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The hydroid of the medusa *Lizzia blondina* Forbes, 1848

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Abstract
A tiny, filiferan hydroid living within the sponge *Haliclona simulans* (Johnston, 1842) could be identified as the so far unknown polyp stage of the hydromedusa *Lizzia blondina* Forbes, 1848. This finding is based on two lines of evidence: (i) the direct observation that sponge pieces with the hydroid release young *Lizzia blondina* medusae, and (ii) 16S rRNA gene sequences obtained from DNA samples extracted from mixed sponge–hydroid samples were identifiable as either *Lizzia blondina* or sponge-related. Histological examination of the hydroid showed that it is colonial, and the individual polyps are connected through stolons which penetrate deeply into the sponge tissue. The polyps only protrude temporarily and partially for the purpose of feeding. The hydroid can retract its tentacles and the hypostome in an introvert-like pouch, becoming thus almost invisible on the sponge surface. The association of the *Lizzia blondina* hydroid with the sponges of the genus *Haliclona* Grant, 1841 is likely a rather specific relationship.

Keywords Cnidaria · Hydrozoa · Life cycle · Sponges · 16S rRNA gene barcodes

Introduction

Lizzia blondina Forbes, 1848 is a tiny medusa (about 1 mm, Fig. 1) which can be regularly found in the plankton of the European coasts from Norway to the Mediterranean. It has also been reported outside Europe, e. g. in the NW Atlantic, western Africa, New Zealand and China (see "Taxonomy" section). Owing to the rapid proliferation by asexual budding, this medusa may at times be exceedingly abundant. The medusa can be kept in jars quite easily, and despite its small size, it feeds well on *Artemia* nauplii. However, attempts to obtain the polyp stage by breeding failed as the larvae did not metamorphose (own, unpublished observations).

Kramp (1926) suspected that the polyp lives mainly along the southern and western coasts of the British Isles, and the distribution in the Danish waters let Kramp think that the polyp lives only in areas with soft, clayey bottoms. Rees (1941: 138) and Russell (1953: 148) thought that perhaps the hydroid *Trichydra pudica* Wright, 1857 is its polyp stage. This was later disproven when Edwards (1973) showed that *T. pudica* is the polyp of *Pochella polynema* Hartlaub, 1917 [see Schuchert (2009) for more details].

Until recently (Bouillon et al. 2006), the genus *Lizzia* Forbes, 1846 was thought to be a member of the Bougainvilliidae Lütken, 1850 because both taxa have medusae with oral tentacles. Schuchert (2007), based on morphological and molecular data, showed that the genus *Lizzia* must be transferred to the Rathkeidae Russell, 1953. Genetically, the genera *Lizzia* and *Rathkea* Brandt, 1837 appear quite closely related and Schuchert (2007) suspected that therefore also their polyp stage could be rather similar. Because *Rathkea* polyps are very small, they have only rarely been reported from nature (see references in Schuchert, 2007). Schuchert (2007) also reported on a new *Rathkea*-like polyp which he thought could be the polyp stage of *Lizzia blondina*, but besides the similarity with *Rathkea,* no other evidence could be provided for this hypothesis. This polyp is extraordinarily small, and it lives in the superficial tissues of the sponge *Haliclona simulans* (Johnston, 1842). I discovered this polyp when preparing my revisions of the Corynidae Johnston, 1836 (Schuchert 2001) and was searching for the hydroids of *Slabberia halterata* Forbes, 1846 and *S. simulans* (Bouillon, 1965), both living on and in the sponge

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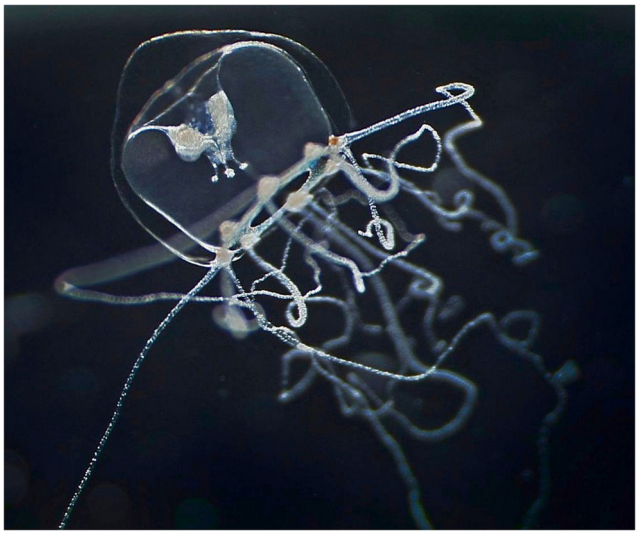

Fig. 1 Adult medusa from the plankton taken off Roscoff, bell diameter about 1 mm

Haliclona simulans (see Bouillon 1971 and Fig. 2). To study these hydroids, pieces of the sponge were cultivated and the *Slabberia* polyps fed regularly with *Artemia* nauplii. During these feedings, it was noticed that not only the two *Slabberia* hydroids captured nauplii but also a smaller and hardly visible polyp which was completely embedded in the sponge tissue. This polyp is very difficult to observe but distinguishable from *S. simulans* because it has filiform tentacles, and the body is white and not pinkish (*S. halterata* grows on the surface of the sponge and is much larger, see Schuchert 2001, 2012). In this report, more details on this polyp are presented, together with evidence that it must be the hydroid stage of *Lizzia blondina*.

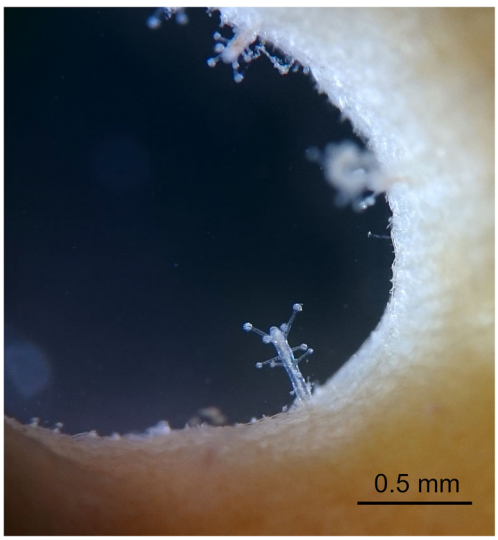

Fig. 2 *Slabberia simulans* on the surface of *Haliclona simulans,* a hydroid regularly associated with the polyp of *Lizzia blondina*

Taxonomy

***Lizzia blondina* Forbes, 1848**

Figs. 1, 3-6

Lizzia blondina Forbes, 1848: 67, pl. 12 fig. 4a–f.

Lizzia blondina—Hartlaub 1911: 145, figs. 131–135—Kramp & Damas 1925: 266, figs. 13–14—Kramp 1926: 52, chart IX—Russell 1953: 145, figs. 69–71, not 72, pl. 7 figs. 1–2, pl. 34 figs. 5–6—Kramp 1959: 105, fig. 78—Kramp 1961: 87—Schuchert 2007: 289, figs. 36–38, revision, synonymy—Wang et al. 2016: 395, figs. 3–8.

Cubogaster gemmascens Haeckel, 1879: 76, pl. 6 figs. 8–12—Kramp & Damas 1925: 266, synonym.

Dysmorphosa minima Haeckel, 1879: 78, pl. 6 fig. 7—Browne 1897: 148, synonym. [not *Cytaeis minima* Trinci, 1903 = *Podocorynoides minima*].

Lizzia claparèdei Haeckel, 1879: 82—Hartlaub 1911: 145, synonym.

? *Lizzia elizabethae* Haeckel, 1879: 83, pl. 4 fig. 12.

? *Lizzia minuta* McIntosh, 1893: 344—Russell 1953: 145.

Dysmorphosa fulgurans A. Agassiz, 1865: 163, figs. 259–260.

Podocoryne fulgurans—Mayer 1910: 139, pl. 12 figs. 5–9, pl. 13 figs. 3–5.

Lizzia fulgurans—Kramp 1959: 105, fig. 80—Kramp 1961: 88.

Dysmorphosa minuta Mayer, 1900: 41, pl. 18 fig. 42.

Podocoryne minuta—Mayer 1910: 140, pl. 14 fig. 1—Kramp 1959: 102, fig. 68—Kramp 1961: 69. – ? Schuchert 1996: 50, fig. 28a–b.

Rathkea spec—Schuchert 2007: 288.

Type Locality: Shetland Islands.

Description: For the medusa, see Schuchert (2007), the polyp is described in this study.

Distribution: The medusa *Lizzia blondina* occurs from Norway to the Mediterranean as well as in the NW Atlantic. It has been recorded as far north as Bergen Norway and Iceland. Beyond the European Seas, it has also been recorded along the coast of western Africa, Florida, New Zealand and along the Chinese coast (Schuchert 2007; Wang et al. 2016).

Biology: The medusa *Lizzia blondina* is a summer species found in nearshore waters. It occurs in the plankton around the British Isles from March to December but more abundantly in May to October. In the Mediterranean, it can be found all year round (Schuchert 2007). Along the Chinese coasts the medusa can be found from April to

August (Wang et al. 2016).

Material and methods

Sampling

Several large, branching colonies of the sponge *Haliclona simulans* (Johnston, 1842) were collected by divers in the Bay of Morlaix (France, Finisterre, approx. 48.6928°N 03.9408°W) in depths of 10–15 m in June 2000, September 2004, September 2006, May 2008, and April 2018. The sponges could be kept in aquaria with running, filtered seawater for up to 1 week. For the examination, parts of the sponge were cut in pieces of 3–4 cm using scissors and placed in glass bowls with 20–50 ml seawater. *Haliclona simulans* is one of the few sponge species that can be kept for several hours in small volumes of seawater without deteriorating the water.

Medusae released from the sponge tissue were cultivated at 18 °C in small (10 ml) glass jars and fed every second day with a newly hatched *Artemia* nauplii.

Some *Lizzia blondina* medusa were also collected in the plankton off Roscoff (France, Finisterre) and Bergen (Norway) using a standard zooplankton net. After examination and documentation, they were preserved in ethanol for later DNA extraction. The medusa *Lizzia blondina* is not particularly common near Roscoff (Teissier 1965) and data on its seasonal presence are missing. A zoo-plankton catch made 18 April 2018 off Roscoff, the time the release from *Haliclona* was observed, did not contain any *Lizzia* medusa (but it was present in September 2006).

DNA work

As attempts to dissect enough intact polyps out of the sponge tissue for DNA extraction were not successful, about 5 mm^2 pieces of the sponge cortex containing the tiny filiferan polyps were directly preserved in 99% ethanol for later DNA extraction. Likewise, for a control, also a sponge piece with the hydroid *Slabberia simulans* was preserved in ethanol. DNA extractions were made as given in Schuchert (2005, 2014). About 600 bp of the large mitochondrial ribosomal RNA (16S) was amplified using the primers SHA (ACGGAATGAACTCAAATCATGT) and SHB (TCGACTGTTTACCAAAAACATA) (Cunningham & Buss, 1993) (30 to 35 cycles, profile: 20 s 94 °C, 45 s 50 °C and 120 s 68 °C). The 16S PCR reactions of the mixed sponge/hydroid extractions resulted in double bands (650 bp and 1100 bp). The gel-separated bands were subsequently cut out of the agarose and purified using the "High Pure PCR Product Purification Kit" according to the manual of the manufacturer (Roche applied science). The purified DNA fragments were then sequenced using the same primers as above (ABI method, service provided by Macrogen Inc.). Sequence comparisons and phylogenetic trees were obtained as described in Schuchert (2005, 2014). All new haplotypes were deposited in the European Nucleotide Archive under the accession numbers LS974807-LS974812. The other Rathkeidae DNA samples or sequences used in this study have either already been mentioned in Schuchert (2007) or are additional samples from the same series. They are identified in Fig. 7 by the code DNA###, referring to the sample number in the DNA collection of the natural History Museum of Geneva (MHNG).

Histology

For the histological examination, 5–7 mm long pieces of sponge branches with embedded hydroids were initially preserved in 4% formaldehyde in seawater. A second fixation with Bouin's fixative (75 parts saturated solution of picric acid, 20 parts of 37% formaldehyde solution, 5 parts glacial acetic acid) was made for 1 day, followed by several washes in 70% ethanol. The sponge spicules (SiO_2) were then dissolved by immersing the tissue samples overnight in 4% hydrofluoric acid in 70% ethanol. After several washes in 70% ethanol, the tissues were dehydrated and paraffin-embedded using standard histological procedures. Serial thin sections (12 μm) were made using a microtome and then stained with haematoxylin and eosin. The two slide series have been deposited in the collection of the MHNG under the accession numbers MHNG-INVE-120690 through 120691.

Taxonomic scopes

The nomenclature of the corynids mentioned in this study (genus *Slabberia* instead of *Dipurena*) follows Schuchert (2010). For the unresolved status of the genus *Lizzia*, see Schuchert (2007).

The following taxa with their authorities and the citations of the original publications (in brackets) are used in this publication: Bougainvilliidae Lütken, 1850 (Lütken 1850); Capitata Kühn, 1913 (Kühn 1913); Corynidae Johnston, 1836 (Johnston 1836); *Cubogaster gemmascens* Haeckel, 1879 (Haeckel 1879); *Dysmorphosa fulgurans* A. Agassiz, 1865 (Agassiz 1865); *Dysmorphosa minima* Haeckel, 1879 (Haeckel 1879); *Dysmorphosa minuta* Mayer, 1900 (Mayer 1900); Filifera Kühn, 1913 (Kühn 1913); *Garveia grisea* (Motz-Kossowska, 1905) (Motz-

Kossowska 1905); *Haliclona* Grant, 1841 (Grant 1841); *Haliclona simulans* (Johnston, 1842) (Johnston 1842); *Lizzia blondina* Forbes, 1848 (Forbes 1848); *Lizzia claparèdei* Haeckel, 1879 (Haeckel 1879); *Lizzia elizabethae* Haeckel, 1879 (Haeckel 1879); *Lizzia* Forbes, 1846 (Forbes 1846); *Lizzia minuta* McIntosh, 1893 (McIntosh 1893); *Pochella polynema* Hartlaub, 1917 (Hartlaub 1917); *Podocorynoides minima* (Trinci, 1903) (Trinci 1903); *Rathkea* Brandt, 1837 (Brandt, 1837); *Rathkea octopunctata* (M. Sars, 1835) (Sars 1835); Rathkeidae Russell, 1953 (Russell 1953); *Slabberia simulans* (Bouillon, 1965) (Bouillon 1965); *Slabberia halterata* Forbes, 1846 (Forbes 1846); and *Trichydra pudica* Wright, 1857 (Wright 1857).

Results

The polyp

While the polyps of *Slabberia simulans* are relatively easy to see (Fig. 2), the tiny hydroids with filiform tentacles are very difficult to observe. They are two times smaller, almost completely embedded in the sponge, and retract completely into the sponge tissue upon the slightest mechanical disturbance. Once contracted, they are hardly visible as they not only withdraw into the sponge tissue but also the tentacles are contracted to become invisible (see below).

The hydroids are best seen when small pieces of the sponge, preferably bifurcations of branches, are examined at high magnification and with a strong lateral illumination (Fig. 3a, b).

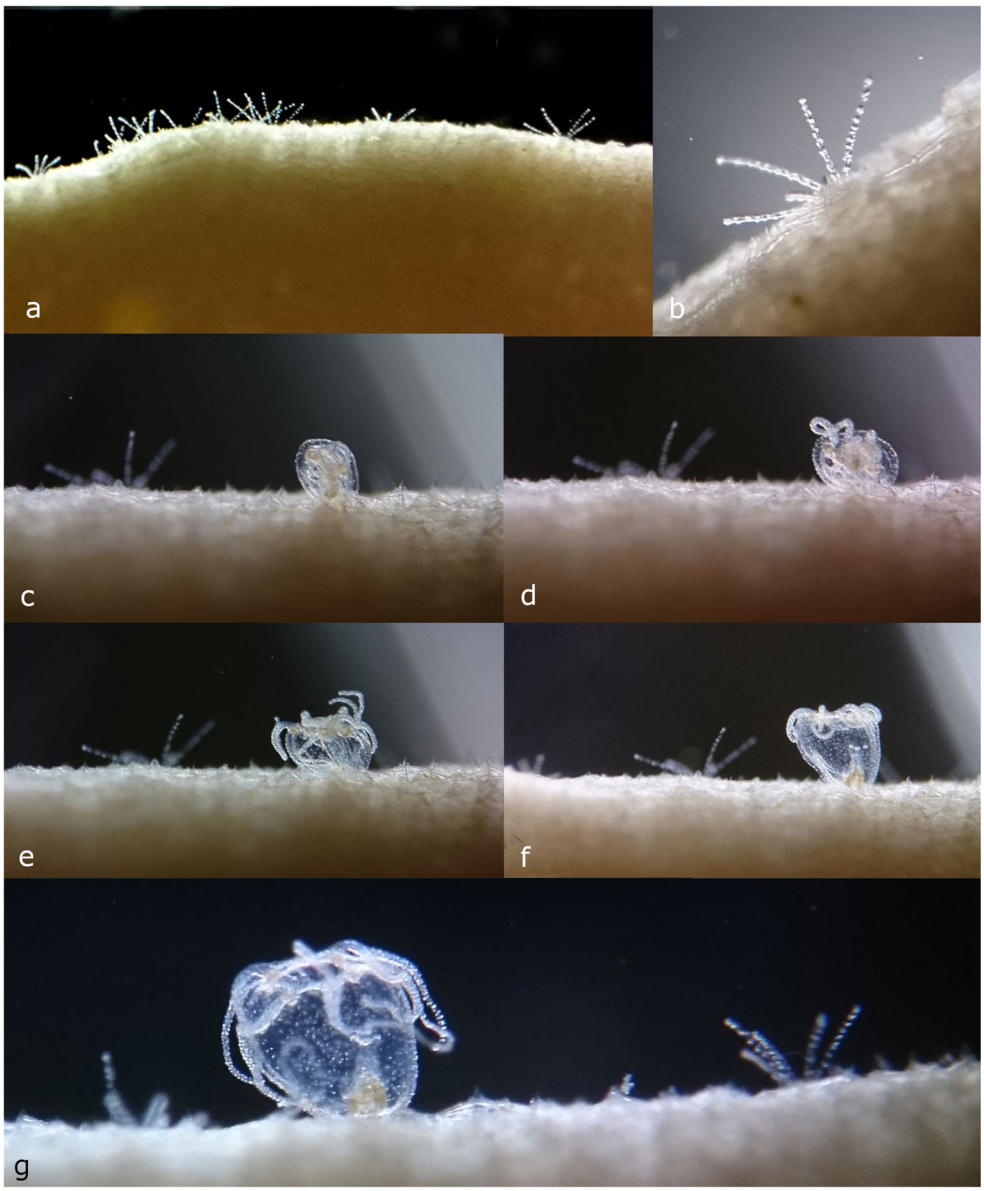

Fig. 3 Polyp of *Lizzia blondina* in the sponge *Haliclona simulans*. **a** Side view of expanded tentacles of polyps embedded in the cortex tissues of the sponge. **b** Higher magnification of one polyp with five tentacles. **c–f** A young medusa squeezes itself out of the sponge tissue: required time was 6 min. **g** The young *Lizzia* medusa is ready to swim away

Dissecting individual polyps out of the sponge tissue proved to be exceedingly difficult, as the tough spongine skeleton combined with the embedded ridged silica needles almost invariably destroy the polyp tissue when the sponge tissue is removed mechanically. The only way I had some success in isolating a single polyp was to cut off some superficial sponge tissue containing the hydroids and then carefully removing the softer sponge tissue lying underneath the hydroids (Fig. 6f).

The observations of living polyps embedded on the sponge, the dissected polyps, and the histological sections permitted to obtain the following description of the polyp (Figs. 3, 5 and 6).

The hydroid reside in tubular cavities close to the sponge surface into which can retract completely (Fig. 6a). The body is about 0.2–0.3 mm in length and 80–100 μm wide, fusiform, without perisarc membrane, with a conical hypostome surrounded by four or five relatively short, filiform tentacles with scattered small clusters of nematocysts. The tentacles can extend at least to 0.3 mm in length, but they also can contract to mere stumps that are hidden in an introvert-like sac together with the whole hypostome (Fig. 6a, b).

The base of the polyp is continued as a stolon without a distinct transition. The stolons are branching and penetrating into the deeper layers of the host: their diameter is about 50 μm. A periderm covering the stolons was not observable (Fig. 6e).

Nematocysts (native): microbasic euryteles (2.5–3.0)×(6–6.5) μm; desmonemes (2–2.5)×(4–4.5 μm).

Colour: white and transparent colourless.

Medusa buds develop at the base of the polyp body and are never visible from outside (see below under "Histological observations" section).

The medusa release and development

While examining *Haliclona* pieces for the hydroid described above, I found that several pieces from independent sponge colonies released medusae that were clearly identifiable as young *Lizzia blondina*. The medusa release from *Haliclona* was only observed in material collected in April 2018 but not in the previous material collected in later months of the year. More than 12 such medusa were obtained, and in at least four cases, it was possible to directly observe the release from the sponge tissue (Fig. 3c–g). The release of the medusa starts with the extrusion of a small part of the umbrella from a small opening in the sponge (Fig. 3c). At this stage, the umbrella is not yet fully expanded. Through contraction of the umbrellar musculature, more and more of the medusa bell wiggles out, usually ending with a fully expanded medusa which adheres with its aboral end to the surface of the sponge (Fig. 3g). The whole liberation process lasts about 10 min and was only observed in regions where also the hydroids occurred. The medusae detach soon afterwards and start to swim freely. The diameter of the newly liberated medusa is 0.4 mm, and the exumbrella is covered with scattered nematocysts and there is a funnel-like depression in the jelly above the manubrium, both typical traits for newly liberated anthomedusae. There are eight marginal bulbs each bearing one tentacle only. The manubrium resides on a shallow peduncle and bears two opposite bulges on its side (likely lipid reserves). No medusa buds are present yet. The four conspicuous capitate oral tentacles are unbranched and insert close to the mouth opening. Even at this young stage, the medusa is clearly identifiable as *Lizzia blondina* ('*minuta*' stage, see Schuchert 2007). *Lizzia* medusae can be cultivated easily in small bowls, and they feed eagerly on newly hatched *Artemia* nauplii (one every second

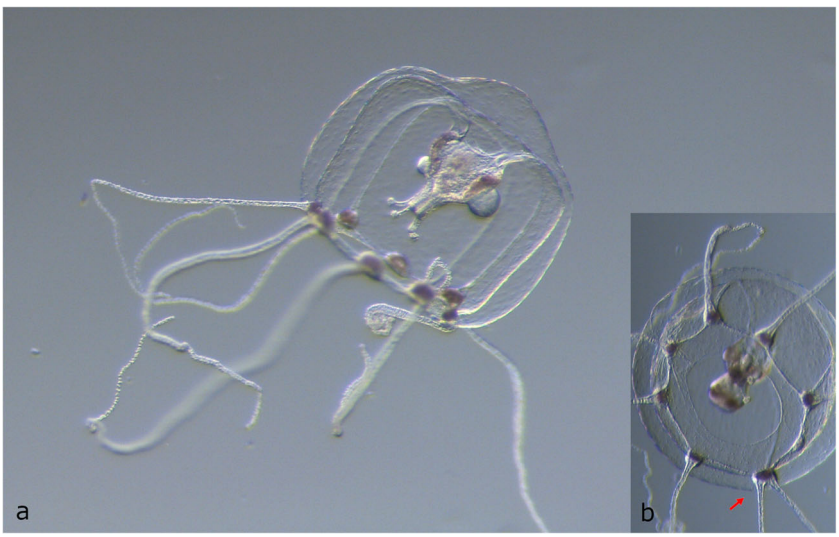

Fig. 4 **a** Side view of a medusa liberated from *Haliclona simulans* after 10 days in culture: the apical process has developed, as well as a pair of medusa buds on the manubrium. Bell diameter about 1 mm. **b** Oblique oral view, the second perradial tentacles develop (arrow)

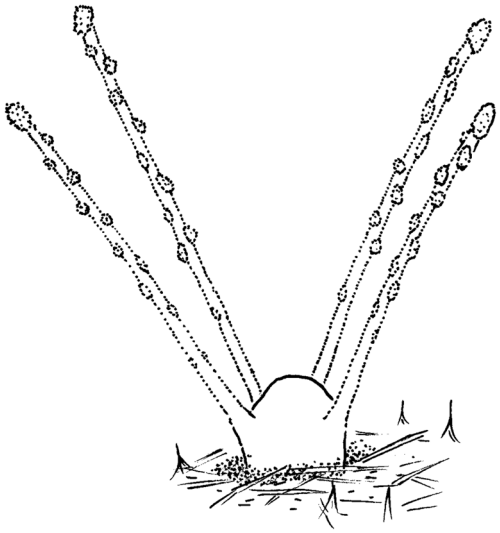

Fig. 5 Drawing of the polyp of *Lizzia blondina* with extended tentacles protruding out of its sponge host

day). They soon develop a shallow apical process, a gastric peduncle, second-generation medusa buds on the manubrium and some of the perradial bulbs get a second tentacle (Fig. 4a, b). After about 8 days, the second-generation medusae start to be liberated. At this stage, the medusae are unambiguously identifiable as *L. blondina*. One of them was nevertheless used to determine its 16S sequence (Fig. 7). The nematocysts of the fully grown medusa were microbasic euryteles (7–9)×(3.5–4) μm and desmonemes (4.5–5)×(3) μm (comp. Russell 1938).

Histological observations

Serial histological section of two sponge pieces which had liberated *Lizzia* medusae permitted to visualise the polyp and the stolons in the sponge tissue and also to observe some additional details, notably medusa buds.

The polyps reside in tubular cavities of the sponge cortex, delimited by a thin cellular layer of the sponge, the pinacoderm (*pi* in Fig. 6a). When fully contracted, the polyps withdraw their hypostome and the tentacles into an introvert-like pouch formed by a thin, annular fold of the epidermis (*ef*, Fig. 6a, b).

The hypostome consists of a thin epidermis covering a thick, dark staining, columnar gastrodermal layer (hs in Fig. 6a, c). Stolons penetrate deeper layers of the sponge (Fig. 6a, e) and seem not to have a periderm covering. The body wall of the polyps often contains one to two large, spherical vesicles resembling follicles (*vs* in Fig. 6d, f). They were also seen in the histological sections and can also be found in the stolons. Their identity and function could not be clarified.

Although more than 20 sectioned hydranths could be seen, only three medusa buds were found and unfortunately, not always in complete series of sections (Fig. 6b, c). It was therefore not possible to obtain a comprehensive understanding of the whole development, and some of the following interpretations might be incorrect. The medusa development takes place in a sac-like diverticulum (*mbc* in Fig. 6b, c) at the base of the polyp. Whether its cavity remains in connection with the gastric cavity of the polyp—as usually seen in other hydrozoans—could not be ascertained. The development of the entocodon (Fig. 6b; see Bouillon et al. 2006 for more details on this structure) starts at the distal point of the diverticulum and leads to a darkly staining cell mass. In the further development, a very dense inner mass (*dcm*, Fig. 6b, c) separates from an outer, somewhat lighter cell mass (lcm, Fig. 6b, c). Both masses start to form cavities (Fig. 6c). More advanced medusa buds could not be found in the available material.

The specimens used for the histological examination were chosen from sponge regions lacking *Slabberia* hydranths. However, even these pieces apparently contain some *Slabberia* stolons deeply embedded in the sponge tissue. They are thicker and darker, they have a perisarc layer, and most importantly, the presence of occasional stenoteles permits to distinguish them from the *Lizzia* stolons.

Molecular identification

Three independent tissue samples (see Table 1) from superficial layers of the sponge *Haliclona simulans* containing also the small filiferan hydroid were used to extract DNA. Care was taken to select pieces lacking any visible *Slabberia* hydroid which often grow together with this hydroid. As a control, also a sponge piece was used which apparently lacked the filiferan hydroid but clearly had embedded *Slabberia simulans* (Fig. 2). Concomitantly, also a *Lizzia* medusa that was released from the sponge was used to obtain DNA and its 16S sequence. It was identical to a previously published 16S of *Lizzia blondina* (nucleotide archive accession number AM411417).

The 16S PCR reactions using a mixture of sponge and hydroid DNA resulted in all four cases in the amplification of two distinct bands, a weak band of about 650 bp—the typical size for hydrozoans—and a much more intense of about 1100 bp (Table 1, figure not shown). The PCR products were subsequently isolated from the agarose gel and directly sequenced. Of the three sequenced 1100 bp fragments, two proved to be identical and a third was a slightly different haplotype (2.3% divergence, LS974811 & LS974812). A comparison with existing sequences in GenBank (BlastN, Johnson et al. 2008) did not yield any perfect matches, but

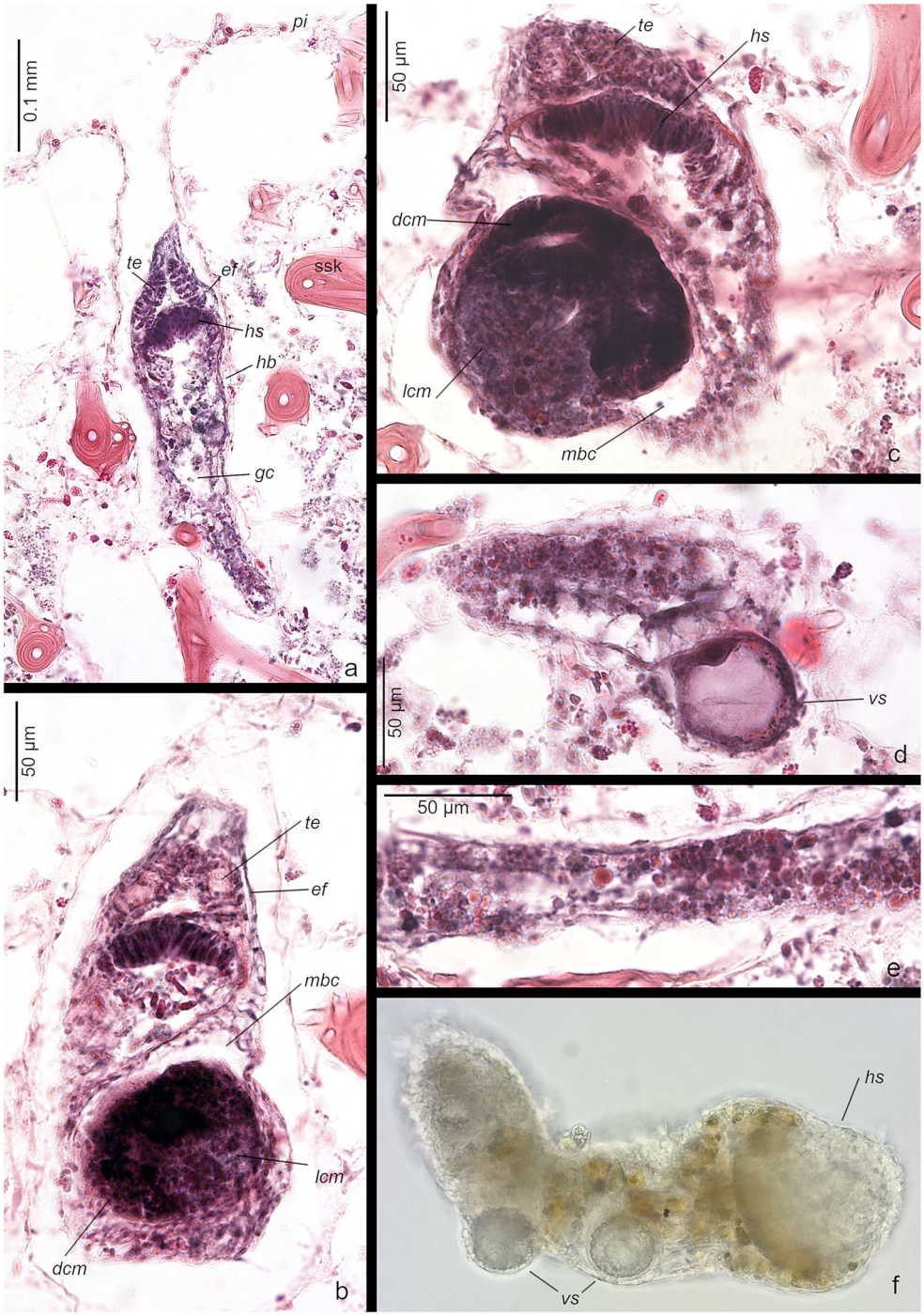

Fig. 6 Polyp of *Lizzia blondina*, histological sections of a branch of *Haliclona simulans* and a whole mount. **a** Longitudinal section of a hydranth. **b** Hydranth with an early stage of a medusa bud. **c** Hydranth with a later stage of a medusa bud. **d** Tangential section of a hydranth body with vesicle of unknown function. **e** Longitudinal section of a stolon. **f** Living polyp dissected out of its sponge host: the oral part is directed towards right. The tentacles and the hypostome (*hs*) are contracted into an introvert and not visible. There are two vesicles (vs) of unknown function. dcm dark cell mass of medusa bud, ef epidermal fold enveloping the contracted tentacles and the hypostome, gc gastric cavity, hb hydranth body, hs hypostome, lcm lighter cell mass of medusa bud, mbc medusa bud cavity, pi pinacoderm of sponge, ssk spongine skeleton, te tentacles, vs vesicles in hydranth tissue.

so far, no 16S rRNA sequences of *Haliclona simulans* have been deposited. However, the highest sequence identities were always found with sponge 16S sequences (e.g. DQ915601 *Amphimedon queenslandica*, KT253771 *Haliclona* cf. *caerulea*). The sequences obtained from *Haliclona simulans* did not show contiguous matches with the GenBank sequences: a large portion in the middle of the fragment was not aligneable. Perhaps the fragment is derived from a nuclear copy. As the sequences of the larger fragment nevertheless obviously originated from the sponge genome and they were thus not pertinent to the questions of the present study, no further characterisation was attempted.

The sequences of the smaller fragments, however, could all unambiguously be attributed to hydrozoans (Table 1, Fig. 7). The sponge piece with *Slabberia simulans* gave a *S. simulans* sequence, while the three with the filiferan hydroid resulted in sequences that were identical or very similar to *Lizzia blondida* medusa from the same region (Fig. 7, Table 1).

Table 1 Sequencing results of DNA isolated from sponge tissues with embedded hydroids. The standard PCR using the primers SHA/SHB resulted always in two bands, one of about 650 bp and one of about 1100 bp. The resulting sequences were identified using BlastN searches in GenBank. Accession numbers of new sequences are marked by an *

Isolate number	Type of material	Date collected	650 bp fragment result and identity	1100 bp fragment result and identity	Interpretation
DNA828	Filiferan hydroid in *Haliclona simulans*	18.09.2006	Identical to AM411423 [=*L. blondina*]	Not sequenced	Embedded polyp is *L. blondina*
DNA1333	Filiferan hydroid in *Haliclona simulans*	13.04.2018	LS974808*, has 1 bp difference to AM411423 [=*L. blondina*]	Identical to LS974811, is similar to Porifera 16S	Embedded polyp is *L. blondina*
DNA1338	Filiferan hydroid in *Haliclona simulans*	13.04.2018	Identical to AM411423 [=*L. blondina*]	LS974812*, is similar to Porifera 16S	Embedded polyp is *L. blondina*
DNA1335	*Slabberia simulans* hydroid in *Haliclona simulans*	13.04.2018	LS974809*, has 1 bp difference to AY512547 [=*S. simulans*]	LS974811*, is similar to Porifera 16S	Embedded polyp is *S. simulans*

Discussion

Marine hydroids, and especially members of the Anthoathecata, are often found in association with sponges (Puce et al. 2005). The sponge *Haliclona simulans* of the Channel Coast is nevertheless particular as it can host at least three different hydroid species: the two capitates *Slabberia halterata* and *S. simulans* (see Bouillon 1971, as *Dipurena* spp.), plus a tiny, filiferan hydroid identified here as the polyp stage of *Lizzia blondina* (for the scopes of the taxa Filifera Kühn, 1913 and Capitata Kühn, 1913 see Bouillon et al. 2006 and Nawrocki et al. 2010).

The identification of the hydroid of *Lizzia blondina* is based on two lines of evidence: (i) the direct observation that sponge pieces hosting the hydroid release *Lizzia blondina* medusae and (ii) 16S gene sequences obtained from DNA samples made from mixed sponge–hydroid samples were identifiable as either *Lizzia blondina* or sponge-related. These results confirm an earlier hypothesis of Schuchert (2007) postulating that due to the close phylogenetic relationship of *Lizzia blondina* and *Rathkea octopunctata* (M. Sars, 1835), also their polyp stages will likely be similar, viz. very small, sessile hydranths with few filiform tentacles.

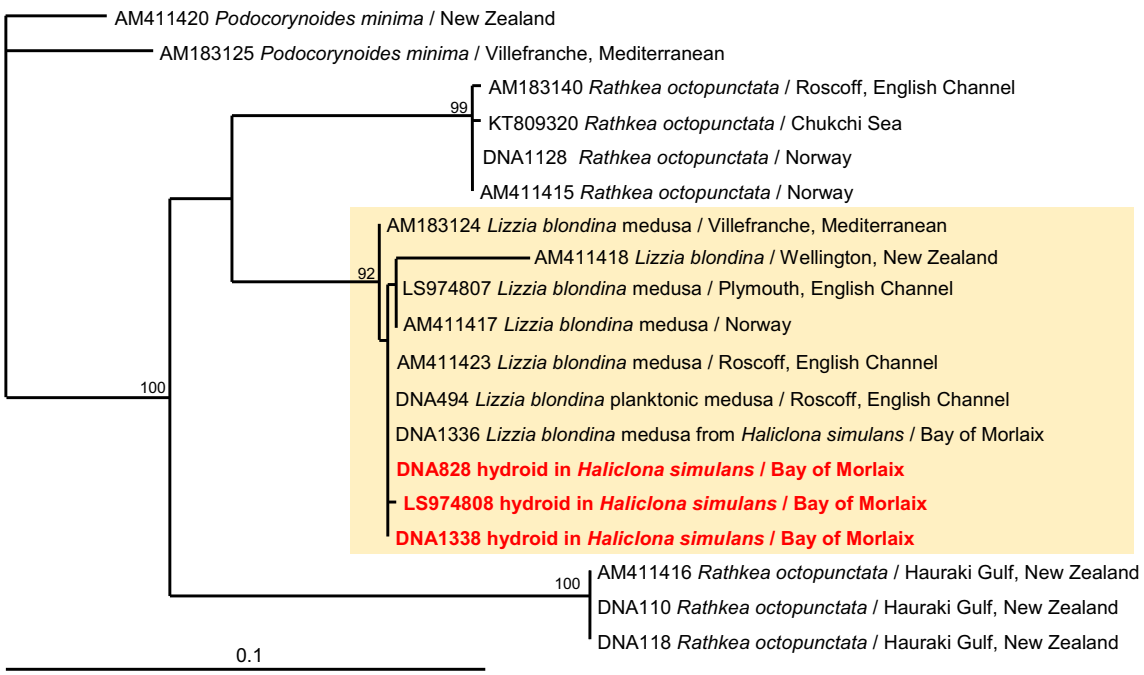

Fig. 7 16S maximum likelihood phylogenetic tree of Rathkeidae species obtained with PhyML (GTR+G model) and based on 589 bp positions of the mitochondrial 16S gene. Node-support values are bootstrap values of 100 pseudoreplicates (shown only if > 70%). For more details, see text. The samples are based on the medusa stage if not indicated otherwise. The sample legends start either with the GenBank accession numbers or the DNA isolate number, followed by the identification/specimen origin. Samples starting with the DNA isolate number represent sequences that had already identical ones in GenBank and which are also present in the tree (see also Table 1)

The *Lizzia blondina* polyps live deeply embedded in their sponge host and only protrude temporarily and partially for the purpose of feeding (Figs. 3 and 5). According to Puce et al. (2005), this mode of life suggests an exclusive and obligatory relationship, possibly a real symbiosis, evolved by co-evolutionary processes. If so, then the distribution of the host sponge should also match the distribution of the medusa *Lizzia blondina* (see section "Taxonomy"). *Haliclona simulans* occurs in the region from the British Isles south to the Canary Islands and the Mediterranean (de Weerdt 1986), thus a distribution which matches the one of *Lizzia blondina* quite closely, except for the northern part. There are, however, other, closely similar nominal *Haliclona* species present in more northern region of Europe (de Weerdt 1986). *Haliclona* Grant, 1841 is a very species rich genus (Van Soest et al., 2018), and its distribution is cosmopolitan (Nicole Boury-Esnault, pers. com.). So, *Lizzia blondina* hydroids outside the NE Atlantic could be associated with other *Haliclona* species than *H. simulans*). Perhaps they are even not *Lizzia blondina* but sibling species. Although the sample of *Lizzia blondina* from New Zealand falls into the same clade as the European ones (Fig. 7), it has a rather long branch indicating a marked divergence. Recent genetic results from other symbiotic anthoathecate hydroids indeed suggest that host- or distribution-related species-level diversity seems to be frequent in this group (e.g. Montano et al. 2017; Maggioni et al. 2017).

As the New Zealand sample belongs to a distinct lineage, this largely excludes a human-mediated introduction as one might suspect by the disjunct distribution of *Lizzia blondina*. In this context, it is worth noting that also the 16S sequences of *Rathkea octopunctata* from the Northern Hemisphere were quite distinct from the ones collected in New Zealand (Fig. 7). The sequences indicate that they are separate lineages and hence likely also distinct biological species (comp. *e. g.* Montano et al. 2017; Maggioni et al. 2017; Postaire et al. 2017; Miglietta et al. 2018; Boissin et al. 2018). In fact, *Rathkea octopunctata* medusae from New Zealand differ also slightly in at least one morphological detail: their oral tentacles are more deeply cleft and the ends much longer than those seen in animals from the NE Atlantic. As also *Podocorynoides minima* (Trinci, 1903) shows quite some divergence (Fig. 7), it is necessary that all three species shown in the 16S tree of Fig. 7 are re-examined in more detail using more specimens, markers and populations.

The *Lizzia blondina* polyp resembles polyps of the free-living *Rathkea octopunctata*, but besides its occurrence in a sponge, it has also two traits which distinguish it from the latter (comp. Schuchert, 2007). Its tentacles are shorter, and when contracted, they and the whole hypostome are withdrawn into an introvert-like pouch formed by a thin epidermal membrane (Fig. 6a, b), giving the impression that the polyps lack tentacles (Fig. 6f). Although rare, this introvert formation is not unique. It has also been described for another Anthoathecata, *Garveia grisea* (Motz-Kossowska, 1905) (see Schuchert 2007: 260, fig. 24B), and likely serves to protect the tentacles from physical damage. The tentacles of the *Lizzia* polyps are withdrawn upon the slightest mechanical disturbance, e.g. by minimally shaking the jar the sponge specimen is examined in or simply by touching the sponge with a needle. This behaviour might also explain why the *Lizzia* polyps were not recognised or mentioned in Bouillon (1971) while examining the often co-occurring *Slabberia simulans*. It seems, however, that the polyps were seen by Bouillon as his Fig. 1 shows three tentacle-free polyps that are almost entirely embedded in the sponge tissue; this is in contrast to the more superficial *Slabberia simulans* polyps. They likely represent *Lizzia blondina* polyps.

The medusa development observed in the serial sections also compares favourably with the one of *Rathkea octopunctata* described by Bouillon & Werner (1965) (see also Tardent 1978). In both species, the medusa development takes place in a small, sac-like diverticulum at the base of the hydranths. The development of the internal medusa anlagen starts at the distal point of the diverticulum with the development of a darkly staining cell mass. In the most advanced stages observed in this study, this cell mass then differentiated into a very dense inner mass (*dcm*, Fig. 6b, c) and an outer, somewhat lighter cell mass (*lcm*, Fig. 6b, c). Both masses start to form cavities (Fig. 6c). Comparing this stage with the medusa-bud development described by Bouillon & Werner (1965), the denser layer (Fig. 6a, b) was interpreted as the future gastrodermal system, while the lighter part will likely become the subumbrellar tissues and the tentacles. Unfortunately, it was not possible to find more advanced stages to prove these assumptions. New detailed studies have to address the problem, which was, however, beyond the scope of the present study.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the author.

Sampling All the sampling for this study was done by the Marine station of Roscoff and is covered by the necessary permits granted to the station.

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