

The zoogeography of extant rhabdopleurid hemichordates (Pterobranchia: Graptolithina), with a new species from the Mediterranean Sea

Running title: A new species of rhabdopleurid pterobranch

Elena Beli^{A,B,G}, Giorgio Aglieri^A, Francesca Strano^A, Davide Maggioni^{C,D}, Max J. Telford^E, Stefano Piraino^{A,F,G,§}, and Christopher B. Cameron^{B,G,§}

^ADipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Provinciale Lecce - Monteroni 73100 Lecce, Italy.

^BDépartement de sciences biologiques, Université de Montréal C.P. 6128, Succursale Centre-ville, Montréal, Quebec, H3C 3J7, Canada.

^CDepartment of Biotechnology and Biosciences, University of Milano-Bicocca Piazza della Scienza, 2 - 20126, Milan, Italy.

^DMaRHE Center, University of Milano-Bicocca, Magoodhoo Island, Faafu Atoll, Republic of Maldives.

^EDepartment Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK.

^FCONISMA, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, 00196 Roma, Italy.

[§] Senior Authors

^GCorresponding authors. Email: elena.beli.sbarra@gmail.com; stefano.piraino@unisalento.it; c.cameron@umontreal.ca

Abstract. The early origin and evolutionary radiation of graptolites (Hemichordata: Pterobranchia) is a story told almost entirely in the fossil record, but for four extant species of the genus *Rhabdopleura*. Here we report the discovery of a fifth species, *Rhabdopleura recondita* sp. nov., at a depth range of -2 to -70 m from the Adriatic and Ionian Seas, always associated with bryozoans in coralligenous habitats. This is the first pterobranch record in Italian waters, and the second in the Mediterranean Sea. The new species is characterized by a) tubaria with smooth creeping tubes adherent to the inside of empty bryozoan zooecia; b) erect outer tubes with a graptolite, fusellar-like organization; and c) zooids that extend from a black stolon, which is free from the creeping tube. Each of the paired feeding arms has two rows of tentacles that do not extend to the arm tip. The distal ends of the arms, the collar, and the cephalic shield are replete with black granules. Phylogenetic analyses of individual and concatenated gene sequences of mitochondrial 16S rDNA and nuclear 18S rDNA support the validity of *R. recondita* as a new species. Finally, we discuss the global biogeographic and habitat distributions of the extant *Rhabdopleura* representatives.

Additional keywords: Rhabdopleuridae, colonial hemichordates, morphology, DNA barcoding, integrative taxonomy.

Introduction

Until recently, Graptolithina (Hemichordata: Pterobranchia) was considered an extinct taxon (Maletz 2015), but a cladistic analysis from Mitchell *et al.* (2013) reorganized the class Pterobranchia so that it now includes a clade for extant *Rhabdopleura* spp within the subclass Graptolithina and a clade for extant *Cephalodiscus*. Rhabdopleurids are a small basal clade of graptolites with a fossil record extending back 536 Myr (Mitchell *et al.* 2013). Graptolites are mostly known from the planktonic forms that suddenly appear in the Early Ordovician, followed by a tremendous radiation, before going extinct in the Lower Devonian. Except for a few poorly preserved specimens, the zooids are entirely unknown. Instead the fossils are known from the fibrous tubaria. Following the Lower Devonian extinction of planktonic forms, only a few specimens of benthic rhabdopleurid tubaria have been found in Permian, Jurassic, and Eocene fossils (e.g. Rickards *et al.* 1984; Mierzejewski 1986; Maletz 2015). The origin of the genus *Rhabdopleura* dates back to the Middle Ordovician (Kozłowski 1961; Maletz 2014 and references therein). *Rhabdopleura* has shown little change in that time, and the four extant *Rhabdopleura* species (*R. annulata* Norman, 1921, *R. compacta* Hincks, 1880, *R. normani* Allman, 1869, *R. striata* Schepotieff, 1909) are thus regarded as living fossils (Durman and Sennikov 1993). These species are known to occur pan-globally, especially from deep waters in polar regions. Here we describe a new species, *Rhabdopleura recondita* sp. nov., collected from shallow-water in the Mediterranean Sea.

In the framework of a faunistic survey of the coralligenous habitats along the SE Adriatic Sea (Strait of Otranto), colonies of a rhabdopleurid pterobranch were first discovered at 20 m depth in March 2009 by the Marine Biology and Zoology Laboratory at the University of Salento, Lecce (see De Vito *et al.* 2010). It was provisionally assigned to *R. compacta* based on similarities of the tubes and zooids. This was the second finding of pterobranch colonies from the Mediterranean Sea, following Laubier's (1964) record of *Rhabdopleura normani* near Cape Abeille (Banyuls, France). Thanks to the year round availability and unexpected abundance of the pterobranch colonies in the Strait of Otranto and neighbouring seas at shallow depths, we were able to describe herein the Italian rhabdopleurid colonies

by integrative morphological and molecular analyses and clarify their taxonomic status as a new species. Based on their morphology, behavioural and distributional data, we assume all rhabdopleurid specimens collected so far along the French and Italian coasts most likely belong to a single pan-Mediterranean species.

Diagnosis of class **PTEROBRANCHIA** Lankester, 1877: 488

Benthic and fixed, or planktonic zooids that are colonial or pseudocolonial. Zooids have a tripartite body plan including a pre-oral cephalic shield (protosoma), a collar (mesosoma) with two or more arms each bearing a double row of ciliated tentacles, and a trunk (metasoma) that houses gonads and a U-shaped gut that has a ventro-posterior positioned, elongated stalk that connects to a mobile germinal disk (pseudocolonial *Cephalodiscus*) or stolon (colonial graptolites) that connects the zooids; external proteinaceous tubarium composed of tubes. *Cambrian*, Series 3, Stage 5 (?*Acadoparadoxides oelandicus* or *Oryctocephalus indicus* or *Yuknessia simplex*) to *Recent*: worldwide in marine environments.

Diagnosis of family **RHABDOPLEURIDAE** Harmer, 1905: 5

Diagnosis (emended). (Maletz 2014: 486) – Colonial pterobranchs with encrusting tubular constructions with irregular fusellar rings or regular zigzag sutures in creeping and erect tubes; resorption porus for the origination of new tubes; erect thecal tubes parallel sided or slowly widening, with unornamented apertures; zooids connected through robust stolon system (black stolon); sicular zooid secretes featureless domal prosicula.

Genera included (modified from Maletz 2014: 486) – ?*Archaeocryptolaria* Chapman, 1919; *Calyxhydra* Kozłowski, 1959; *Chitinodendron* Eisenack, 1938; *Cylindrohydra* Kozłowski, 1959; *Diplohydra* Kozłowski, 1948; *Eorhabdopleura* Kozłowski, 1970; *Epigraptus* Eisenack, 1941; *Fasciculitubus* Obut & Sobolevskaya, 1967; *Graptovermis* Kozłowski, 1948; *Haliolophus* Sars, 1868; ?*Haplograptus* Ruedemann, 1933; *Idiotubus* Kozłowski, 1948; *Kystodendron* Kozłowski, 1959; *Lagenohydra* Kozłowski, 1959; ?*Malongitubus* Hu, 2005; *Palaeokylix* Eisenack, 1932; *Palaeotuba* Eisenack, 1934; *Rhabdopleura* Allman,

1869; *Rhabdopleurites* Kozłowski, 1967; *Rhabdopleuroides* Kozłowski, 1961; *Rhabdotubus* Bengtson & Urbanek, 1986; ?*Sphenoecium* Chapman & Thomas, 1936; *Sphenothallus* Chapman, 1917 (non *Sphenothallus* Hall, 1847: uncertain tubular fossil, see Fatka *et al.* 2012); *Stolonodendrum* Kozłowski, 1948; *Xenotheca* Eisenack, 1938; ?*Yuknessia* Walcott, 1919.

Diagnosis of genus ***Rhabdopleura*** Allman, 1869

Rhabdopleura is the only graptolithinid genus where the zooidal anatomy and zooidal development is known. The zooid body of *Rhabdopleura* is tripartite like those of *Cephalodiscus* and *Atubaria* but possesses only a single pair of arms. In most cases zooids have a life-long connection to the stolon system. Additional minor anatomical differences exist, mostly associated with a reduced body size (Cameron 2005). *Rhabdopleura* females produce only a single egg and gill pores are absent. Sixteen fossil species are described (Chapman *et al.* 1995) and four extant species are currently recognized (Horst 1939): *Rhabdopleura annulata* Norman, 1921; *Rhabdopleura compacta* Hincks, 1880; *Rhabdopleura normani* Allman, 1869; and *Rhabdopleura striata* Schepotieff, 1909. Here we add a new, previously undescribed species, *Rhabdopleura recondita* sp. nov. Beli, Cameron and Piraino.

Rhabdopleura recondita sp. nov. Beli, Cameron and Piraino (Figs 1A–I, 2A–C)

Etymology

The specific name means ‘hidden’, ‘concealed’, because colonies inhabit the interstices of vacant cheilostome bryozoan zooecium, from which the erect pterobranch tubes project.

Type specimens

(Following NMNH – Smithsonian - policy, accession numbers will be provided by Karen Osborn, Curator of Invertebrates, once the manuscript is accepted)

Holotype and paratype specimens are catalogued in the National Museum of Natural History (Smithsonian Institution), Washington D.C.

Holotype. Italy, Otranto (LE), -70 m, 13.v.2016, *R. recondita* colony is in a tube of 4% formalin within two fragments of *Myriapora truncata* (Pallas, 1766) zooecium of about 1 cm and 1.5 cm, encrusted with serpulid tubes and barnacles. The *R. recondita* colony is complete with stolon, zooids and erect tubes. Accession number: XXXX

Paratypes:

1. Italy, Otranto (LE), -18 m, 23.iii.2016, *R. recondita* colony hosted in *M. truncata*, fixed in 96% ethanol. Accession number: XXXX
2. Italy, Otranto (LE), -18 m, 13.iii.2016, *R. recondita* colony hosted in *Schizoretepora serratimargo* (Hincks, 1886), fixed in 4% formalin. Accession number: XXXX
3. Italy, Otranto (LE), -18 m, 13.v.2016, *R. recondita* colony hosted in *S. serratimargo*, fixed in 96% ethanol. Accession number: XXXX
4. Italy, Otranto (LE), -18 m, 10.v.2016, *R. recondita* naked zooid, fixed in 4% formalin. Accession number: XXXX
5. Italy, Otranto (LE), -18 m, 23.iii.2016, *R. recondita* naked zooid, fixed in 96% ethanol. Accession number: XXXX

Material examined

Colonies of *Rhabdopleura recondita* were collected by SCUBA diving or by gill nets from 2013 to 2016 at 2-70 m range depth at different subtidal pre-coralligenous and coralligenous habitats around the Salento Peninsula, SE Italy (Table 1).

Rhabdopleura recondita colonies were most commonly found in the interstices of the vacant calcareous zooecium of the cheilostome erect bryozoans *Myriapora truncata*, *Schizoretepora serratimargo*, and more rarely in the encrusting bryozoans *Celleporina caminata* (Waters, 1879) and *Reptadeonella violacea* (Johnston, 1847) (Table 2).

Bryozoan zooecium debris were collected from the sea floor, or less commonly attached to vertical walls. The bryozoan community and more generally, the biogenic coralligenous community at Otranto is diverse and abundant (e.g. molluscs, serpulids, madreporarians). Sympatric taxa that were found on the zooecium debris with *R. recondita* included coralline algae, sponges, hydrozoans, polychaetes, nudibranchs, crustaceans and ciliates.

The zooecium fragments with the pterobranch colonies were placed in plastic containers, transferred to the laboratory at the University of Salento, Lecce, and placed into an aquarium at 18 °C, the seawater temperature recorded at the time of collection. The specimens were then observed by the means of a stereomicroscope and pictures obtained with a Sony digital camera.

Pterobranch zooids, or partial zooids were fixed either in RNAlater, frozen with dry ice and stored at -80 °C, or in 99% ethanol for transcriptome and DNA sequencing, respectively. DNA from ethanol-preserved zooids was extracted following the protocol of Zietara *et al.* (2000), or using DNeasy Tissue extraction kits (Qiagen, Valencia, CA). Two different molecular markers were amplified: (i) an approximately 600-bp portion of 18S from nuclear rRNA and (ii) an approximately 600-bp portion of the mitochondrial 16S rRNA. The PCR amplification were set up using the same protocols and primers described in Halanych (1995) and Palumbi (1996) and all products were purified and directly sequenced in forward and reverse directions using an ABI 3730xl DNA Analyser (Applied Biosystem, Foster City, CA, USA). The sequences obtained in this study were deposited in GenBank with the accession numbers: LT714188- LT714195, KU873083, KU873084. Sequences were aligned with other sequences of *Rhabdopleura* species and outgroups retrieved from GenBank (Table 3), using MAFFT 7.110 (Kato and Standley 2013) with the E-INS-i option and the obtained alignments were run through Gblocks (Castresana 2000; Talavera and Castresana 2007) to remove ambiguously aligned regions using the default 'less stringent' settings. The sequences were combined in a concatenated dataset and jModeltest 2.1.4 (Darriba *et al.* 2012) was used to determine the appropriate molecular models. Bayesian inference (BI) and maximum likelihood (ML) were used to infer phylogenetic relationships for both single-locus and multi-locus datasets. BI analyses were performed using MrBayes 3.2 (Ronquist *et al.*

2012). Four parallel Markov Chain Monte Carlo runs (MCMC) were run for 3×10^6 generations. Trees were sampled every 100th generation and burn-in was set to 25%, based on checking the parameter estimates and convergence using Tracer 1.6 (Rambaut *et al.* 2014). ML trees were built with Garli 2.01 (Zwickl 2006). Non-parametric bootstrap values were calculated from 1000 replicates, each based on five heuristic search replicates; the resulting trees were read into the SumTrees 4.0.0 program in the DendroPy 4.0.0 package (Sukumaran and Holder 2010) to obtain bootstrap support values and to map them on the best ML tree. The genetic distances (uncorrected p-distance, 1000 bootstraps) within and among rhabdopleurids clades were also estimated for each locus using MEGA 6 (Tamura *et al.* 2013).

External zooid morphology

Colonies of *Rhabdopleura recondita* grew within the empty zooecium of cheilostome bryozoans, with pterobranch zooids and stolons occupying the available vacant interstices (Fig. 1C–I). The zooids were tripartite (Figs 1A, 2A, B) with an anterior ciliated cephalic shield (protosoma), involved in locomotion, grasping the edge of the tube when feeding and in the secretion of the tubes. Posterior to the cephalic shield the collar (mesosoma) included a pair of dorsal anterior extended arms, each bearing paired rows of ciliated tentacles with a length of $166 \pm 35 \mu\text{m}$ ($N = 20$). Zooids were $616 \pm 115 \mu\text{m}$ in total length (including arms); the metasoma was $246 \pm 48 \mu\text{m}$, and the mesosoma with collar and arms was $367 \pm 91 \mu\text{m}$ ($N = 20$). Both cephalic shield (especially the leading edge) and the arms (particularly the distal tips) were replete with dense, black pigment spots (Fig. 1A–C). Observations with the optical microscope revealed sparse yellow granules on the metasoma surface, on arms and tentacles and on the contractile stalk, possibly bodies involved in the secretion of mucus for filter feeding (Stebbing and Dilly 1972) and tube/stolon construction (Fig. 1B). Distally, the two arms lacked tentacles for about one third of their length (Figs 1A, B, F, G, 2A, B), and terminate with slightly bulbous tips. When relaxed, zooids stretched the non-tentacled arms' parts, and the tips in particular, up to more than half the total arms' length (Fig. 1G). The elongated non-tentacled distal tips of the arms, is the most striking and

unique feature of *R. recondita* zooids. As a zooid emerges from a tube, the black pigmented arm tips are the first structures to exit, so may function to detect light. The arms developed from zooid buds (and the buds from branches of the stolon); the non-tentacled, distal-most part appeared first, whereas tentacles number and length increased by basal growth (Fig. 1C). The number of tentacles in adult zooids was rather variable: in shallow water colonies (2-20 m depth) 11 to 15 tentacles per row were counted, but this number increased up to 24 per row in colonies living under low hydrodynamic conditions (70 m depth). The trunk (metasoma) was light brown and globular shaped. The U-shaped gut, visible through the ectoderm, occupied almost the entire body cavity (Figs 1A, B, 2B). The anus was located dorsally on the metasoma, posterior to the collar whereas the contractile stalk extended ventrally from the trunk to a black stolon (Figs 1B, D, 2B). Pterobranchs are unusual in that colonies may be male, female or dioecious, but in our case gametes were not found and so the gender of individual zooids was not determined.

Tube and stolon morphology

Rhabdopleura recondita occupied the interstices of bryozoan zooecium and so it did not construct typical tubular-shaped, creeping tubes with zig-zag shaped fuselli. Instead, the cavities of the bryozoan host were lined with thin, fragile and transparent, smooth tissue. Notwithstanding its unusual structure, not described in other graptolite species, we call here this smooth structure as ‘creeping tube’ to avoid introducing another term in the already linguistically rich graptolite literature. Unlike the creeping tubes of congeneric species, *R. recondita* creeping tubes showed a smooth internal and external surface texture, lacking half-ring fuselli (possibly because they develop within the protected inner cavities of the bryozoan zooecium). Inside the lacunae of the bryozoan zooecium the colony produced new zooids by asexual budding (Fig. 1C, E). Septa separating individual zooids were not found. Erect tubes projected outward from the bryozoan zooecium pores ranged $670 \pm 406 \mu\text{m}$ (N = 20) in length from the outer edge of the bryozoan zooecium (Figs 1D, F–H, 2C). They were transparent or slightly opaque, perhaps darkening with age. The erect tubes were characterized by a number of annular flared fuselli (13 ± 9 , N = 20) that had $37 \pm 11 \mu\text{m}$ of space between them (Fig. 1F). Differently from

the smooth creeping tubes, the erect tubes had smooth internal and rough external surfaces. The diameters of the distal outer tubes were $222 \pm 57 \mu\text{m}$ ($N = 20$).

The zooid stalk was connected to a sclerotized black stolon (Figs 1E, I, 2C), from which other zooids were similarly attached. According to Schepotieff (1907) the stolon of rhabdopleurids is produced by expanding outgrowths of the terminal zooid. However, this observation was never confirmed by other authors (Mitchell *et al.* 2013; Maletz and Steiner 2015). At first, the stolon is soft (*gymnocaulus*) and later it becomes a sclerotized, inflexible black stolon (*pectocaulus*) (Lankester 1884). We found no evidence for the occurrence of *gymnocaulus* in *R. recondita* colonies, but presume one preceded the abundant *pectocaulus*. The *pectocaulus* of *R. recondita* was apparently not adherent to the ‘creeping tube’, but was free and ‘loose’ (Figs 1E, I, 2C). Zooids branched at irregular distances along the creeping *pectocaulus* to which they were attached by a contractile stalk (Fig. 1B). This irregular spacing accommodated the variable positioned exit holes from inside the zooecium. Along the length of the stolon thickened pigmented globules were frequently found (Figs 1I, 2C). The origin and construction of these is unclear, but they may be equivalent to the ‘dormant buds’ (see Urbanek and Dilly 2000) where buds develop (cf., Stebbing 1970a; see Urbanek and Dilly 2000).

Molecular 16s and 18s rRNA gene trees

The general topology of the phylogenetic trees based on BI and ML analyses were almost identical, and therefore only the Bayesian topology is shown. Both single-locus and concatenated analyses recovered trees mostly concordant with each other (Figs 3, S1A, B) and with recent hemichordate phylogenies (Cameron 2005; Cannon *et al.* 2013, 2014; Simakov *et al.* 2015). *Rhabdopleura* and *Cephalodiscus* confirm to be monophyletic clades within the class Pterobranchia, supporting the phylogenetic reconstruction proposed by Mitchell *et al.* (2013). *Rhabdopleura recondita* sp. nov. constitutes a fully-supported monophyletic clade, well-separated from all other species included in the analyses. According to genetic distance estimations (Table 4), *R. recondita* shows a low intra-specific genetic diversity for both markers, whereas most species are highly divergent with each other, especially regarding 16S rRNA sequences, with an average among-species genetic distance of

22.8 (\pm 2.9) %. The only exception is represented by *Rhabdopleura normani* from Bermuda and *Rhabdopleura* sp. 2 from the Gulf of Mexico, for which the divergence over nuclear sequences is extremely low and is exceeded by the intraspecific diversity of *R. normani*.

Remarks

Interspecific differences of living rhabdopleurids

Besides the 18S rRNA and 16S rRNA sequence differences (Fig. S1A, B), several morphological characteristics distinguish *R. recondita* from the other four known species.

Rhabdopleura normani is lemon-yellow in colour with two kinds of epidermal pigments: reddish brown, mainly in the cephalic shield and tentacles, and light green mainly in the anterior margin and dorsal cephalic shield (Lankester 1884; Stebbing 1970a, 1970b). Like most rhabdopleurids, its pectocaulus is embedded in the lower wall of the creeping tube (Lankester 1884). Differently, *R. recondita* is characterized by dense aggregations of dark black granules throughout the cephalic shield and collar, with sparse yellow spots visible to the optical microscope, followed by a light brown-coloured trunk. Also, the creeping tube of *R. recondita* is not a regularly arranged tubular shape because it lines the irregular internal surface of the host bryozoan zoecium, and its pectocaulus is not fused to the creeping tube. The stolon ramifications are relatively long compared to those of *R. normani* whose stolons produce short branches immediately attached to the contractile zooid stalk. Further, *R. recondita* colonies reach only few mm in width while those of *R. normani* reach some centimetres. The depth range of *R. normani* is wide, ranging from 5 to 896 m (Stebbing 1970b: table 2). *Rhabdopleura recondita* was sampled in relatively shallow water (2-70 m) but we suspect this is the upper end of a much deeper range.

The zooids of *Rhabdopleura compacta* are slightly shorter (505 μ m) than those of *R. recondita* (616 \pm 115 μ m). The elongated, non-tentacled, black pigmented, distal tips of the arms is the most striking feature of *R. recondita* zooids, a unique

distinguishing character that readily differentiates this species from other described *Rhabdopleura*, and not previously recorded in any of the congeneric species (Stebbing 1970a, 1970b; Sato *et al.* 2008; Urbanek and Dilly 2000). The bathymetric range of *R. compacta* is from 21 m (Stebbing 1970b) to 100 m (Jullien 1890). *Rhabdopleura compacta* from Plymouth is a similar lemon-yellow colour to *R. normani* (Stebbing 1970a, 1970b). The erect tubes of *R. compacta* are very similar to those of *R. recondita*, with flared fuselli. The mean distal tube diameter of *R. compacta* is $183 \pm 12 \mu\text{m}$, and *R. recondita* is $222 \pm 57 \mu\text{m}$. The distance between successive rings of the erect tubes is $30 \pm 9 \mu\text{m}$ in *R. compacta* and $37 \pm 11 \mu\text{m}$ in *R. recondita*. Additional traits measured for which there is no comparative information in *R. compacta* were the lengths of the erect tubes ($670 \pm 406 \mu\text{m}$) and the number of outer fuselli rings of the erect tube (13 ± 9), both measured from the bryozoan pore to the distal lip of the tube.

Rhabdopleura striata colonies are 7-8 cm long (Schepotieff 1909), much bigger than the millimetre-sized *R. recondita*. *Rhabdopleura striata* has a tube width of 1 mm and the height of erect tubes are 10-12 mm as opposed to a 0.222 mm tube diameter and 0.670 mm erect tube height of *R. recondita*. The zooids of *R. striata* are 1-1.5 mm in length whereas those of *R. recondita* are 0.616 mm. The colour of *R. striata* zooids are dark brown. Erect tubes in *R. striata* regularly branch on the same side of the creeping tube and are longitudinally striated, while those of *R. recondita* branch irregularly, exit the bryozoan zooecium at all directions, and lack longitudinal striations.

Rhabdopleura annulata was described based on the tubarium structure. The erect tubes branch irregularly from the creeping tube and tubes are a light brown colour. *Rhabdopleura annulata* was named for the clearly marked fusellar rings of the erect tubes, which differ from the gently flared annulations of *R. recondita*. In contrast to *R. recondita*, and similarly to *R. normani*, the stolon is embedded in the lower wall of the creeping tube, and transverse septa separate the zooids. The erect tube diameter is $222 \mu\text{m}$, like the $222 \pm 57 \mu\text{m}$ of *R. recondita*, and average distance between successive rings is $50 \mu\text{m}$ versus $37 \pm 11 \mu\text{m}$ for *R. recondita*. The erect

tubes have 9 to 47 fusellar rings, versus 13 ± 9 for *R. recondita*. Norman (1921) supposed that erect tubes with more fuselli may be those of older zooids, but it may also be due to food availability or differences due to the *in situ* water velocity where the tubes were secreted.

Zoogeography of living rhabdopleurids

Nearly a century has passed since the last description of a *bona fide* new species of living *Rhabdopleura* (*R. annulata* Norman 1921). *Rhabdopleura recondita* should be regarded as the fifth known species, though other rhabdopleurids have been found. Cannon, Swalla & Halanych (2013) sequenced rhabdopleurids from Iceland and the Gulf of Mexico but no descriptions were provided. Laubier (1964) collected one from Cape Abeille, near Banyuls-sur-mer, in the French Mediterranean and provisionally identified it as *R. normani* based on similarity of the zooids. It was collected between 25-40 m depth, in coralline algae and was found inside the zooecium of the bryozoan *Dentiporella sardonica* (Waters, 1879) (Laubier 1964). In this latter respect Laubier (1964) recognized this species as unusual. No figures were provided and no type specimen was deposited in a museum, but based on its Mediterranean locality, its use of a dead bryozoan zooecium as habitat, and the peculiarity of ‘creeping tubes’, we suspect that Laubier (1964) had discovered *R. recondita* and not *R. normani*. Further sampling needs to be done to determine whether the range of *R. recondita* extends from the Italian coasts of the SE Adriatic and Ionian Seas to Cape Abeille, France, if not the entire Mediterranean basin. Regional and ocean-scale basins in fact may be key to understanding the global distribution of *Rhabdopleura* species (Fig. 4). *Rhabdopleura normani* apparently has the widest distribution, having been collected throughout the North Atlantic basin, including the Norwegian Sea (Sars 1874; Hincks 1880; Nordgaard 1900; Burdon-Jones 1954; Stebbing 1970*b*), North Sea (Norman 1869; Allman 1869; Hincks 1880; Lankester 1882, 1884; Herdman 1892; Schepotieff 1904; Schepotieff 1907; Grieg 1914; Burdon-Jones 1954; Stebbing 1970*b*), Barents Sea (Kluge 1948), Greenland Sea (Kluge 1948), Labrador Sea (Norman 1903; Burdon-Jones 1954), Bay of Biscay (Köehler 1896; Burdon-Jones 1954), Celtic Sea (Stebbing 1970*b*: table 2), Gulf of Lion (Laubier 1964; Stebbing 1970*b*: table 2), the Azores (Jullien

1890, Jullien and Calvet 1903; Burdon-Jones 1954; Stebbing 1970*b*) and Bermuda (Barnes 1977; Dilly 1985). It is moreover present in the South Atlantic (including the Argentine Sea) (Lopez-Gappa 1987), Fiji (Dilly and Ryland 1985) and Antarctic Ocean (Johnston 1937; Lopez-Gappa 1987). This widely discontinuous distribution could represent the relict populations of a formerly wide spread species if indeed it is absent from the intervening waters. Alternately, the disjunct populations may be polyphyletic species and derived by parallelism from a common antecedent once wide spread, or even by convergence from separate antecedent species. This is in the realm of possibility because: (1) *Rhabdopleura* species are defined and distinguished from each other by a very small number of features, (2) these traits are frequently those of tubaria that might be expected to converge on similar form depending on the attachment substrate, water flow and the availability of food, (3) development is via a non-feeding and short lived planula-like larva that presumably contribute little to the distribution of a population and (4) *Rhabdopleura* has shown little change in over a half billion year history.

Rhabdopleura compacta is found in the English Channel (Jullien 1890; Burdon-Jones 1954; Stebbing 1968, 1970*b*), and in the Irish Seas North Channel, off County Antrim (Hincks 1880; Burdon-Jones 1954; Stebbing 1970*b*), but also in the Gulf of Mexico (WoRMS: <http://www.marinespecies.org/>) (Fig. 4). Its disjunct distribution may be influenced by the Gulf Stream that passes from the Gulf of Mexico to the Northeast Atlantic, and is thus expected to occur in between.

Rhabdopleura striata is known from a single collection made in the coral reefs of North Sri Lanka. Rarity is not an adequate explanation in its one-locality distribution. Lack of systematic search in contiguous habitats may play some role. Though a lack of suitable habitats, if for example coral reefs are required, may also explain its rarity.

Rhabdopleura annulata is known from the waters around Indonesia (Norman 1921), South Australia including Tasmania and New Zealand and thus represent a real species (Norman 1921; Johnston 1937; Hyman 1959), seemingly widespread in the South Pacific.

The fossil record for *Rhabdopleura* extends back to a little less than a half billion years and thus it is one of the oldest genera on the planet. Phylogenetically,

rhabdopleurids are basal graptolites that have survived 5 major extinction events, including the Lower Devonian events that wiped out the more derived, spectacularly speciose, planktonic graptolite forms (Mitchell *et al.* 2013). The deep sea habitat of rhabdopleurids may have served as a refuge from the five major extinction events, the ability to form buds from cells protected in the black stolon, and the capacity for clonal reproduction have contributed to *Rhabdopleura* longevity. On the other hand, rhabdopleurids have rarely been found even in deep-sea samples where they might otherwise be expected. This may be due to the difficulty of finding small sized pterobranchs that have cryptic association with biogenic substrates (e.g. corals, bivalve shells, bryozoan zooecia). Another interpretation of the limited distribution of *R. recondita* and *R. striata* and the disjunct distributions of *R. normani* is that they are relicts of once wider ranging species, and the prevalent patchiness manifested by the large number of seemingly suitable localities lacking any *Rhabdopleura*, between the sites where they occur, suggests a declining group, but not one expected to disappear anytime soon. The abundance of populations in the proximity of Britain and Europe suggests that sampling frequency could be an important factor explaining their distribution. In much more conspicuous groups, like sponges, knowledge on species richness and biogeography appears to be strongly biased by collection and taxonomy efforts (Van Soest *et al.* 2012). Similarly, we may find more rhabdopleurids with the increased use of ROV sampling, and more attention paid to small animals on calcium carbonate substrates.

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Figure Legends

Fig. 1. *Rhabdopleura recondita*. *A*, general zooid anatomy: protosoma with the cephalic shield (cs), mesosoma with arms, tentacles and collar, and the trunk, or metasoma; *B*, zooid compressed between two slides for optical microscope observation, cephalic shield (cs), arms, tentacles and trunk are visible, a contractile stalk (cst) on the anterior of the trunk is evident; *C*, developing zooid within bryozoan zooecium (bz) with concentrated black pigment on arms and cephalic shield; *D*, external view of a colony from 70 m depth, with abundant erect tubes growing outside the zooecium of the bryozoan *Schizoretopena serratimargo*; *G*, *R. recondita* zooid in its erect tube, the conspicuous long naked arms tips are visible; *H–I*, sections of a calcified skeleton of a dead *Myriapora truncata* (Bryozoa) colonized by *R. recondita*, with erect tubes project outward from the bryozoan zooecia apertures, and pterobranch zooids inside, a pigmented globule (pg) is visible. Scale bars: *A–C*, *E–I* = 200 μm ; *D* = 3 mm

Fig. 2. *Rhabdopleura recondita* zooid and colony drawings. *A*, General zooid subdivision in protosoma, mesosoma and metasoma in frontal view; *B*, zooid lateral view with the U-shaped gut visible through the metasoma epidermis, anus and contractile stalk (cst) are indicated; *C*, section of a bryozoan zooecium colonized by *R. recondita* with the stolon running along the spaces leaved empty by the bryozoan zooids, the pigmented bodies (pg) are indicated.

Fig. S1. Bayesian Inference trees of the (A) 18S rRNA obtained under the HKY+G+I model and (B) 16S rRNA obtained under the GTR+G+I model. Numbers at nodes represent Bayesian posterior probabilities and maximum likelihood bootstrapping values, respectively. Trees were rooted with the deuterostome *Branchiostoma lanceolatum*.

Fig. 3. Bayesian Inference tree of the concatenated 16S and 18S rRNA datasets. Numbers at nodes represent Bayesian posterior probabilities and maximum likelihood bootstrapping values, respectively.

Fig. 4. Map of the global distribution of *Rhabdopleura* species (after Burdon-Jones 1954 and Stebbing 1970*b*). Where more than one sampling was performed, we indicate the location where the most precise collection location was reported. See text for references. The map was created with R software (<https://www.r-project.org/>).

Table 1. Sampling data for *Rhabdopleura recondita* colonies.

Table 2. Information on the *Rhabdopleura recondita* hosts.

Table 3. Specimens included in the analyses and associated GenBank accession numbers.

Table 4. Pairwise comparisons of genetic distance (uncorrected p-distance in %) of 16S and 18S rRNA sequences within and between rhabdopleurid clades. Standard deviations are in parentheses. n. c. not calculated.