New observations on reproduction in the branching polychaetes *Ramisyllis multicaudata* and *Syllis ramosa* (Annelida: Syllidae: Syllinae)

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Reproduction in the shallow-water, sponge-dwelling, branching syllid *Ramisyllis multicaudata* exhibits several features unique among syllids and among annelids in general. We describe and illustrate the segmental asymmetry which intervenes between regions of symmetry, only found in branching annelids. We describe the morphology of the stolons and of the stolon stalks, which are unique to branching syllids but differ in detail from those of the other known branching syllid, *Syllis ramosa*. We also illustrate newly found paddle-shaped chaetae, which might indicate that these stolons do swim. We list the number of branch points and termini in an effort to gauge the extent of branching in a worm contained in a small sponge. The species appears to have separate sexes, but this cannot be proven. We compare R. multicaudata and S. ramosa as originally described and find new morphological differences between the branching syllid from Japan described as S. ramosa and R. multicaudata. We also compare the known stolons of branching syllids to those of other genera of the 'ribbon clade', a group including *Trypanobia* and *Trypanosyllis*, now known to be close relatives of R. multicaudata. *Ramisyllis multicaudata* is the first member of the ribbon clade, and one of few Syllinae, known to have sexually dimorphic stolons.

INTRODUCTION

Ramisyllis multicaudata Glasby, Schroeder & Aguado, 2012 (Syllidae: Syllinae) is commensal with an undescribed species of *Petrosia* Vosmaer, 1885 (Demospongiae: Petrosiidae), a marine sponge living in coastal waters of northern Australia (Glasby *et al.*, 2012). *Petrosia* species, also called stony sponges, are hard and strong, but brittle. They are very slow growing (some species exhibiting little growth in 20 years) and repair of damage is also slow (Maldonado & Riesgo, 2009). Each sponge usually hosts only a single mature worm, which branches extensively through the excurrent canals of the sponge; the worm's presence is indicated only from the many tails visible on the surface of the sponge (Glasby *et al.*, 2012).

The branches of the worm emerge at right angles from the body axis in a pattern referred to as random branching asymmetry (Glasby *et al.*, 2012). The species also exhibits 'segmental asymmetry' in some parts of the body due to the different-length paired dorsal cirri of each segment (explained further in Results); although the branching asymmetry can be explained by cohabiting with an asymmetrical host,

reasons for the segmental asymmetry are not so clear. The only other branching annelid, the deep water species *Syllis ramosa* McIntosh, 1879, exhibits the same two types of asymmetry; it differs most notably from *R. multicaudata* in parapodial morphology, shape of non-natatory chaetae, and in the branching process. In the former, branching is initiated by segment addition at a parapodium, whereas in the latter segments are added from a region between the parapodia (Glasby *et al.*, 2012), suggesting a slightly different position of the segment addition zones (SAZ) in each species (Aguado *et al.*, 2015a).

Like other Syllinae, *R. multicaudata* reproduces by schizogamy, which involves the formation of a series of new gamete-bearing segments (stolons). These regions form heads, which usually include sensory structures. Once the stolons are developed (or close to developed), they detach from the main body of the worm (the stock) and seek another stolon of the opposite sex. However, we are not yet certain that the stolons of *R. multicaudata* leave the sponge. After detachment, the posterior segments of the stock are usually regenerated; the regenerated segments may then, in turn, form a second stolon, which is not necessarily of the same sex as the first (see Heacox, 1980 for *Typosyllis pigmentata* (Chamberlin, 1919) (as *T. pulchra* (Berkeley & Berkeley, 1938)). The degree of head development in the stolon varies in different species (Malaquin, 1893; Schroeder & Hermans, 1975) and this development may continue after the separation of the stolon from the rest of the

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body. Some species of the genera *Trypanosyllis* Claparède, 1864 and *Parahaplosyllis* Hartmann-Schröder, 1990 produce a number of simultaneous stolons in close sequence (collateral budding), which may appear as a sort of bush at the posterior end of the worm (e.g. *T. crosslandi* Potts, 1911; *T. gemmipara* Johnson, 1901 (Johnson, 1902); *Parahaplosyllis kumpol* Álvarez-Campos, San Martín & Aguado, 2013). Additionally, some species of the genus *Trypanobia* Imajima & Hartman, 1964 produce stolons that grow simultaneously from the dorsum of several posterior segments (successive budding) (e.g. *T. asterobia* (Okada, 1933)).

Trypanosyllis and *Parahaplosyllis* were thought to be related to *Ramisyllis* based on an initial comparison of DNA sequences (Glasby *et al.*, 2012); they were also shown to be related to *Eurysyllis* Ehlers, 1864 and *Xenosyllis* Marion & Bobretzky, 1875, but not close to species of *Typosyllis* Langerhans, 1879 or *Syllis* Savigny in de Lamarck, 1818. The relationships among these genera were later confirmed by other molecular phylogenetic studies which identified *Trypanobia* as the sister group of *Ramisyllis* (Aguado *et al.*, 2015a). Collectively, the group including *Eurysyllis, Xenosyllis, Trypanosyllis, Parahaplosyllis, Trypanobia* and *Ramisyllis* is referred to as the 'ribbon clade' because all, except for the commensally adapted *Ramisyllis*, have flattened bodies. Other genera, such as *Plakosyllis* Hartmann-Schröder, 1956 and *Nuchalosyllis* Rullier & Amoureux, 1979 share similar characteristics but their relationships have yet to be assessed in a molecular phylogenetic analysis. All members of the ribbon clade reproduce by schizogamy and several are gemmiparous (i.e. stolons develop simultaneously). Gemmiparity in the ribbon clade includes both collateral and successive budding, which may indicate one or several SAZs, respectively (Aguado *et al.*, 2015a). Unfortunately, the other branching syllid, *S. ramosa* has not been included in a molecular phylogenetic analysis, and hence it is uncertain up to now if the branching body pattern has developed independently in the two species or if it arose in a common ancestor.

In this study we provide further information on the unique morphology of the stolons and of the stolon stalk of *R. multicaudata*, adding to the initial observations of Glasby *et al.* (2012). In addition, we compare the form of its stolons and reproductive biology with other members of the ribbon clade, and the only other known branching annelid, *S. ramosa*.

MATERIALS AND METHODS

Material studied comes mainly from the vicinity of Channel Island, Darwin Harbour (12°33.2′S 130°52.4′E), from low water spring tide level, during collecting events between 2006 and 2016 and lodged at the Museum & Art Gallery Northern Territory (NTM) as follows: Paul Schroeder, personal collection no. QD4 (CG06/07), coll. C. Glasby, 8 November 2006, one adult from one *Petrosia* sp.; NTM W26330, W26332 (CG15/01), coll. C. Glasby, 20 March 2015, 22 detached male stolons, 12 detached female stolons from four *Petrosia* sp.; NTM W26331 (CG16/04), coll. C. Glasby & A. Malpartida, 10 March 2016, 12 detached female stolons, 15 attached female stolons from four *Petrosia* sp.

Observations on *Ramisyllis multicaudata* were all carried out on worms that were dissected out from the sponge using light microscopes as follows: Nikon SMZ 1500 stereo microscope and a Nikon Eclipse 80i compound microscope

with differential interference optics. Images were obtained using a microscope mounted Qimaging Micropublisher 5.0 RTV and a tripod mounted DSLR Canon 5D MkII with MPE–65 macro lens. Measurements were made using a calibrated slide (0.1 mm). Chaetiger counts for the stolons do not include the modified first chaetiger, in which the dorsal cirri appear almost like tentacular cirri.

Comparative data for *Syllis ramosa* came from McIntosh (1879, 1885, pl. XXXIII, figures 11–13, pl. XV$_A$, figures 17 & 19; pl. XXXIV$_A$, figures 9, 10 & 12) and Imajima (1966) and specimens of the same lodged at the Natural History Museum, London (BMNH) and the Oxford University Museum of Natural History, Oxford (OUM), as follows: Syntypes BMNH 1885: 12: 1: 150–154, *Challenger* Expedition, M79 – specimen from Challenger Station 209, 95 fathoms, near Cebu, Philippines, M85 – specimen from Challenger Station 192, 140 fathoms, Arafura Sea, SE of Ceram; M85 – male stolon, probably detached, but may include a bit of the stalk (pl. 34A, figure 13) from a sponge in the Oxford Museum of unstated provenance, provided by Professor Mosely. In addition, we examined one specimen of *Syllis* cf. *ramosa* from Sagami Bay, Japan, 100 fathoms, which is lodged in the National Science Museum, Tokyo (NSM unreg.).

RESULTS

Segmental asymmetry

Ramisyllis multicaudata exhibits regional areas of asymmetry revealed by the dorsal parapodial cirri, which as in the post-proventricle segments of many syllids, alternate between long and short forms. The long dorsal cirri in the posterior (distal) region of the present specimens contain vivid white pigment (other worms living with white *Petrosia* have purple coloured dorsal cirri), as seen in photos of living animals (Figure 1). In this figure, most of the white cirri on each segment are opposite one another. However, in one section of the worm the white dorsal cirri are opposite short, unpigmented dorsal cirri indicating a symmetry shift. This type of asymmetry, referred to as 'segmental asymmetry', has also been observed in *S. ramosa* and is unique to the branching syllid morphotype.
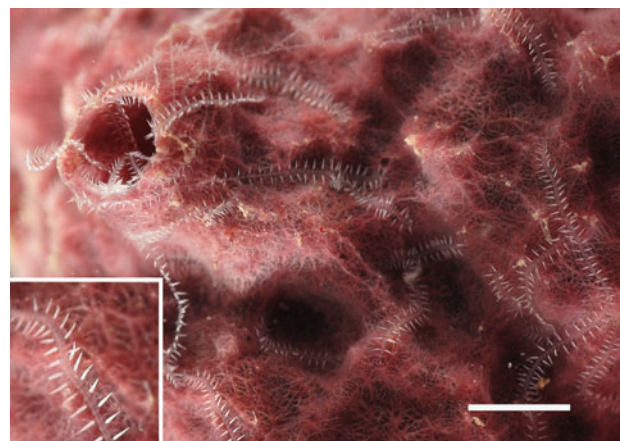

Fig. 1. *Ramisyllis multicaudata* and host sponge *Petrosia* sp. *in vitro* showing tails emerging from excurrent canals. Inset shows dorsal cirri out of phase indicating segmental asymmetry. Scale bar: 5.0 mm.

Fig. 2. Two early stolons of *Ramisyllis multicaudata* attached to a main branch via a stolon stalk (ss), and below leading to a normal tail. Note alternating enlarged dorsal cirri on the main branch which are not present on the stolon or stalks. Scale bar: 0.5 mm.

Description of stolon stalks

Stolon stalk segments resemble the 'intermediate' segments (*sensu* Glasby *et al.*, 2012) of the stock in terms of segmental length, dorsal cirri morphology and chaetal form; in particular, the dorsal cirri do not show the typical alternation pattern of long/thick – short/thin that is present in anterior and posterior segments, including those emerging from the sponge (Figure 2). Stolon stalks branch from the stock in the midbody (a zone proximal to the production of terminus-producing branches); they include from 6–25 segments (Figure 3A, B). Each stalk produces a stolon, and we have seen no sign of oogenesis in the stalk.

Female stolon development is initiated when posterior (distal) segments (the future stolon) begin to swell with the onset of vitellogenesis (Figure 2). The proximal segments fail to develop gametes and are soon differentiated from the developing stolon, remaining as the stalk. There does not

appear to be a clear relationship between the number of stalk segments and the maturity of the stolon; stalks with about 8 segments can carry immature (Figure 2) or near fully mature stolons (Figure 3B).

Stolons sometimes occur in yoked pairs with one stalk branching from another (Figure 4), although unbranched stalks with well-developed stolons and no branches are usually present as well. At least some of these yoked pairs arise as an initial branch from the axial segments, which proceed to generate a stolon at its growing end; but another branch also forms from this stalk, which also produces a stolon. This newer stolon starts to develop after the first stolon has already developed somewhat, and hence is at an earlier developmental stage (Figure 4). Once this sequence has been recognized, it is possible to detect stalk pairs from which a mature stolon has already departed. The abandoned stalks show a characteristic terminus distinct from the growing tip of a new stolon or of a developing axial branch; it is smaller in diameter and appears as a short stubby appendage on the end of the stalk (Figure 4). This appendage develops a pair of anal cirri (Figure 5); the beginnings of an anal opening can be seen more clearly on better developed stubs.

Description of stolons

Male stolons ranged between 2.0–2.8 mm long, 0.50–0.65 mm wide (max.) for 18–22 chaetigers. Female stolons ranged between 3.15–3.50 mm long, 0.60–0.75 mm wide (max.) for 17–19 chaetigers. All specimens of *R. multicaudata* examined were observed to have developing stolons attached to stolon stalks in the midbody region within the sponge. The stolons of any adult specimen were consistently either male or female.

One specimen (QD4) from a sponge which measured ~5.3 × 4.2 cm, was dissected out as completely as possible. It had about 375 branches and over 500 termini, including 36 stolons, all female, most of which contained a full complement of oocytes. Three other sponge specimens that were systematically searched for the presence of worms also showed that, within any host sponge, attached stolons were all of one sex or all of the other. Thus the available evidence indicates that the worms have fixed separate sexes (see Discussion).

The head of the stolons is deeply bifid, has two pairs of eyes and lacks antennae and palps. Male and female stolons differed significantly in appearance (Table 1). The first chaetiger
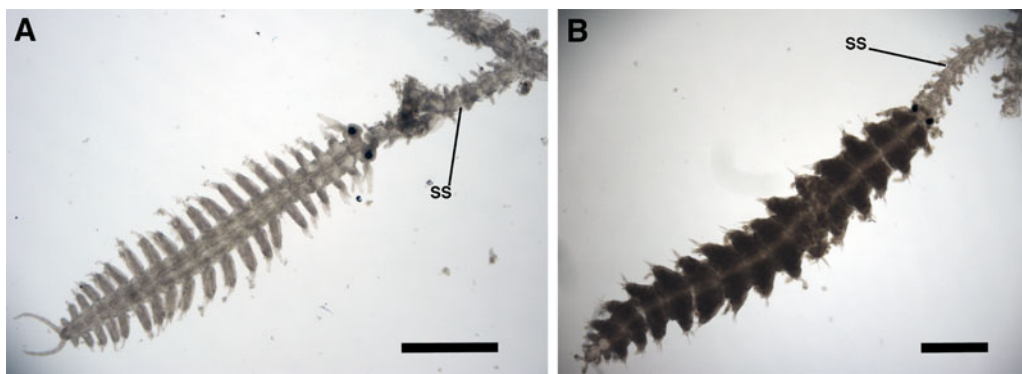

Fig. 3. Near mature stolons of *Ramisyllis multicaudata* and attached stolon stalks (ss), (A) male; (B) female. Scale bars: 0.5 mm.
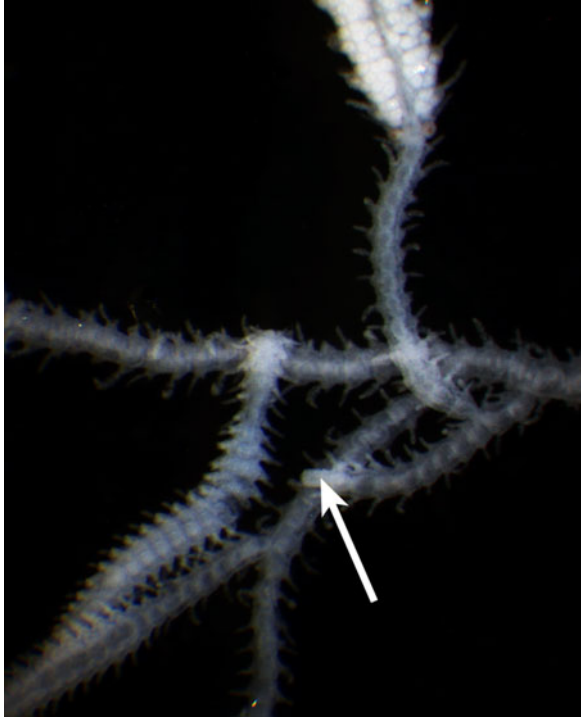
Fig. 4. *Ramisyllis multicaudata*, female stolons in yoked pairs. Note terminus at stolon detachment point. Scale bar: 0.5 mm.

of both male and female stolons is slightly modified, almost fused to the head, and carries dorsal cirri, a neuropodium bearing chaetae and ventral cirri (Figures 5 & 6A, B). The first pair of dorsal cirri of the male is 2–3 times as long as those on the subsequent stolonal segments (in the female it is about the same length as the later dorsal cirri). Female stolons are laden with oocytes, usually well-developed and
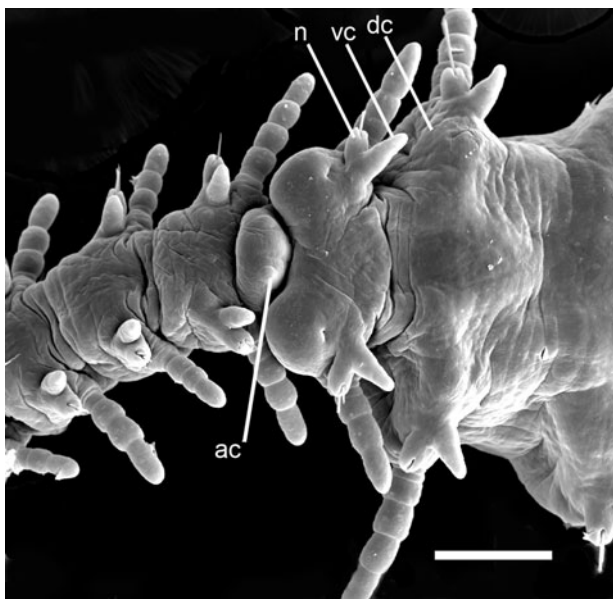

Fig. 5. *Ramisyllis multicaudata*, Scanning electron micrograph of developing female stolon attached to stalk segments of parent-stock, anterior end, ventral view. Note minute developing anal cirri (ac), dorsal cirrus (dc), neuropodia (n) and ventral cirrus (vc) of first chaetiger. Scale bar: 0.1 mm. Modified after Glasby *et al.* (2012).

readily seen by the time the stolon and its branch are easily distinguished from somatic branches. One female stolon (Figure 6B) contained between 250 and 300 oocytes; oocytes develop in the coelom between chaetigers 1–4 and 13–14 (Figures 6A & 7B). In one specimen (CG 16–03 sponge 2) containing about 10 isolated mature female stolons, very few oocytes were remaining, suggesting that spawning had occurred inside the sponge prior to collection (see Discussion).

The body of males is slimmer in most cases than that of the females and the parapodia are relatively better developed, including the presence of a lanceolate neuropodial lobe, which is absent in the female (Figure 7; Table 2). Further, the dorsal cirri of the male are unarticulated whereas those of the female are articulated. The ventral cirri of both sexes are unarticulated and lingulate. Fully mature males had their anterior three segments filled with pale material (Figure 3A) – interpreted here as sperm masses – which often caused these segments to appear slightly swollen. Histological sections of less mature, presumed, male stolons (without such apparent sperm localization) showed only gonia at immature stages in the coelom. Since no gametes have been observed in segments of the stalk (above), we believe that gametogenesis occurs within the stolon (spermatogenesis could be easily missed).

Mature stolons of both sexes have, in addition to typical neurochaetae of the stock (Figure 8A, B), paddle-like notochaetae which differ from the capillary types of other sylline stolons (Figure 8A, B). The chaetae are arranged in two distinct chaetal bundles (each with about 10 notochaetae) adjacent to a thin notoacicula. The paddle-like chaetae are simple, obliquely truncate and distally tapered to a thin, drawn-out tip, very transparent, and difficult to see until one is aware that the notochaetal bundle diffracts light; thus a small rainbow in the parapodium of a preserved specimen under a dissecting microscope indicates the presence of this notochaetal bundle, even though the individual chaetae cannot be easily distinguished (Figure 6B, segment 4). Paddle chaetae appear to develop late in the formation of the stolon, since they are not detectable in those still attached to their stalks or in recently detached male stolons. Their shape and late occurrence suggests a swimming function, in which case they are similar to the natatory chaetae of other Syllidae.

One female stolon of 18 chaetigers had three heads anteriorly (Figure 9); we suggest that this represents aberrant growth rather than regeneration of lost anterior segments, because the specimen otherwise conforms to the 17–19 fixed chaetiger number of females, and the general pattern of segmental oocyte development (anterior and posterior region free of oocytes). The posterior-most head and following segments (including the bulb-like vestigial gut) had the appearance of a normally developed female.

Comparison of stalks and stolons between *R. multicaudata* and *S. ramosa* (pooled data from Japanese and Philippine specimens)

There appear to be few, if any, differences in the form of the stolon stalk between the two species. In both species, chaetiger width is less than adjoining branches of the stock, and the parapodia and chaetae are the same form as those of the adjoining stock segments. *Ramisyllis multicaudata* has 6–25

Table 1. Morphological differences between male and female stolons of *Ramisyllis multicaudata*.

	Female	Male
Chaetiger number	17–19	18–22
Prostomium colour	Yellow	Unpigmented
Eyes – relative sizes of dorsal *vs* ventral pairs	Dorsal pair slightly smaller than ventral pair	Dorsal pair about $\frac{1}{2}$ size of ventral pair
Vestigial gut, chaetiger 2	Present, bubble-like	Absent
Parapodia – relative length	About $\frac{1}{2}$ width of body	About width of body
Neuropodial lobe	Lanceolate neuropodial lobe absent	Lanceolate neuropodial lobe present
First pair dorsal cirri (head cirri) – length	About same length of later dorsal cirri	2–3 times length later dorsal cirri
Dorsal cirri – form	Articulated; extend well beyond parapodial tips	Smooth; just reaching parapodial tips
Dorsal cirri – colour	Pigmented	Unpigmented
Ventral cirri – form	Similar length to parapodial lobe	3–4 times longer than parapodial lobe
Pygidial cirri – form	Short (about 3× pygidium width), beaded	Long (about 7–9× pygidium width), beaded
Gamete/gonia distribution (immature)	Chaetigers 4–13 (approx.)	Spermatocytes throughout coelom
Gamete distribution (mature)	Chaetigers 1–14 (approx.)	Localization of mature sperm in anterior 3 chaetigers

Table 2. Comparison of key variable developmental features of *Ramisyllis multicaudata* with other ribbon clade members.

	Type of schizogamy	Regeneration of posterior segments while stolon attached to stock	Stolons – dimorphism	Stolons – natatory chaetae (presence; type)	Stolon type
Eurysyllis	Scissiparity	Absent	Not described	Present: capillary	Acerous
Parahaplosyllis	Collateral budding (gemmiparity)	Present, ventral to stolon	Not described	Absent	Dicerous
Ramisyllis	Branching gemmiparity	Present, ventral to stolon	Dimorphic (see Table 2)	Present: paddle	Acerous
Trypanobia	Successive budding (gemmiparity)	Not described	Not described	?	Dicerous
Trypanosyllis	Collateral budding (gemmiparous species); scissiparity	Present or absent	Not described	Absent, except in *T. crosslandi*	Acerous
Xenosyllis	?	?	?	?	?

Data for *Ramisyllis* from this study; those for other taxa from Potts (1913), Gravier & Dantan (1928), Okada (1933), Aguado & San Martín (2009), Aguado *et al.* (2012, 2015a, c) and Álvarez-Campos *et al.* (2013). Note that *Trypanosyllis* is very probably not monophyletic, and the gemmiparous species might well be different from those that are scissiparous.
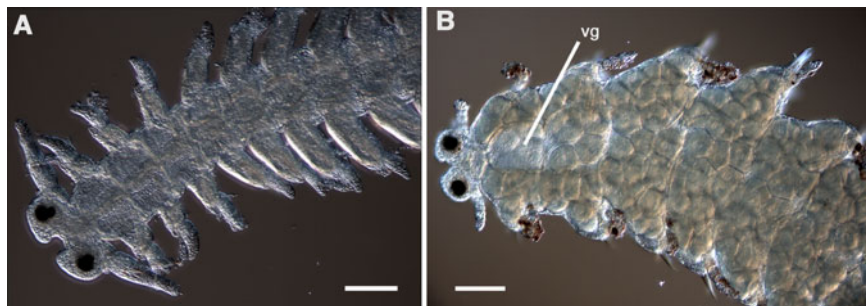
Fig. 6. *Ramisyllis multicaudata* Anterior end of male stolon (A) and anterior end of female stolon (B) photographed using differential interference optics (Nomarski). vg, vestigial gut bulb. Scale bars: 0.1 mm.
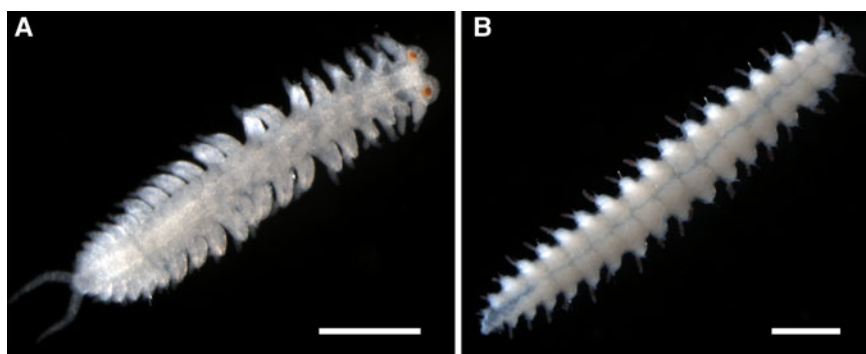
Fig. 7. *Ramisyllis multicaudata*, (A) male stolon, dorsal view; (B) female stolon, ventral view. Scale bars: 0.5 mm.
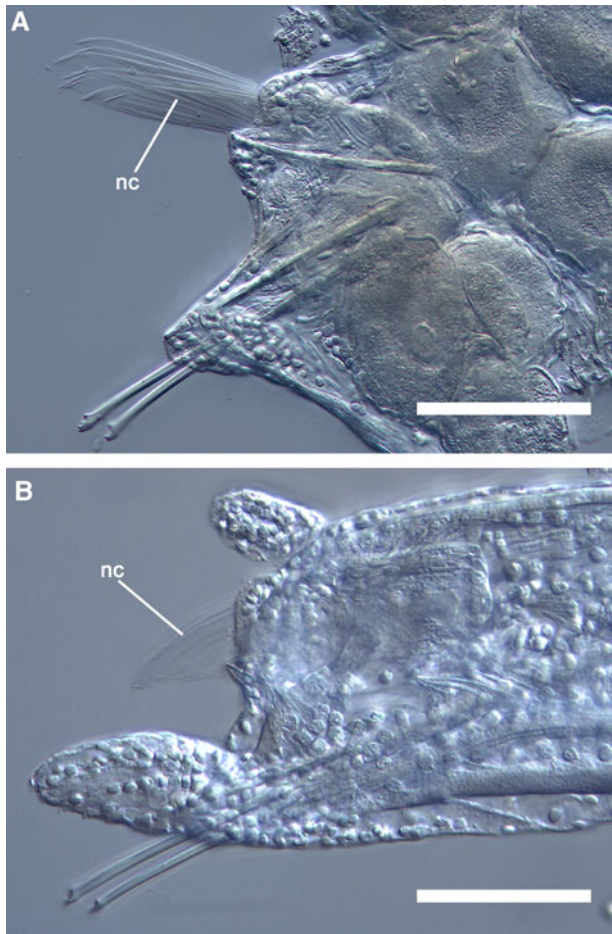
Fig. 8. *Ramisyllis multicaudata* midbody parapodium of stolons (dorsal side up), showing presumptive natatory chaetae (nc). (A) male; (B) female. Scale bars: 0.1 mm.

Fig. 9. Aberrant polycephalic female stolon of *R. multicaudata* photographed under a stereo microscope using top and bottom oblique-angle lighting. Scale bar: 0.5 mm.

cirri, 0 (male), 3–4 (female) compared with 4–5 (male), 13 (female) (Table 3).

DISCUSSION

Gametogenesis and stolon aberrance in *Ramisyllis multicaudata*

The site of gametogenesis in stolon-forming Syllinae is variable. Compared with *R. multicaudata* where gametogenesis takes place within those segments that form the stolon, the gametes in *Haplosyllis spongicola* (Grube, 1855) originate in the stock and are later transferred to the stolon (Wissocq, 1966); in *Typosyllis antoni* Aguado, Helm, Weidhase & Bleidorn, 2015b they are developed within a large number

stolon stalk chaetigers compared with the slightly greater maximum range of 4–33 for *S. ramosa* (Table 3).

The stolons of the two species have very similar head morphologies with two pairs of similar-sized eyes and lack of palps and antennae. However, there are several differences between the two species not associated with the head. Compared with *S. ramosa*, the female stolons of *R. multicaudata* are smaller (3.15–3.5 cf. 9 mm), have fewer chaetigers, 18–22 (male), 17–19 (female) compared with 29–33 (male), 29 (female), and have fewer articulates in the dorsal

Table 3. Comparison of key morphological differences in the stolons and stolon stalks of *Ramisyllis multicaudata* and *Syllis ramosa*.

	R. multicaudata	*S. ramosa*
Stolon stalks		
Chaetiger number	6–25	4–33
Stolons		
Chaetiger number (male)	18–22	29–33
Chaetiger number (female)	17–19	29
Length (female; mm)	3.15–3.5 mm	9 mm
Number dc articulations (male)	unarticulated	4–5
Number dc articulations (female)	3–4	13
Natatory chaetae – form	paddle-like; tip not bifid (male and female)	paddle-like; tip bifid (female)

Dc, dorsal cirri. (*S. ramosa* data from observations of syntype material (BMNH, OUM) and Japanese specimens (NSM), and from the literature – McIntosh 1879, 1885; Imajima, 1966; all specimens of *S. ramosa* assumed to be conspecific).

of posterior segments including those that are later transformed into the stolon; when the stolon is completely developed, it detaches, but some posterior segments full of gametes still remain for later stolonizations (Aguado et al., 2015b; MTA, personal observation).

The finding of a multi-headed stolon in *R. multicaudata*, while noteworthy, is not unique. The development of aberrant polycephalic stolons in other Syllinae has been explained by the absence of the proventricle (artificially removed in lab conditions) (Weidhase et al., 2016 and references therein). However, we cannot confirm that the specimen of *R. multicaudata* that developed one aberrant stolon had lost the proventricle. In contrast, this specimen possessed several stolons with all except one showing normal development. This might suggest that other factors, apart from the proventricle and its role in controlling reproduction, are involved in stolon development in *R. multicaudata*. Such polycepahlic stolons have been produced experimentally in the genera *Trypanosyllis* (Junqua, 1957) and *Typosyllis* (Weidhase et al., 2016).

Stolon morphology in Syllinae

The morphology of stolons across the Syllinae is poorly known and based mostly on those attached to the parent stock rather than free swimming forms. Thus, comparison of late-developing stolon features such as the appearance of presumptive natatory chaetae, maximum number of chaetigers, eye development, and pigmentation patterns, all of which may occur or be completed after stolon detachment, is currently not possible. Despite this limitation, several authors including Malaquin (1893) and Potts (1911) have attempted a classification of sylline stolons based on head morphology. Five stolon types are currently recognized, in order of increasing complexity: acephalous (no head), acerous (a head but no head appendages), dicerous (palps only), tetracerous (palps and lateral antennae), and pentacerous (palps, lateral antennae and a median antenna). Note that the appendages of dicerous stolons, referred to here as palps because of their position on the head and because they are not apparently articulated, is not universally agreed upon. Potts (1911) originally referred to them as lateral tentacles, and subsequent authors have called them antennae. However, we prefer to confine the concept of stolon antennae to the articulated appendages of tetracerous and pentacerous stolons, which is in keeping with the homologies of prostomial antennae established by Rouse & Fauchald (1997) for Phyllodocida in general. The nature of the paired appendages on the head of dicerous stolons needs further study. Notwithstanding these somewhat confused concepts, it seems clear that the stolons of *R. multicaudata* are of the acerous type. This is indicated by the deeply bilobed anterior prostomium, two pairs of eyes, absence of antennae and palps (Potts, 1911; San Martín, 2003); additionally they show sexual dimorphism.

Comparisons with *Syllis ramosa*

As in *R. multicaudata*, the stolons of *S. ramosa* are most probably acerous. Most of McIntosh's illustrations of heads show the absence of antennae and palps, characteristic of acerous stolons. His figure 10 in pl. XXXIV$_A$ shows a stolon with a small pair of 'antennae', characteristic of a dicerous stolon; however, McIntosh (1885: 201) seems to express some

doubt about the species identity of the stolon, as its supposedly young stage (small oocytes) did not match its high number of segments (31).

The presence of a branch bearing a developing stolon at its tip is obviously a feature of branching syllids, since to our knowledge such a structure has only been found in *R. multicaudata* and *S. ramosa*. If these taxa are not closely related as the current taxonomy suggests, the branching body pattern might have evolved independently. Both species are schizogamous; however, because multiple stolon producing stalks may appear at the same time, in this sense the species are gemmiparous (see Garwood, 1991 for descriptions of typical examples of these two systems of stolonization). Aguado et al. (2015a) used the term 'branching gemmiparity' to more accurately describe this type of reproductive mode.

McIntosh (1885) suggested that *S. ramosa* from the type locality in the Philippines was viviparous. We have found no sign of viviparity in either his material (from the BMNH) or that from Japan. Therefore, *S. ramosa* was erroneously included in the Schroeder & Herman's (1975) list of viviparous species. Since the available Japanese specimens do not include stolons, the following observations come from Okada (1937). In the Japanese *S. ramosa*, the stolon stalk is composed of a variable number of segments, all of which are typical in their structure. They thus differ from McIntosh's (1885, pl. XXXIII, figure 11) description of the *Challenger* material, in which the stolon stalks are formed of four segments, each of which has a pair of small 'tentacles', which might represent the dorsal cirri of rudimentary parapodia. Okada (1937) doubted that the condition described by McIntosh (1885) actually occurs, suggesting that the two might be different species; however, not enough information is available to answer this question. McIntosh (1879, 1885) did not report how many such stalk-stolon combinations he observed. He notes that some male stolons have gametes in eight segments. McIntosh (1885, pl. 33, figure 11) illustrates the attachment of a developing female stolon of his *S. ramosa* to the main axis of one of the branches. It is attached by a segmental stalk four segments long, on which the parapodia are either incompletely developed or (in the two segments closest to the body) absent.

Comparisons with other members of the ribbon clade and other Syllinae

Like other members of the ribbon clade, *R. multicaudata* appears to have acerous stolons (see Aguado et al., 2012, although the stolons of *Parahaplosyllis* were not known at that time). Okada (1933, 1937), Álvarez-Campos et al. (2013) and Aguado et al. (2015c) take a different view on the nature of the head appendages thus coming to the conclusion that both acerous and dicerous stolons are present in members of the ribbon clade. Clearly further comparative studies are required to elucidate the nature of these stolon head appendages.

At present, we can find no exclusive common reproductive characteristics among the ribbon clade taxa. Features such as stolons lacking regionalization, absence of brooding, and embryonic development not connected with the parental body, are simply plesiomorphic absences found in most Syllinae. The reproductive features that are variable within the clade and currently unique to *Ramisyllis*, such as

branching gemmiparity, presence of presumptive natatory chaetae in late stolons and dimorphic males/females (Table 1) may also be present in other Syllinae; however, whether they represent convergences or homologies is uncertain. For example, natatory chaetae somewhat resembling those in *R. multicaudata* have been found in the only known specimen of *Nuchalosyllis lamellicornis* Rullier & Amoureux, 1979, although these are pointed rather than truncate (Aguado & San Martín, 2009). Nothing is known of reproduction in this species, nor were such chaetae observed in a second species of *Nuchalosyllis, N. maitieae* Fukuda & Nogueira (2013). This taxon also resembles other ribbon clade members in terms of general body form (flattened body), and therefore would be a valuable addition to any future phylogenetic analysis of the group. *Ramisyllis* appears to have separate sexes producing only male or only female stolons; sequential hermaphroditism is also a possibility and will be the subject of further investigation (below). We do not know whether stolons of both sexes leave the sponge to mate in open water, or if one is visited by the other inside a sponge. We have never observed developing larvae or embryos inside a sponge. Males appear to be less swollen and have fewer sperm-filled segments compared with typical sylline stolons such as *Typosyllis pigmentata* (PCS, personal observation), which spawn in open water and are usually as stuffed with gametes as are the females. Such a strategy would suggest that spawning occurs within the sponge. An intra-sponge spawning hypothesis is also supported by the finding of mature female stolons inside the sponge having very few eggs remaining inside the coelom. A population study using DNA microsatellites (or the whole genome) to compare adults, detached stolons and juveniles from different sponges would contribute to elucidating parentage as well as to assessing the probable spawning site.

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