

Supplementary data

Character analysis

Because ten Hove and Kupriyanova [59] provided a detailed illustrated description of morphological features used in taxonomy of serpulids, here we focus the character analysis on the coding decisions and comparisons with the coding in the earlier studies. Character numbers in the description below correspond to the character summary in Appendix A.

General body (1-2). The body is symmetrical in the outgroups and most serpulids but is asymmetrical in conjunction with tube coiling in spirorbins and *Spiraserpula* (char 1). Sabellids, fabriciids and serpulids have well delineated thoracic and abdominal regions, marked by chaetal inversion (char 2). In spirorbins, however, chaetal inversion is incomplete *sensu* Rzhavsky et al. [45]. Both body symmetry and chaetal inversion were used as binary (A/P) characters in Kupriyanova [58] and Kupriyanova et al. [33].

Radiolar crown (3-7). The radiolar crown bears radioles with paired ciliated pinnules in sabellids, fabriciids and serpulids. The lobes of the radioles are completely separated in serpulids and fabriciids but are fused in sabellids (char 3). The base of the radioles in sabellids and serpulids may be joined together by an inter-radiolar membrane (char 4), but this has never been recorded for fabriciids. Although the membrane is very high in some taxa, such as, for example, in *Pyrgopolon* where it unites radioles for up to half of their length, it is less developed and absent in other taxa [59]; no attempt was made here to distinguish multiple states of the character based on the membrane length. This character was used in both Kupriyanova (2003) [58] and Kupriyanova et al. (2006) [33]. Generally, the absence/presence of char 4 is a constant character within a genus; however, Bastida-Zavala and ten Hove (2003) [81] mention the presence of an inter-radiolar membrane in two out of eight specimens of *Hydroides trompi* Bastida-Zavala and ten Hove, 2003, while presence never has been recorded for any of the other species of *Hydroides*.

Ten Hove and Kupriyanova (2009) [59] reviewed of the types of eyes (simple ocelli, ocellar clusters, and compound eyes) in serpulids and the documented distribution of eyes in the group. They conclude that, although the eyes may ultimately be useful in some taxonomic decisions, these characters have not been systematically studied for most serpulids. Unfortunately, the pigment associated with ocelli tends to fade in ethanol-preserved material, so eyes are difficult to find, and as a result, they are not commonly mentioned in species descriptions. Here we score radiolar eyespots (char 5) as absent or present, without distinguishing the types of the ocelli or their position. The character was used in [33, 58]. Eyes are absent in fabriciids, but are known in some sabellids including the outgroup, *Schizobranchia*, used here. Bok et al. (2017) [82] provided the most recent overview of sabellids eyes.

Arrangement of radioles (char 6) was coded as a multistate character (semi-circular, pectinate and spiral) according to [59]. The character was not used in previous studies. External unpaired finger-like projections known as stylodes (char 7) is a feature found in the monotypic *Dasynema* only. They are different from the paired stylodes found in the sabellid genera *Pseudobranchiomma* and *Branchiomma* [59]. Because *Dasynema* was not included in any previous analysis, the character has not been used before.

Tubes (8-21). Whereas most sabellid tubes are made of mucus and muddy or sandy sediment, serpulids build tubes of crystalline calcium carbonate and a mucopolysaccharide matrix using calcium glands located on the collar and the secretory epithelium on the ventral side of the thorax (e.g. Vinn et al. 2009 [83], reviewed by Ippolitov et al. 2014 [84] and Kupriyanova et al. 2020 [7]). This major dichotomy is scored as tube material (char 8). Tube additions are molded by collar folds when the worm is in feeding position at the entrance of the tube (reviewed in [7]). The shape and outer sculpture of the resulting tube apparently depend upon the degree of rotation of the worm and the shape of the collar folds. The presence or absence of longitudinal keels on the tubes (char 9) is used as a character for the first time in this study. It is additional to the characters 10-13 “tube cross-section” (semi-circular, triangular, quadrangular, and trapezoid) that were used in [33] a multi-state character.

A “granular hyaline overlay on the tube” (char 14) [59] is a character not been used before and is very rarely reported in literature. We treat the presence of a very thin granular overlay separately from a proper outer hyaline (transparent) layer on the top of the opaque internal layer. This character (tube wall transparency, char 15) was a binary character in [33], but here a third state (completely hyaline) is introduced to accommodate *Placostegus*, *Vitreotubus*, and *Neomicrorbis*, which were not included in earlier analyses.

Tubes are coiled either in a tight or in ascending spiral in most spirorbins, the feature that gave the name to the group. The shape of serpulid tubes can very variable even within a species, so we treat this variability (char 16) as a binary character with states “straight or irregular” and “coiled”. Asexual budding can result in chains of tubes (*Filogranula*) or in combination with and gregarious larval settling in intertwining “colonies” (in taxa such as *Salmacina*, *Filograna*, and *Filogranella*), treated here as a separate character (char 17), as also was done in [33]. All serpulid larvae settle and initially attach to hard substrate, but some detach and can inhabit soft sediments unattached (*Ditrupa*, *Bathyditrupa*, *Spirodiscus*), which is treated here as the character “adult tube attachment” (char 18).

The internal tube structures (char 19) have never been used before and refer to a synapomorphy that unites species in the genus *Spiraserpula*. Another new character introduced here is the presence of tabulae (char 20), the transverse tube elements that may partition the oldest parts of the tube as a response to tube damage [59]. Although the tube of serpulids is usually uniformly white, some taxa rarely have completely or partly pink, orange, mustard, bluish or purple tubes; tube colouration was treated as a separate character (char 21).

Operculum (22-38). The presence of an operculum (char 22), a modification of the distal part of a radiole, is typical for many serpulids, but is unknown in the outgroups. Serpulids such as *Serpula*, *Spiraserpula*, *Crucigera*, and *Hydroides* are characterized by the presence of a pseudoperculum (char 23), an underdeveloped operculum carried on the side of the opercular crown opposite to the functional operculum. However, the pseudopercula on shortened opercular peduncles typical for the *Serpula*-*Crucigera*-*Hydroides* clade [81] appear to be different from pseudopercula on a long pinnulated radiole found in *Vermiliopsis* and *Metavermilia*. A new character pseudoperculum on radiole (char 24) was added to accommodate this.

Unlike [33], we do not score opercular types based on the general shape because most of them, or at least the basal opercular ampulla, fall in the conical or globular category. The only exception was done for

the very distinct funnel-shaped *Serpula*-type opercula found in *Serpula*, *Crucigera* and *Hydroides* (char 32). Serpulid opercula differ by the structure of the reinforcements, mainly of the endplates. The operculum may be lacking reinforcement (soft and membranous, as in *Filograna* or *Apomatus*), reinforced with chitinous structures (e.g., *Metavermilia*, *Vermiliopsis*, *Ficopomatus*), reinforced with calcium carbonate deposits and spines (e.g., *Spirobranchus*, *Galeolaria*), or with a thickened cuticle as in *Serpula*, *Crucigera*, and *Hydroides* [59]. Here the presence or absence of opercular reinforcements is treated as a separate character (char 25), and the type of reinforcement as separate binary characters (char 26-28), while in [33] it was coded as a single multistate character. Also, in contrast with [33], we treat the presence of chitinous spines (char 29) as a binary character separate from the shape of the chitinous opercular endplate (char 30 multistate), because the spines may not be associated with an endplate, as for example, in *Ficopomatus enigmaticus*. For the opercular structure, we also have characters based on basal bosses below the operculum (char 31) that are found in *Crucigera* and *Janita* only (contrary to the use by ten Hove [57]). To distinguish opercula within the *Serpula*-*Crucigera*-*Hydroides*, we coded *Serpula*-type operculum (a funnel made of fused radii) with or without a complex distal verticil of chitinous spines as found in *Hydroides* (char 33).

The types of calcareous opercular reinforcement are coded here as a multistate character (char 34). The movable spines of *Galeolaria* and non-movable spines of *Spirobranchus* are treated here as a separate character (char 35), in contrast to the coding in [33]. Unlike calcareous spines, which are directed upwards, calcareous talons (char 36) are extensions of the opercular calcareous distal plate that are directed downwards, inside the opercular ampullae. They are commonly found in spirorbins, whereas in other serpulids well-developed talons are found only in *Pyrgopolon* and in some species of *Spirobranchus* (see e.g., Simon et al. [85]).

As in [33], an opercular constriction (char 37) is coded as a binary character to distinguish the condition where the operculum gradually merges into the opercular peduncle from that when it is clearly distinct from the opercular peduncle. Finally, opercular ontogeny (char 38) was used as a new binary character with the states “direct” and “indirect”, with attention paid to the restrictions discussed in [59].

Peduncle (39-44). In some serpulids the radiole that bears the operculum is identical to others (e. g., *Filograna*, *Apomatus*, *Zibovermilia*), but in most it is modified into a smooth opercular peduncle. Unlike Kupriyanova et al. [33], who coded the opercular peduncle shape as single binary character (with the states “normal radiole” and “modified into opercular peduncle”), here we distinguish two characters. The first (char 39) distinguishes a normal radiole, though bearing an operculum, from modified ones. The second (char 40) distinguishes the presence or absence of pinnules on the distinct peduncle. This is because the operculum-bearing radiole may be a clearly modified peduncle much thicker than normal radioles, but still bearing pinnules, as in *Bathyditrupa* and *Spirodiscus*.

The opercular peduncle may have distal wings (e.g., *Spirobranchus*, and *Galeolaria*), which is treated as a binary character (char 41), as done in [33]. A binary character for proximal peduncular wings (char 42) is a new character for serpulid systematics [59].

Which radiole is ontogenetically modified to become the peduncle has been a matter of debate [59]. The insertion of the opercular peduncle (char 43) in either of three known positions is coded here as observed in the examined material. This character was not used in [33] but was used in [58]. The

peduncular shape in cross-section (char 44) was not used in [33] either, but was in [58], though in a different formulation. Both peduncle width (char 45) and surface texture (char 46) are new characters.

Collar (47- 53). The base of the radiolar crown of sabellids, fabriciids and serpulids is surrounded by a peristomial collar. In serpulids the collar (char 47) is usually sub-divided into two dorso-lateral and one medio-ventral lobe, but may be also un-lobed (e.g., *Ditrupa arietina*). The occurrence of elongated tonguelets (char 48) located between the dorso-lateral and ventral lobes of the collar were used in the same way as in previous studies [33, 58].

In almost all Sabellida, the collar segment (chaetiger 1) lacks neuropodial uncini and bears only notopodial chaetae (termed collar chaetae). It appears that uncini are secondarily lost in the collar segment, the biramous condition being original in annelids. Secondary loss of collar chaetae occurs incidentally in some species of *Spirobranchus* and *Pyrgopolon*. Collar chaetae are consistently absent in the genera *Ditrupa* (see above), *Marifugia*, *Placostegus*, and *Rhodopsis*). The presence or absence of these chaetae (char 49) is a standard feature in descriptive serpulid taxonomy [59].

The presence of special chaetae in the bundle of collar chaetae, in addition to simple limbate ones, has been another standard feature in serpulid systematics. These special chaetae are further treated (char 50) here as having either distal or basal modifications if compared with typical limbate chaetae. The distally modified special collar are found only in the genus *Ficopomatus*, they have coarse curved teeth alongside the distal part of chaetae. Basal modifications of special collar chaetae are coded as three binary characters (char 51-53). They refer to bayonet-type, with one or two large basal bosses at the base of the distal limbate zone, as in e.g., *Serpula*, *Crucigera*, and *Hydroides* (char 51); fin-and-blade (the basal boss ("fin") made of relatively few teeth of intermediate size, as in e.g., *Chitinopoma* (char 52); 'Spirobranchus-type' with a basal boss consisting of very numerous tiny hair-like spines (char 53).

Thorax and thoracic membranes (54-67). The dorso-lateral collar lobes in serpulids continue as thoracic membranes (char 54), a feature found only in serpulids, though the degree of thoracic membrane development varies markedly [7, 59]. They may end at any thoracic segment, or may continue to the end of the thorax, often fusing ventrally and forming an apron. Here we treat the length of thoracic membranes as one multistate character (char 55) and reduce the length diversity to four states, where state 4 is the development of an apron. The structure was used in a different way as a character in the analyses in [33, 58].

Thoracic notochaetae are simple, narrow and limbate in all serpulids, but in posterior thoracic segments of some genera they may be supplemented by very characteristic 'Apomatus' chaetae (char 56). This character has been widely used in serpulid taxonomy and was included in all previous analyses.

The thoracic neurochaetae and abdominal notochaetae of sabellids, fabriciids and serpulids are uncini, arranged in a torus side by side in a transverse line relative to the long axis of the body. Depending on the number of vertical rows of teeth in each uncinus, the uncini are usually termed saw-shaped (one row of teeth), saw-to-rasp-shaped (one row at one end to several rows at the opposite end), or rasp-shaped (several rows of teeth). The number of rows of uncinal teeth is coded here as three binary characters (char 57-59). The subset of terminals used in [33, 58] only allowed for the first two categories.

Sabellid and fabriciid uncini, unlike serpulid uncini, may have a main tooth (fang or peg *sensu* [59]) surmounted by a broader series of smaller teeth and a proximal handle. The anterior tooth (main fang) of sabellid uncini is considered homologous to that of serpulid uncini. The latter was coded as simple or bifurcate in [33, 58]. However, subsequent SEM examinations [59] revealed a previously undocumented variability and, in particular, showed that “bifurcate” anterior teeth are flattened gouged underneath structures. As a result, the shapes of serpulid anterior teeth of uncini (char 60-63) are re-formulated.

The number of teeth as seen in profile in thoracic uncini [59] has a fairly continuous variation, and although commonly reported character in descriptive taxonomy, it has been avoided in [33, 58]. In the present study, we subdivided the number of teeth into three states: < 8, 8-19 and > 20 (char 64).

The number of thoracic segments (char 65) is also fairly constant in most serpulids and has been used in both traditional taxonomic diagnostics and previous cladistic analyses. Usually, the thorax consists of seven thoracic chaetigers (i.e., six segments with both chaetae and uncini plus the collar segment with collar chaetae only). Rarely, serpulids have six (e.g., *Laminatubus alvini*, *Hyalopomatus*) or five (*Bathyditrupa hovei*) thoracic chaetigers including the collar segment with notochaetae. In spirorhynchans the number of thoracic chaetigers normally does not exceed four, although *Neomicrorbis* has 5-6 chaetigers. However, when collar chaetae are absent altogether, the number of chaetigerous segments no longer equals the number of thoracic segments. For example, *Spirobranchus* has seven thoracic segments, a collar chaetiger and six uncinigerous chaetigers; *Laminatubus* has six thoracic segments, a collar chaetiger and five uncinigerous chaetigers; however, some species of *Spirobranchus* lacking collar chaetae as adults (e.g., *S. kraussii*) have six uncinigerous segments. To avoid any confusion, we included in the matrix only the number of uncinigerous thoracic segments (char 65).

The situation is more complex in taxa such as *Filograna*, *Filigranella*, *Salmacina* and *Turbocavus* where number of thoracic segments varies from six to 12. Thus, we introduce a variable number as separate character (char 66), which has not been done in previous studies.

Thoracic uncinigerous tori are generally positioned similarly along the lateral side of the thorax, but in some taxa they are widely separated in front, gradually approaching one another posteriorly, so that the posterior thoracic tori may touch each other, forming a triangular depression on the ventral side of the thorax. This triangular depression in the thorax (char 67) is a feature that has not been used in previous studies.

Abdomen (68-75) The presence of an achaetous region in the anterior abdomen (char 68) is a character used for the first time to phylogenetic inference among serpulids. Detailed examination of abdominal chaetae with SEM [59] revealed a previously unreported diversity of ultrastructure within what was traditionally referred to as “geniculate chaetae”. As a result, we distinguish five characters for abdominal chaetae (char 69-73) as opposed to three used in [33]. The description of these types is detailed in [59]. SEM examination showed that only *Bathyditrupa hovei* possesses simple capillary chaetae (char 69) and only *Paumotella* has acicular chaetae (char 73). The majority of serpulid abdominal chaetae can be grouped into three major categories: flat trumpet-shaped chaetae (char 70), true trumpet-shaped chaetae (char 71), and flat geniculate chaetae (char 72). The final two binary abdominal characters, the presence of posterior glandular pad (char 74) and the posterior long capillary abdominal chaetae (char 75) have not been used in previous studies.

Characters not used

Some features used in taxonomic descriptions of serpulids were not used as characters for this analysis. The number of radioles is an unreliable character. The lower limit of the number of radioles is meaningless because all juveniles have fewer radioles than adults, while the upper limit is commonly a function of size. The long filiform ends of radioles (filamentous tips) sometimes mentioned in serpulid descriptions have not been systematically documented and the feature is variable on the infraspecific level [59]. Similarly, we did not use body size as a character.

The presence of mouth palps (dorsal lip outgrowths) is another feature not commonly used in serpulid systematics. Although the binary character was also previously used in [33, 58], it still is poorly understood and apparently in serpulids all “palps” may not be homologous [59]. Similarly, tube ultrastructures are highly variable in serpulids and can be potentially used for phylogenetic inferences, but they are still poorly documented [84].