533–**1240.9**–1968/ 15–**24.3**–39

–, not referred; n.f., not found.

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Table 4. *Distribution of polymorphic sites in the ITS1-5.8S-ITS2 DNA sequences for five specimens of* Pachymatisma normani *and seven specimens of* P. johnstonia.

Species	Genbank accession number	Locality	Positions of mutations					
			153	615	672	711	749	755
P. normani	EF577048	Korsfjord	T	C	C	C	T	G
	EF577049		.	.	.	.	.	.
	EF577050		.	.	.	.	.	.
	EF577051		.	.	.	.	.	.
	EF647867	Skagerrak	.	.	.	.	.	.
P. johnstonia	EF577052	Rathlin Island	A	.	T	.	C	.
	EF577053		.	.	T	T	.	T
	EF577054		.	T	T	.	.	T
	EF577055		.	T	T	.	.	T
	EF577056		.	T	T	.	.	T
	EF647868	Roscoff	.	T	T	Y	.	T
	AF062601		?	.	T	T	.	T

?, missing data; Y, T/C.

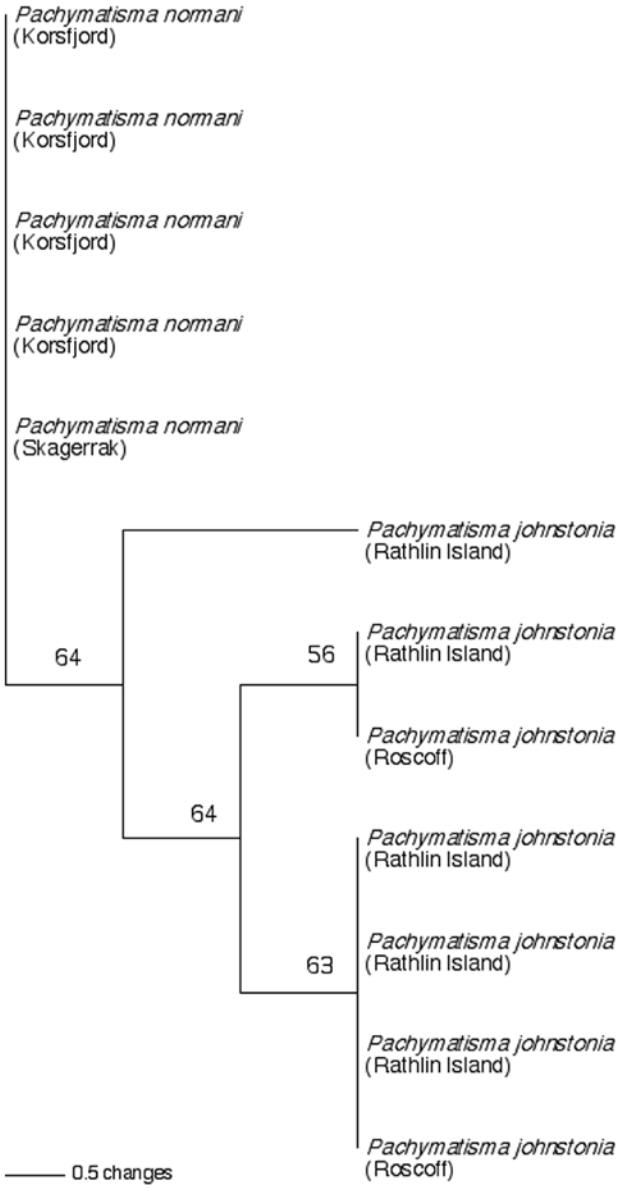

Figure 5. Unrooted maximum parsimony tree of 12 *Pachymatisma* ITS1-5.8-ITS2 sequences. A single tree was found (length=6 steps, CI=1.00). Bootstrap values are indicated above the branches.

Molecular results

Twenty COI sequences were included in a 658 character alignment. Pair-wise comparisons revealed one single polymorphic position between the two species. The observed change is a transversion (T-G) at the third position of a codon (nucleotide 489 in our alignment). This results in a synonymous substitution since both GGT and GGG code for glycine. ITS1-5.8S-ITS2 sequence amplifications usually gave multiple bands, one bright band and 1–2 other faint longer ones. The shortest and brightest band whose size corresponded to previous sponge ITS1-5.8S-ITS2 sequences was gel extracted. Twelve ITS1-5.8S-ITS2 sequences were included in an 836 bp alignment. ITS boundaries were determined by comparison with *P. johnstonia* (AF062601) and other poriferan sequences in Genbank. ITS1, 5.8S and ITS2 in *P. johnstonia* and *P. normani* are respectively 301, 161 and 240 bp long. Only six polymorphic sites were detected, all substitutions: 1 in ITS1 and 5 in ITS2 (Table 4). No mutations were found in 5.8S. All *P. normani* sequences were strictly identical whereas *P. johnstonia* sequences were polymorphic. Chromatograms indicated that intragenomic variation may exist, although these could be sequencing artefacts due to secondary structure (Wörheide et al., 2004). An MP analysis gave an unrooted unique tree of 6 steps (CI=1.00) with four parsimony informative characters (Figure 5). *Pachymatisma normani* and *P. johnstonia* form two distinct clades.

DISCUSSION

External morphology

Outer shapes of *Pachymatisma normani* and *P. johnstonia* are both massive but are clearly different. Adult *P. johnstonia* have a more regular, often elongated, hemispherical shape with bloated ramifications (Figure 2A&C). *Pachymatisma normani* is either more compressed with a 'dirty' and irregular appearance or clearly cone shaped. Although radially arranged massive sponges are considered to have a fairly conservative morphology (Burton, 1928) the two morphotypes of *P. normani* could be environmentally induced. Furthermore, it should also be emphasized that the

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unusual stalked shape of the *P. normani* specimen UiB-PC105 has never been observed in *P. johnstonia*. Since the Norman Collection was made of dry and alcohol preserved specimens (Sollas, 1880), we can suspect that Sollas never saw a fresh specimen. Unfortunately, no drawing or description of the morphology of the type exist so we do not know if he had a complete specimen or just a broken part. Topsent, to our knowledge, never saw a *P. normani* sample. He could only rely on spicule measurements and cortex comparisons to put *P. normani* in synonymy. However, the overall morphology of sponges cannot be neglected, and sibling species are often easier to distinguish when alive (Knowlton, 1993). We therefore stress the fact, as others did before us (e.g. Burton, 1932), that although outer shape of sponges may be subject to phenotypic plasticity, it can still serve as a taxonomic character of importance. In addition to the overall shape, oscule distribution is different. Oscules in *P. johnstonia* are often lined up along the edges of oblong ridges, sometimes in high numbers (Figure 2B,C). This aligned pattern of oscules has never been observed in *P. normani*. The fact that *P. normani* has a lower density of oscules could be an adaptation to a low-wave force environment as observed in *Halichondria* cf. *panicea* Pallas, 1766 (Palumbi, 1986). Concerning the colour, external colour of *P. johnstonia* can be grey to purple when exposed to light, otherwise whitish. Live *P. normani* specimens are never truly white like some of our *P. johnstonia*. According to Bowerbank (1864, p. 51) deeper living specimens of *P. johnstonia* can become pink-reddish but following authors have never confirmed this. He might, like Hansen (1885), have confused *P. johnstonia* with *Isops phlegraei* Sollas, 1880, another Geodiidae, for which we did observe reddish deep-water specimens (H.T. Rapp & P. Cárdenas, unpublished data). The issue of the ectosymbionts calls for further studies insofar as we have had the impression that *P. normani* had a richer and relatively denser ectosymbiont fauna than *P. johnstonia*. Ectosymbionts (*Spirorbis* sp., hydrozoans, bryozoans) were seldom found on *P. johnstonia*, its surface being otherwise fairly smooth. Hispid areas made of oxeas, which have only been observed in *P. normani*, might provide a good substrate for invertebrate larvae. As for the cortex thickness, the single external pretext put forward by Sollas (1888) to define *P. normani*, our results show that it is not a relevant character. Variation in thickness not only exists between species but also within specimens. However, *P. normani* tends to have a thicker cortex, probably due to its larger sterrasters (Topsent, 1894).

Spiculation

Strongyloxeas

Strongyles are abundant in *P. johnstonia* specimens while oxeas are far more numerous in *P. normani* specimens. *Pachymatisma normani* had also numerous fused oxeas (Figure 4C) which were already described by Sollas (1882). These fused oxeas were not observed in *P. johnstonia*.

Triaenes

The triaenes of *P. johnstonia* have been described as orthotriaenes (Sollas, 1888; Hanitsch, 1890; Topsent, 1894; van Soest et al., 1981; Uriz, 2002) because clads are often bent and can become horizontal after the bend. Our observations suggest that all intermediate forms exist between orthotriaenes and plagiotriaenes in *P. johnstonia*. Meanwhile, in *P. normani*, we have only observed plagiotriaenes. Deformed triaenes in both species are fairly common and can sometimes have bifurcated/polyfurcated rhabdomes or clads. Nevertheless, true dichotriaenes were only observed in *P. normani* (UiB-PC105) and never in *P. johnstonia*.

Oxyasters I and II

Sponge morphologists have always observed a single category of oxyasters in *P. johnstonia*, across a wide range: 22 to 63 μm (Table 2). The only exception is Chombard (1998, p. 71) who noticed a second category of oxyasters. There are large spiny and irregular to regular oxyasters with 4–7 slender actines, and smaller spiny regular oxyasters with 8–12 actines each measuring around 23 μm. The difference between young oxyasters I and oxyasters II is sometimes difficult to perceive and we have mostly relied on the number of actines to decide on the nature of the oxyaster. The oxyasters II were consistently observed in *P. johnstonia* samples, albeit in low numbers (2–3/slide were found). They were not found in the cortex as Chombard (1998) states, but in the choanosome. In *P. normani* three putative oxyasters II were found, all in the same young specimen (UiB-PC105). It should be noted that they could be in fact young oxyasters I since they had only eight actines. Further investigation is needed to confirm the absence of oxyasters II in *P. normani*. We did not find any oxyasters II in the deep *P. johnstonia* sample, and raise the question whether the presence of oxyasters II could be controlled by depth and environment.

Spicule measurements

Our measurements corroborate most previous measurements for *P. johnstonia* and *P. normani* (Tables 2 & 3). Between the two species, the microrhabds, oxyasters and megasclere measurements show some partial size overlap, but are on average larger in *P. normani*. Indeed, the mean measurements of oxyasters, strongyloxeas and ortho/plagiotriaene cladomes were consistently higher in *P. normani*. Concerning the length of the sterrasters, the overlap in size between the two species is very narrow. The length of the sterrasters varied between 82 and 140 μm (overall average of 107.6 μm) in *P. johnstonia*, while it varied from 128 to 228 μm (overall average of 174.3 μm) in *P. normani*. There is no size overlap concerning the width and thickness of the sterrasters. The sterrasters of *P. normani* are always wider and thicker than the ones of *P. johnstonia* (Figure 4A,B). Within *P. johnstonia* and *P. normani*, the deeper specimens clearly had a more robust spiculation. Therefore, the larger spicules of *P. normani* are probably largely due to its deep-water habitat. Larger spicules is also what prompted Topsent (1928) to doubt the validity of *P. normani* when he examined the deep *P. johnstonia* specimen from Belle Îsle. But apart from the longer sterrasters (140 μm), the rest of the spicule measurements for this specimen match the ones commonly reported for *P. johnstonia*. It should be noted that extremely large and deformed microrhabds (up to 150 μm in length instead of 20 μm) were observed in the deep *P. johnstonia* specimen

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from Mingulay Reef (Figure 4D). These have never been observed before and could be induced by depth.

Thickness of sterrasters

This is the first study to measure the thickness of *Pachymatisma* sterrasters: our results show that sterrasters are flattened in both species (Figure 4A,B). Flattened sterrasters had previously been noticed by Bowerbank (1864) but later authors have seldom emphasized this character. This observation gains a significant meaning when one considers the sister-group relationship of *Pachymatisma* and *Erylus* obtained with a 28S molecular phylogenetic study (Chombard et al., 1998). *Erylus* is known to have flattened sterrasters called aspidasters while the rest of the Geodiidae are considered to have globular sterrasters. Some *Erylus* (e.g. *Erylus topsenti* Lendenfeld, 1903, *Erylus polyaster* Lendenfeld, 1907, *Erylus geodioides* Burton & Srinivasa Rao, 1932 and *Erylus fibrillosus* Lévi & Lévi, 1983) even have sterraster-like aspidasters, which resemble the *Pachymatisma* sterrasters. Still, one important difference classically sets these two genera appart: *Erylus* has uniporal pores while *Pachymatisma* has cribriporal pores. But our observations also imply that the limit between uniporal and cribriporal pores is not obvious. Both types of pores were observed in *P. normani* (Figure 3). It seemed to us as if when the pores would increase their surface area, they would go from a uniporal stage to a cribriporal stage by additional piercing of the thin ectocortex. Thus, the uniporal/cribriporal character might not be consistent in *Pachymatisma*. In the Geodiidae, doubts have been previously raised on the value of pore and oscule characters, with respect to the *Geodia-Sidonops-Isops* complex (Laubenfels, 1936; Koltun, 1966; van Soest & Stentoft, 1988; Hajdu et al., 1992; Silva, 2002). Our observations confirm the weakness of these characters to define Geodiidae genera and challenge the monophyly of *Erylus* with respect to *Pachymatisma*.

Molecular support

The single polymorphic site found between the COI sequences of *P. johnstonia* and *P. normani* is perfectly coherent with previous molecular results on sponges and supports the morphological differences observed. Among the Porifera, one cannot give a fixed level of genetic divergence to distinguish taxonomic species due to a great variability of rates of evolution between groups (Solé-Cava & Boury-Esnault, 1999). Heim et al. (2006) have found one base pair difference between *Aplysina aerophoba* and *Aplysina cavernicola* (both from the Mediterranean Sea) but 73 base pair difference between *Suberites massa* and *Suberites domuncula* (Mediterranean Sea). One can also find intraspecific COI variation (Duran et al., 2004b; Duran & Rützler, 2006) or no variation at all between species (Schröder et al., 2003; Addis & Peterson, 2005; Heim et al., 2006). It should also be noted that these two species, unlike some other sponges, would be perfectly barcoded with the help of the COI Folmer fragment (Erpenbeck et al., 2006). There are currently no data on mutation rates in Porifera but strong similarities between the rate of evolution of coral mtDNA and that of sponges is acknowledged (Shearer et al., 2002; Lavrov et al., 2005; Wörheide, 2006). Hellberg (2006) estimated the synonymous substitution rates of coral COI to be 0.00055 substitutions/site/10^6 years, similar to that found in most plants. If we extrapolate that rate to our *Pachymatisma* species, the single synonymous substitution observed in COI would mean that the two species diverged 2.7 MY ago.

Our ITS1-5.8S-ITS2 phylogenetic tree (Figure 5) corroborates the COI results. Site number 672 separates the sequences in two monophyletic clades corresponding to the two *Pachymatisma* species. ITS sequences are identical for *P. normani* and slightly polymorphic for *P. johnstonia*. The absence of ITS diversity for *P. normani* coupled with an overlap between intra- and inter-species variations might be an indication of a recent bottleneck speciation effect. Our preliminary investigation also suggests that ITS sequences would be good nuclear markers for future population genetic studies of *P. johnstonia*, provided that one checks for intragenomic variation (Wörheide et al., 2004).

CONCLUSION

This study shows that despite similar spicule types and spicule size overlaps, there are enough differences to regard *Pachymatisma normani* and *P. johnstonia* as distinct species: (i) the outer-morphologies are distinct, and this is especially obvious in adult specimens; (ii) compared to *P. johnstonia*, *P. normani* has larger spicules in general, especially larger sterrasters, a majority of oxeas, an absence of oxyasters II and no orthotriaenes; (iii) they have different depth distributions; (iv) clearly separated geographical ranges; and (v) we found consistent genetic differences in two independent sequences: COI (mitochondrial), ITS1-5.8-ITS2 (nuclear). Since the holotype is supposedly lost (Clare Valentine, personal communication) a specimen of *Pachymatisma normani* from the type locality has been designated as neotype and deposited in the collections of the Museum of Bergen under the number ZMBN 77858 (Figure 2E,F; M9622, M9624, M9625, M9626; EF564322 (COI); EF577051 (ITS1-5.8S-ITS2)). According to known distribution (Figure 1) and habitat preferences, *P. johnstonia* and *P. normani* are allopatric species. The geographical border between the two species may be the Norwegian Channel, separating the Shetlands from western Norway.

The genus Pachymatisma

Species described under the genus name of *Pachymatisma* (modified and updated from Vosmaer, 1933):

- ***P. johnstonia*** (Bowerbank in Johnston, 1842)
- *P. listeri* Bowerbank, 1858 from Madeira is insufficiently known. According to the short description (Bowerbank, 1858, p. 290; 1862, p. 815), it has aspidasters and dichotriaenes. Like Boury-Esnault & Lopes (1985), we are pretty confident that it is an *Erylus*.
- ***P. areolata*** Bowerbank, 1872 from the Red Sea. Collected and redescribed by Burton (1926) and Lévi (1967) in South Africa.
- *P. contorta* Bowerbank, 1873 from the Fiji Islands. Sollas (1888, p. 271) did not observe any microrhabds in the type-slide. Since it was described as having uniporal pores by Bowerbank, Sollas concluded it was an *Isops*.

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- *P. inconspicua* Bowerbank, 1873 from the South Pacific. Since Bowerbank did not describe any microrhabds, Sollas (1888, p. 260) moved this species to the genus *Cydonium*, synonym of *Geodia*. Vosmaer (1933, p. 117) affirms it is *Geodia cydonium*, but we think it is highly improbable when one considers that *G. cydonium* is a north-east Atlantic–Mediterranean species.
- ***P. normani*** Sollas, 1888 from Korsfjord, Norway. Synonymized by Topsent (1894, 1928) with *P. johnstonia*. Resurrected in this study.
- *P. apiarium* (Schmidt, 1870) as *Caminus apiarium* from Florida. Sollas (1888, p. 268) had tentatively moved this species to the genus *Isops* after re-examining a type-slide and not finding any spherules or microrhabds. Lendenfeld (1903, p. 92), without giving any reason, decided to move the species to *Pachymatisma*. We think it is better to keep it in the *Isops* genus until further observations.
- *P. intermedia* (Schmidt, 1868) as *Stelletta intermedia* from Algeria. It has been tentatively moved to the genus *Erylus* first (Sollas, 1888, p. 241) then *Pachymatisma* (Lendenfeld, 1903, p. 90). But after redescribing the type, Topsent (1938) synonymyzed this species with *Geodia conchilega*.
- *P. monaena* Lendenfeld, 1907 from South Africa. It is a synonym of *P. areolata* according to Burton (1926) and Lévi (1967).
- ***P. bifida*** Burton, 1959 from the Maldives.
- *P. geodiformis* van Soest & Stentoft, 1988 from Barbados. After re-examination of the holotype (ZMA-POR 5269) we confirm, as stated by van Soest & Stentoft (1988), that this species has uniporal pores (see Morphobank pictures: M9721, M9722). It should therefore be moved to the genus *Erylus* as suggested by Adams & Hooper (2001). We also noted that it had slightly flattened sterrasters (M9723). After *Erylus topsenti* Lendenfeld, 1903, *Erylus polyaster* Lendenfeld, 1907, *Erylus geodioides* Burton & Srinivasa Rao, 1932 and *Erylus fibrillosus* Lévi & Lévi, 1983, this is the fifth *Erylus* species to have sterraster-like microscleres.

We propose the following key for the four species of *Pachymatisma* here considered as valid.

Key for the species of Pachymatisma

1. Presence of strongylasters.....*P. areolata*
— Absence of strongylasters.....2

2. Mean length of microrhabds >50 μm and mostly dichotriaenes..... *P. bifida*
— Mean length of microrhabds <50 μm and very few dichotriaenes..... 3

3. Mean length/width/thickness of sterrasters <140/110/80 μm and mostly strongyles..... *P. johnstonia*
— Mean length/width/thickness of sterrasters >140/110/80 μm and mostly oxeas..... *P. normani*

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