JCZ: Zoologischer Anzeiger - A Journal of Comparative Zoology 325 (2026) 222–236

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

A phylogeny reconstruction of substrate selection by tube-dwelling worms within the genus *Spirobranchus* (Polychaeta: Serpulidae)

Roeland J. van der Schoot [a,b], Dagmar Wels [a], Charles H.J.M. Fransen [a], Harry A. ten Hove [a], Bert W. Hoeksema [a,b,*]

[a] *Naturalis Biodiversity Center, P.O. Box 9517, Leiden, 2300 RA, the Netherlands*
[b] *Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, Groningen, 9700 CC, the Netherlands*

ARTICLE INFO

Handling Editor: Maikon Di Domenico

Keywords:
Coral-association
Host specificity
ITS2
Cytochrome b
Cryptic diversity
Phylogeography

ABSTRACT

Large tube-dwelling polychaetes of the genus *Spirobranchus*, popularly known as Christmas tree worms, are predominantly known as coral associates on tropical shallow reefs. In temperate seas, species are generally smaller and mostly occur on hard substrates, as solitary individuals or colonial aggregations. Coral-associated *Spirobranchus* species have been recorded from a broad host range and occasionally from non-coral substrates, indicating a generalist strategy in terms of host selection. Other *Spirobranchus* taxa display preferences for abiotic substrates or scleractinian host species. This raises the question whether their substrate preferences are phylogenetically determined and if there is a biogeographical component herein. This study infers phylogenetic relationships from 165 newly sequenced specimens analysed with ITS2 and *cytochrome b* markers, complemented by publicly available sequences, and integrates substrate types and host-coral taxa by projecting them onto the resulting trees alongside their geographic records. This represents one of the largest phylogenetic datasets available for *Spirobranchus*. Using a family-level framework, coral association vs. abiotic substrate is evaluated as a sessile strategy in an evolutionary context. Two principal *Spirobranchus* clades were recovered, both represented by Atlantic and Indo-Pacific species, with coral association occurring as a polyphyletic trait next to alternative substrate preferences. The Indo-Pacific *Spirobranchus corniculatus* and the Atlantic *Spirobranchus giganteus* occupy broad ranges of host species, with *S. giganteus* appearing to be the most generalist of the two. The most common species on Caribbean reefs, *S. giganteus,* reveals minimal genetic variation, whereas *S. corniculatus,* which is dominant on Indo-Pacific reefs, comprises multiple lineages that correspond to their geographic localities.

1. Introduction

Coral-associated invertebrate species form an important component in the biodiversity on coral reefs. The number of described species was 869 in 2011 (Stella et al., 2011) and has since then increased due to the discovery of new cryptic species and associations across various phyla, such as Annelida, Arthropoda, Cnidaria, Ctenophora, and Mollusca (Gittenberger and Gittenberger, 2011; Alamaru et al., 2017; Montano and Maggioni, 2018; Rauch et al., 2019; Hu et al., 2020; Mehrotra et al., 2020, 2024; Maggioni et al., 2022; Jia et al., 2023; Britayev et al., 2025; Kolbasov et al., 2025). Because many reef taxa are undescribed, species records for coral reefs substantially underestimate true diversity (Fisher et al., 2015), a problem that also applies to coral-associated invertebrates, many of which are cryptic because of their small size, camouflage and/or hiding behaviour (Hoeksema, 2017; Montano, 2020, 2022).

Coral associates form a vulnerable group as their existence depends on the survival of their hosts (Goulet and Goulet, 2021; van der Schoot and Hoeksema, 2022, 2024). Among coral-associated invertebrates, the genus *Spirobranchus* Blainville, 1818; Serpulidae Rafinesque, 1815) is a well-known, extensively described and globally occurring group of tube-building polychaetes. Half of its species that occur in the tropical zone have been described as completely or partly coral-associated (van der Schoot and Hoeksema, 2024). They are popularly known as Christmas tree worms, owing to their colourful branchial crowns consisting of two conical whorls.

Coral-associated *Spirobranchus* species, both in the Indo-West Pacific and in the Atlantic, are known to occur in association with a wide variety

* Corresponding author. Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA, Leiden, the Netherlands.
E-mail address: bert.hoeksema@naturalis.nl (B.W. Hoeksema).

https://doi.org/10.1016/j.jcz.2026.10.003
Received 26 April 2026; Received in revised form 11 October 2026; Accepted 13 October 2026
Available online 14 October 2026
0044-5231/© 2026 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

of scleractinian coral species and are therefore considered host generalists (Perry et al., 2018b; Hoeksema et al., 2019b; Idris et al., 2022; van der Schoot and Hoeksema, 2024). Several coral-associated *Spirobranchus* species, both Atlantic and Indo-Pacific, have been observed living on dead coral or non-coral substrate (Nishi, 1996; van der Schoot et al., 2016; Perry et al., 2018b; Hoeksema et al., 2019b; Idris et al., 2022) implying a facultative symbiosis with live coral. For example, *Spirobranchus giganteus* (Pallas, 1766) was observed living in octocorals, ascidians, sponges and zoantharians as secondary hosts that had overgrown their primary scleractinian hosts (Hoeksema et al., 2015, 2020; García-Hernández and Hoeksema, 2017). Despite their generalist character, host-coral preference has been documented repeatedly (Hunte et al., 1990b; Rowley, 2008; Montebon and Yap, 2009; Idris et al., 2022). Settlement experiments with *Spirobranchus* larvae show a preference for certain coral species as well as the presence of conspecifics, which in the present study is referred to as host selection (Marsden, 1987; Marsden and Meeuwig, 1990; Marsden et al., 1990). This mechanism is considered to be driven by chemical cues (Marsden and Meeuwig, 1990), as observed in the serpulid species *Galeolaria caespitosa* Lamarck, 1818; Watson et al., 2017).

Non-coral-associated species of *Spirobranchus* are most common in temperate regions, either living solitarily on hard substrate, such as rock and shells, or overgrowing each other due to gregarious settlement, thereby producing serpulid reefs consisting of their calcareous tubes (Gosselin and Sewell, 2013; Nishi et al., 2022). Both sessile life forms have been observed within the same species (ten Hove and van den Hurk, 1993; Smith et al., 2012), indicating that environmental factors may determine settlement, selection of substrate type, and subsequent development (Chan et al., 2014; Gosselin et al., 2019; Wolf, 2024). Within the family Serpulidae the association with live coral and the formation of aggregates are both not unique to *Spirobranchus*, since several other serpulid species have been reported to be associated with living corals (van der Schoot and Hoeksema, 2024) or are known to display gregarious settlement (Watson et al., 2017; Giangrande et al., 2020).

The most common coral-associated *Spirobranchus* species are *S. giganteus* in the West Atlantic Ocean and *Spirobranchus corniculatus* (Grube, 1862) in the Indo-West Pacific Ocean (Rowley et al., 2026). Both have been recorded from a wide range of host-coral species and occasionally also from non-coral substrates (Scott, 1987; Hunte et al., 1990a; Dai and Yang, 1995; Floros et al., 2005; Hoeksema et al., 2019b; Idris et al., 2022; van der Schoot and Hoeksema, 2024). With regard to host corals, they seem to favour massive growth forms with small polyps in a shallow environment (Dai and Yang, 1995; Rowley, 2008; van der Schoot and Hoeksema, 2022). Both species have also been reported to cause injuries to their hosts, the size of which differs among host coral species, depending on their polyp morphology (Hoeksema et al., 2019a, 2019b; van der Schoot and Hoeksema, 2025). Phylogenetic studies have demonstrated several distinct clades for *S. corniculatus* that are not coherent with opercular morphology or geographic locality of the specimens, which therefore led to the synonymizing of both *Spirobranchus gaymardi* (Quatrefages, 1866) and *Spirobranchus cruciger* (Grube, 1862) with *S. corniculatus* (see Willette et al., 2015). The much-studied *S. giganteus,* the type species of its genus, has been largely neglected in modern phylogeny studies despite its wide distribution and common occurrence in the tropical West Atlantic region. To date, only one specimen has been sequenced (Seixas et al., 2017), which was included only once in a phylogenetic analysis with its congeners (Nishi et al., 2022).

The kind of substrate to which polychaetes are attached or in which they are embedded has been an underexplored topic (Martin and Britayev, 1998, 2018; Molodtsova et al., 2016). In descriptions of *Spirobranchus* specimens, the substrate is often only briefly noted as "live coral," without specifying its identity or clarifying whether the worm tube was truly embedded in the coral skeleton or situated adjacent to it (van der Schoot and Hoeksema, 2024). As a first step towards resolving these knowledge gaps, this study presents a phylogeny reconstruction of substrate selection in *Spirobranchus* using newly collected specimens and their documented host taxa or substrate of a subset of worm species in combination with available sequences and substrate data retrieved from the literature.

This work centres, amongst others, on the evolutionary position of *S. giganteus* in relation to a selection of congeneric taxa and the intraspecific genetic diversity within *S. giganteus* itself. Its phylogeny is analysed in relation to substrate use by various individuals in the context of host specificity, with a focus on the selection of host-coral taxa alongside their biogeographic range. A phylogenetic relationship with substrate use is expected, as some *Spirobranchus* spp. are well known as coral-dwelling species, whereas others have never been reported from live coral (van der Schoot and Hoeksema, 2024). The relationship with host coral taxa was also analysed for two other frequently sampled nominal species, i.e., *S. corniculatus* and *S. giganteus*. Given the broad taxonomic range of their recorded host corals this relationship is expected to be weak (van der Schoot and Hoeksema, 2024).

In addition, a family-wide macro perspective is used as a starting point in which the substrate type of *Spirobranchus* spp. is compared to that of other serpulid species, with a discussion on the evolutionary origin and history of coral association as a trait. Information about host specificity of species or species complexes improves insight in the implications of coral reef degradation on the survival of coral-associated serpulids.

2. Materials and methods

2.1. Sampling

Samples were collected by means of SCUBA diving at depths of 1–30 m across sites in the Caribbean and Indo-West Pacific using the roving diver method (Hoeksema et al., 2019b, 2022; van der Schoot and Hoeksema, 2022). In total 165 samples distributed over 20 species or lineages within the genus *Spirobranchus* from various localities were included (Table S1, Fig. S3). Koh Tao in the Gulf of Thailand and Curaçao in the Caribbean Sea were more extensively sampled than other sites. Sampling took place at several sites around these islands. Worm tubes were opened using a hammer and chisel, taking particular care to minimize damage to the surrounding coral tissue. The worms were pulled out of their tube with a bending wire. A maximum of five worms was sampled per host species. Worms were preserved in 96% ethanol. After tissue collection for DNA analysis, worms were transferred to 70% ethanol and stored at Naturalis Biodiversity Center (Leiden, the Netherlands).

2.2. DNA isolation and sequencing

DNA was extracted using the NucleoMag 96 tissue kit (Machery-Nagel, Düren, Germany) on a KingFisher Flex system (Thermo Fisher Scientific). DNA extractions were obtained in a final volume of 20 µL and subsequently diluted 1:100 prior to use in PCR reactions. PCR amplification was performed for two markers, the mitochondrial gene *cytochrome b* (*cytb*) and the nuclear Internal Transcribed Spacer 2 (ITS2) region. *Cytochrome b* was amplified using primers cytb424F (5′-GGWTAYGTWYTWCCWTGRGGWCARAT-3′; Boore and Brown, 2000) and cobr825 (5′- AARTAYCAYTCIGGYTTRATRTG-3′; Burnette et al., 2005). The ITS2 region was amplified using newly developed primers designed with Primer3 (Untergasser et al., 2012): M13F-F1 (5′-TGTAAAACGACGGCCAGTAGCTGTCGACGGTAGGGTAG-3′) and M13R-R1 (5′ CAGGAAACAGCTATGACTGTCAATCCTTGCAGTGTCC-3′). The PCR reactions were performed in a total volume of 25 µL, the PCR mixture for ITS2 contained 2.5 µL PCR Coral Load Buffer, 1.0 µL $MgCl_2$ (25 mM), 1.0 µL BSA (Promega), 1.0 µL forward primer (10 pmol/µL), 1.0 µL reverse primer (10 pmol/µL), 0.5 µL dNTPs (2.5 mM), 0.25 µL Taq polymerase (5 units/µL), 16.8 µL extra pure PCR water, and 1.0 µL DNA

extract. The PCR mixture for *cytb* contained 5.0 µL PCR Coral Load Buffer, 1.0 µL $MgCl_2$ (25 mM), 1.0 µL BSA (Promega), 1.3 µL forward primer (10 pmol/µL), 1.3 µL reverse primer (10 pmol/µL), 0.5 µL dNTPs (2.5 mM), 0.5 µL Taq polymerase (5 units/µL), 13.4 µL extra pure PCR water, and 1.0 µL DNA extract. PCR amplifications were performed for 40 cycles, preceded by an initial denaturation step at 94°C for 4 min (3 min for *cytb*) followed by cycles with denaturation at 94°C for 15 s, annealing at 50°C for 30 s and extension at 72°C for 40 s, followed by a final extension at 72°C for 5 min. PCR products were analysed on E-Gel Precast Agarose Gels (E-Gel 96 Gel, 1% agarose). Successfully amplified products were purified and subjected to bidirectional (Sanger) sequencing at BaseClear B.V. (Leiden, the Netherlands) using an ABI3730XL sequencer (Life Technologies).

2.3. Phylogenetic analysis

Sanger chromatograms were processed in PIPEBAR (Oliveira et al., 2018). Quality trimming was performed using a minimum PHRED quality score of 25 and a PHRED offset of 33. Consensus sequences were assembled with a minimum overlap of 25bp and a minimum similarity threshold of 90%. The assembled sequences together with sequences obtained from GenBank, one *cytb* sequence of *Spirobranchus polycerus* (Schmarda, 1861), which was supplied to the first author by Ivison C. Brandão (University of São Paulo, Brazil), and *cytb* and ITS2 sequences of *Galeolaria gemineoa* Halt et al., 2009 and *G. caespitosa* to serve as outgroups, were aligned according the MAFFT alignment algorithm using the MPI Bioinformatics Toolkit (Zimmermann et al., 2018) with a Gap open penalty of 1.53. The *cytb* multiple sequence alignment (MSA) was manually curated in AliView (Larsson, 2014) by removing ambiguous regions at the alignment termini and deleting gaps associated with low-quality base calls in individual sequences, while maintaining the correct reading frame. Ambiguously aligned regions of the MSA of the non-coding ITS2 marker were removed using Gblocks using the following settings "allow gap positions within the final blocks" and "allow smaller finer blocks" (version 0.91b; Castresana, 2000).

Phylogenetic analyses were performed separately for each marker

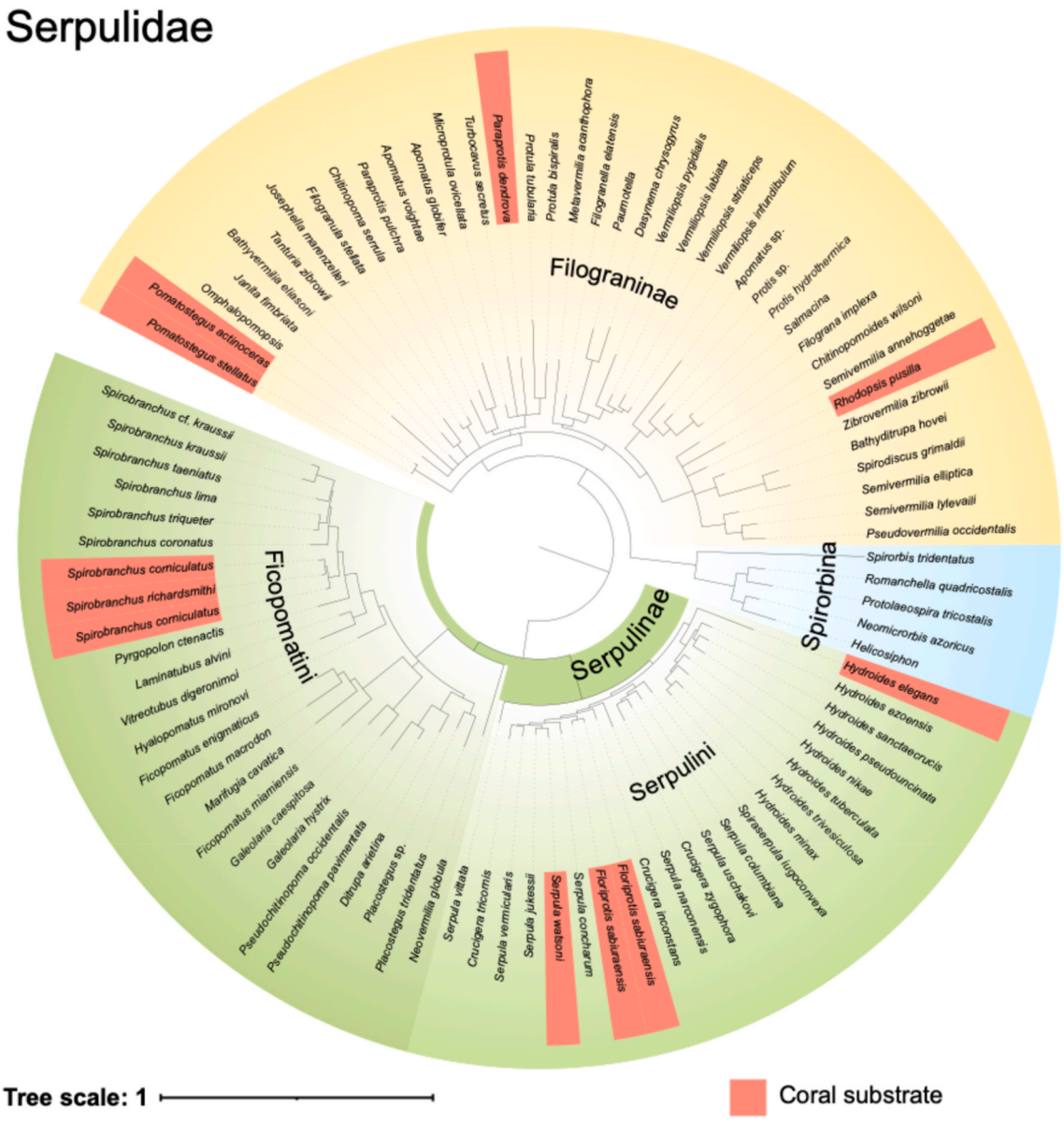


Fig. 1. Phylogeny reconstruction of the family Serpulidae Rafinesque, 1815 based on a combined morphology and molecular dataset as originally presented including supporting values by Kupriyanova et al. (2023). Species for which coral-association was documented are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

using both maximum likelihood (ML) and Bayesian inference (BI). Additionally, a concatenated reconstruction using both methods was made based on *cytochrome b* and ITS2, including only samples for which both markers were available. The ML analyses were performed in IQ-TREE (v3.0.1.; Wong et al., 2026) using 1000 ultrafast bootstrap replicate, bootstrap values (BS) were used as measure of node support. Model selection for the ML analyses was based on the Bayesian Information Criterion (BIC) and performed in IQ-TREE with ModelFinder Plus. BI analyses were conducted in MrBayes 3.2.7a (Ronquist et al., 2012) using the General Time Reversible model with gamma-distributed rate variation and a proportion of invariable sites. Two independent Markov Chain Monte Carlo (MCMC) analyses were run until the average standard deviation of split frequencies reached a value < 0.01, trees were sampled every 1.000 generations. The first 25% trees were discarded as burn-in, the remaining trees were used to generate posterior probabilities (PP) and a consensus tree. Prior to the concatenation analysis both markers were individually aligned (MAFFT), the *cytb* partition was manually trimmed and for the ITS2 partition Gblocks was used as described above in this section. Consequently, the two partitions were combined and used for phylogenetic analysis (BS and BI) with the same settings as described for the analyses for the individual markers. Species were classified as coral-associated when one or more records of their occurrence in live, hard-coral substrate were available; no distinction was made between facultative and obligate associations. For

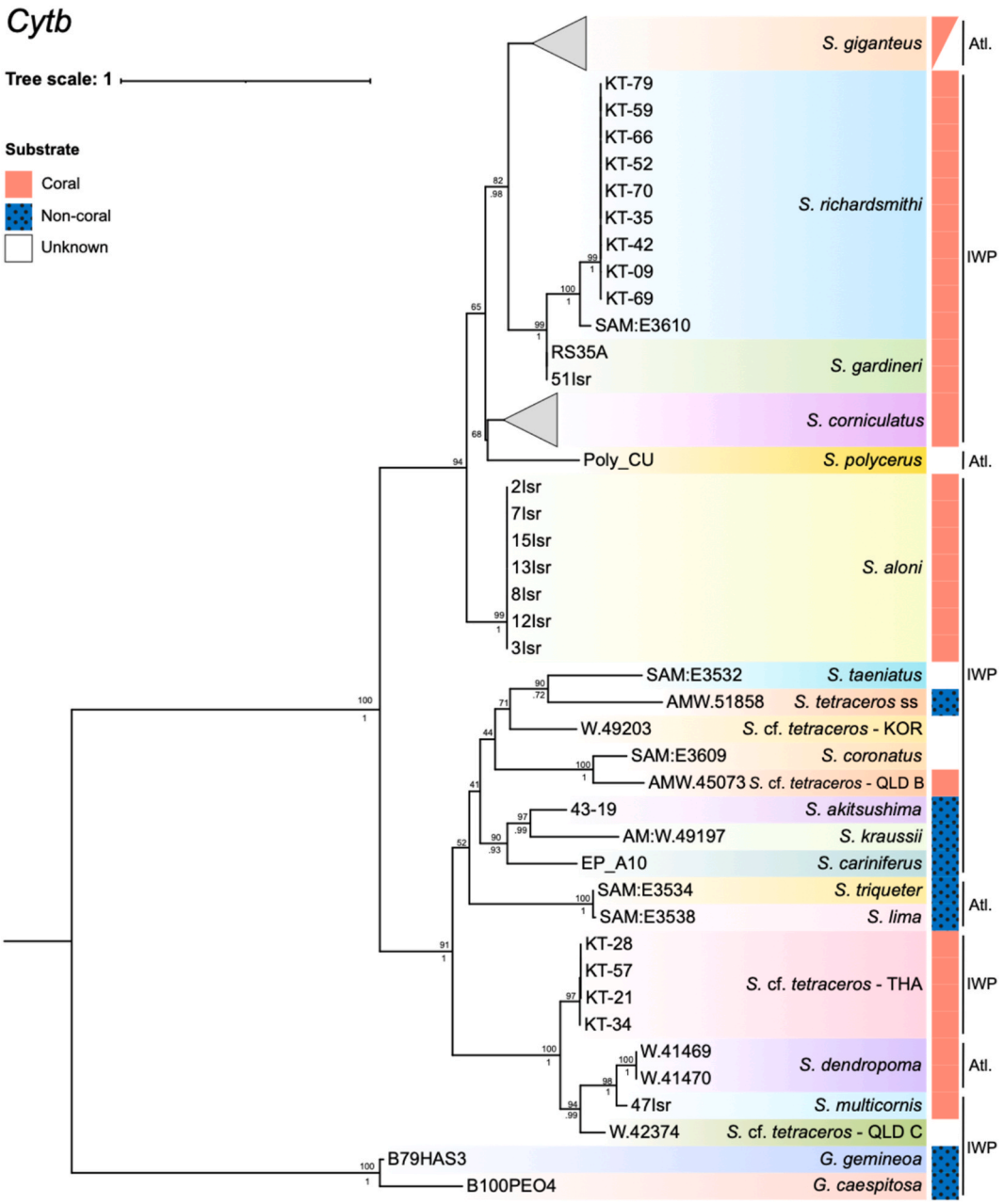


Fig. 2. Maximum likelihood phylogram of the genus *Spirobranchus* and two *Galeolaria* species as outgroup based on *cytochrome b*. Substrate use is indicated per specimen in categories i.e., coral (red), non-coral (dotted blue), and unknown (blank). The ocean the specimens are originating from is indicated, Atl.: Atlantic Ocean, IWP: Indo-West Pacific Ocean. Supporting values are indicated with bootstrap (BS) values obtained from the Maximum likelihood analysis (above) and posterior probabilities (PP) obtained from Bayesian inference (under). At some nodes PP values are not present, depicting an incongruence between ML and BI topology. Accession numbers and metadata of specimens can be found in Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Figs. 5 and 7 showing only selected clades or species groups, the phylograms were derived from the full phylogenetic trees containing all included taxa. These trees were visually pruned in iTOL to improve readability and to focus on the relevant clade, while preserving the topology and branch lengths inferred from the original full-tree analyses.

The phylogram of the family Serpulidae (Fig. 1) was calculated by MrBayes with the molecular and morphology dataset, which is available as supplementary materials (S3) in Kupriyanova et al. (2023). Annotated phylograms were constructed with use of the annotation software iTOL (Letunic and Bork, 2024).

2.4. *Spirobranchus* and host-coral identification

Identification of the worms was performed using a combination of morphology and phylogenetic placement. Specimens with operculum were morphologically identified and used as reference for samples for which no operculum was available. For *S. polycerus*, a detailed picture of the operculum was used instead (Fig. S1). The second method relied on phylogenetic placement relative to previously morphologically identified records. If a sample from this study clustered with a previously published sequence of known species identity, that species identity was assigned to the corresponding specimen from this study. The reference sequences used for these assignments were obtained from reliable taxonomic and molecular studies (Table S1). This approach was applied with particular caution in complex species groups, such as the *S. corniculatus* and *Spirobranchus tetraceros* (Schmarda, 1861) complexes. Where species-level identification remained uncertain, 'cf.' was added to the species name, whereas unresolved lineages were referred to as *Spirobranchus* sp.

Identification of host corals was based on photographs of the host corals and performed with use of the Reef Coral Identification guide (Humann and DeLoach, 2013) for Caribbean species and CoralsOfTheWorld.org (Veron et al., 2016) for Indo-Pacific species. Both Atlantic and Indo-Pacific species were checked for current taxonomic status at

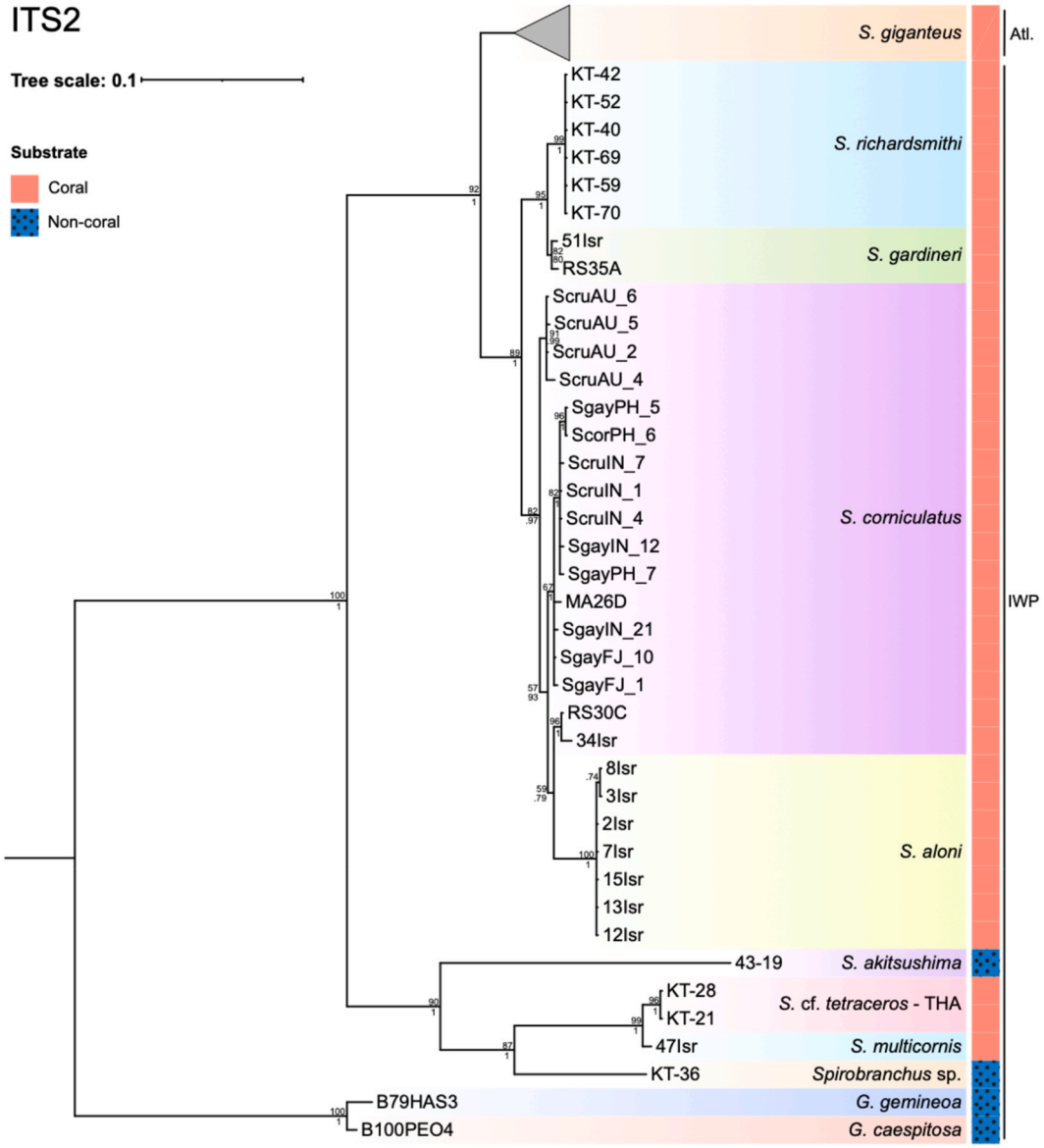


Fig. 3. Maximum likelihood phylogram of the genus *Spirobranchus* and two *Galeolaria* species as outgroup based on ITS2. Substrate use is indicated per specimen in categories i.e., coral (red), non-coral (dotted blue). The ocean the specimens are originating from is indicated, Atl.: Atlantic Ocean, IWP: Indo-West Pacific Ocean. Supporting values are indicated with bootstrap (BS) values obtained from the Maximum likelihood analysis (above) and posterior probabilities (PP) obtained from Bayesian inference (under). For one node the PP value is not present, depicting an incongruence between ML and BI topology. Accession numbers and metadata of specimens can be found in Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

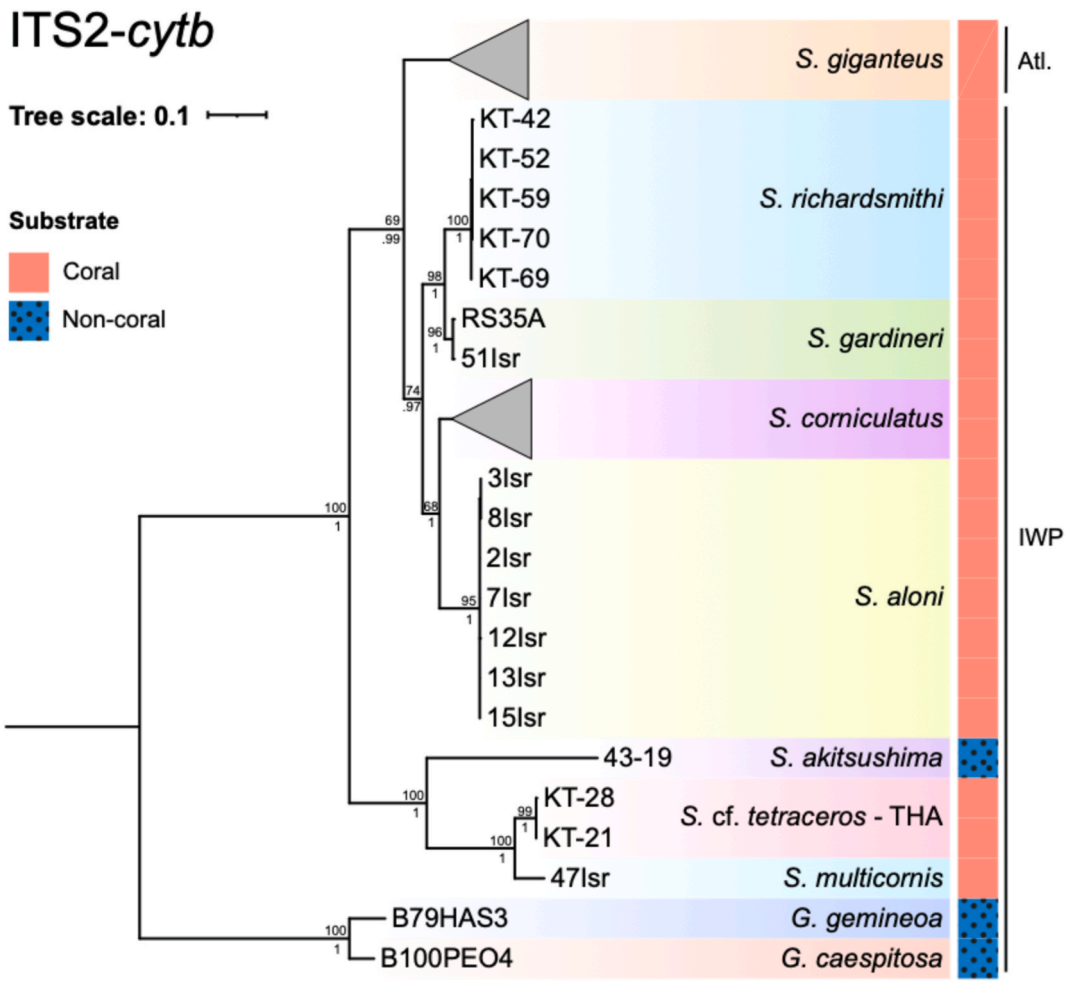


Fig. 4. Maximum likelihood phylogram of the genus *Spirobranchus* and two *Galeolaria* species as outgroup based on the concatenated sequences of ITS2 and *cytochrome b*. Substrate use is indicated per specimen in categories i.e., coral (red), non-coral (dotted blue). The ocean the specimens are originating from is indicated, Atl.: Atlantic Ocean, IWP: Indo-West Pacific Ocean. Supporting values are indicated with bootstrap (BS) values obtained from the Maximum likelihood analysis (above) and posterior probabilities (PP) obtained from Bayesian inference (under). Accession numbers and metadata of specimens can be found in Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the World List of Scleractinia of the World Register of Marine Species (Hoeksema and Cairns, 2025). In cases species-level identification was not possible, only the genus name was assigned.

2.5. Ancestral state reconstruction and phylogenetic clustering analyses

To quantitatively evaluate the distribution of substrate use, host-coral genus, and sampling locality across the *cytb* phylogenies, ancestral state reconstruction (ASR) and phylogenetic clustering analyses were performed in R (R Core Team, 2026).

Ancestral state reconstruction of substrate association was performed on a *cytb* maximum-likelihood (ML) tree reconstructed from one representative sequence per lineage/species. Substrate use was coded as a binary discrete character, coral or non-coral. Lineages or species for which substrate use was unknown were excluded prior to tree reconstruction and were therefore not included in the ASR. The tree was rooted with *G. gemineoa* and *G. caespitosa* as outgroups using the R package 'ape'; after rooting, the outgroups were removed. Trait data were matched to the corresponding tree tip labels. Ancestral states were reconstructed in R using the packages 'ape' and 'phytools'. Alternative transition-rate models for the binary discrete character were compared, and the best-fitting model was selected using Akaike's Information Criterion (AIC). Stochastic character mapping was performed with 'phytools' to visualize uncertainty in ancestral substrate states and to summarize inferred transitions between coral and non-coral substrate association.

In addition to the ASR, five separate permutation-based phylogenetic clustering analyses were performed. For each analysis, a separate *cytb* ML tree was reconstructed using the same alignment, trimming, model-selection, and ML procedures as described above, but including only the sequences for which the relevant trait information was available. First, substrate association was tested across the sampled *cytb* phylogeny using all sequences for which substrate was known and coded as "coral" or "non-coral"; samples with unknown substrate association were excluded. Second, the host-coral genus was tested within *S. corniculatus*, using only *S. corniculatus* sequences with a known host genus. Third, host-coral genus was tested within *S. giganteus*, using only *S. giganteus* sequences with known host genus. Fourth, the sampling locality was tested within *S. corniculatus* as an exploratory clade-by-geography analysis, using only *S. corniculatus* sequences with known locality. Fifth, the sampling locality was tested within *S. giganteus* as an exploratory clade-by-geography analysis, using only *S. giganteus* sequences with a known locality.

All five *cytb* trees used for the phylogenetic clustering analyses were rooted in R with *G. gemineoa* and *G. caespitosa* as outgroups using ape::root(). After rooting, the outgroup taxa were removed from the corresponding analysis trees using ape::drop.tip(). Phylogenetic clustering was assessed using two complementary permutation-based approaches

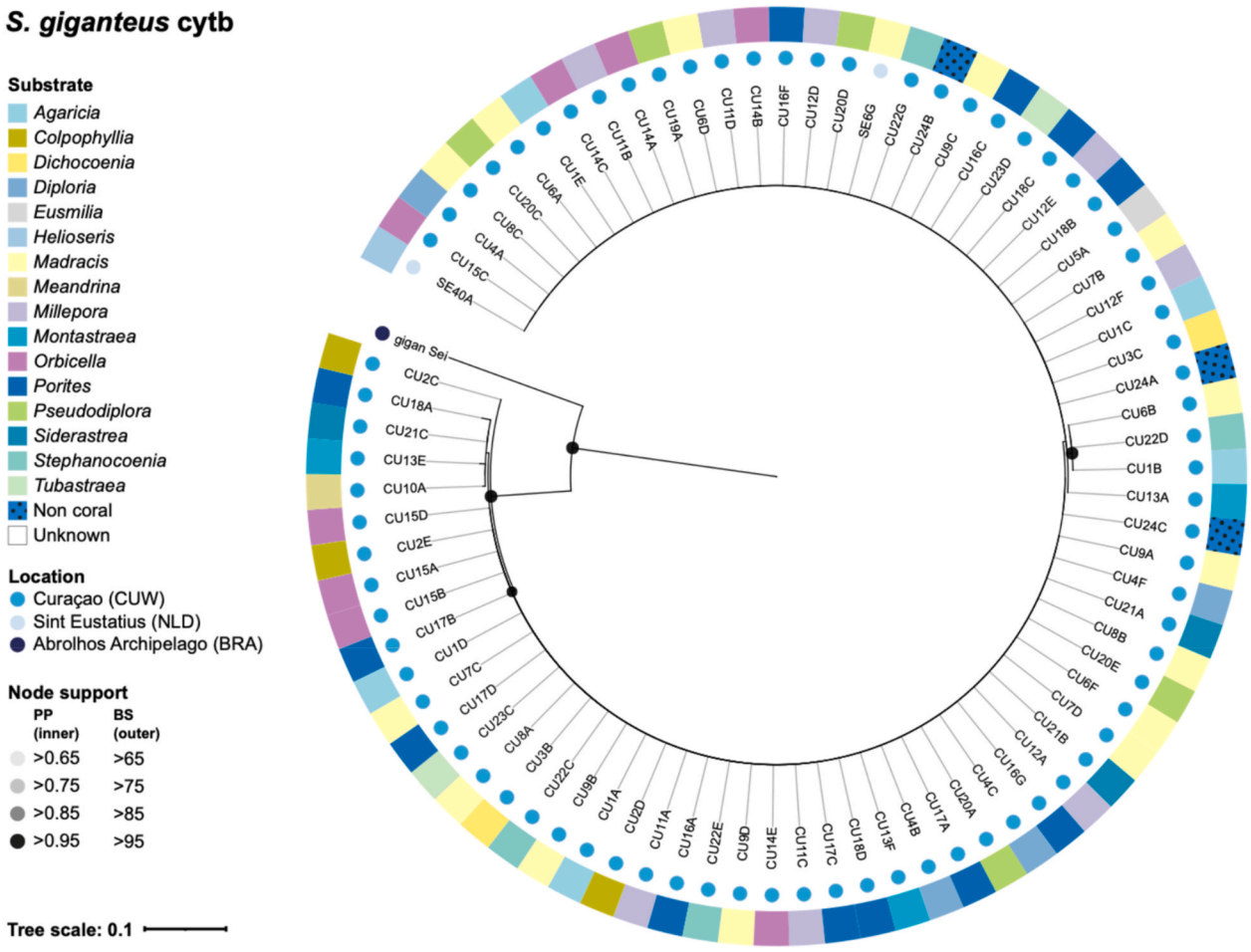


Fig. 5. Maximum likelihood phylogram of *Spirobranchus giganteus* based on *cytochrome b*. Substrate is indicated per specimen in categories i.e., host coral genus, non-coral substrate (dotted blue) and unknown (blank). The locality the specimens are originating from is indicated with coloured circles. Supporting values are indicated with bootstrap values (BS) obtained from the Maximum likelihood analysis (inner circle) and posterior probabilities (PP) obtained from Bayesian inference (outer circle). Accession numbers and metadata of specimens can be found in Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in R with the packages 'ape', 'phangorn', 'dplyr', 'tibble', and 'stringr'. First, a parsimony-based clustering test was used to compare the observed number of trait-state transitions across the tree with a null distribution generated by randomly permuting trait labels among tips. For each permutation, the minimum number of changes required to explain the observed trait distribution was calculated using phangorn::phyDat() and phangorn::parsimony(). Second, the mean patristic distance was calculated among tips sharing the same trait state using pairwise phylogenetic distances obtained with ape::cophenetic.phylo(). This observed mean within-state distance was compared with a null distribution generated by randomly permuting trait labels among tips. For all permutation-based analyses, 9999 randomizations were used.

Additionally, a descriptive post hoc summary was performed to identify which host genera were most frequently represented within the major *S. corniculatus* lineages. For this analysis, each *S. corniculatus* sequence was assigned to a major *cytb* lineage based on the reconstructed tree (Red Sea, Maldives_1, Maldives 2, Japan, Koh Tao_1, Koh Tao_2). Host-genus and lineage data were processed in R using 'dplyr', 'stringr', and 'tidyr', and presented in Tables S9–12.

3. Results

3.1. Coral substrate of Serpulidae

The Serpulidae phylogeny as presented by Kupriyanova et al. (2023) was supplemented with coral substrate records (van der Schoot and Hoeksema, 2024, Fig. 1). Live coral substrate was observed in two out of three subfamilies (Serpulinae Rafinesque, 1815; Filograninae Rioja, 1923) and absent in the five included species of the subfamily Spirorbinae Chamberlin, 1919. The phylogenetic analysis of Kupriyanova et al. (2023) is not exhaustive and includes only a subset of the serpulid species known to be associated with live coral (van der Schoot and Hoeksema, 2024). However, to date, no species of Spirorbinae have been reported from live coral. *Spirobranchus* is the most studied serpulid genus and the only genus within the tribe Ficopomatini Pillai, 1960 documented from live coral. The other tribe in the subfamily Serpulinae (Serpulini Rafinesque, 1815) contains three genera recorded from live-coral substrate (*Floriprotis* Uchida, 1978, *Hydroides* Gunnerus, 1768, and *Serpula* Linnaeus, 1758), which are not closely related. In total 20 species of Spirorbinae and 11 of Filograninae have been reported from live coral. Although *Spirobranchus* and *Hydroides* contain 12 and 7 coral-associated species, respectively (van der Schoot and Hoeksema, 2024), not all of them are closely related within each genus.

Ancestral state reconstruction of substrate association was performed by plotting the substrate type on the *cytb* tree containing one representative sequence per lineage/species, which resulted in a slightly different topology with respect to the complete *cytb* ML tree (Fig. 2 and Fig. S2). After removal of the outgroups, the analysis included 18 ingroup tips, of which 12 were coral-associated and six non-coral-associated. The equal-rates model provided the best fit to the data

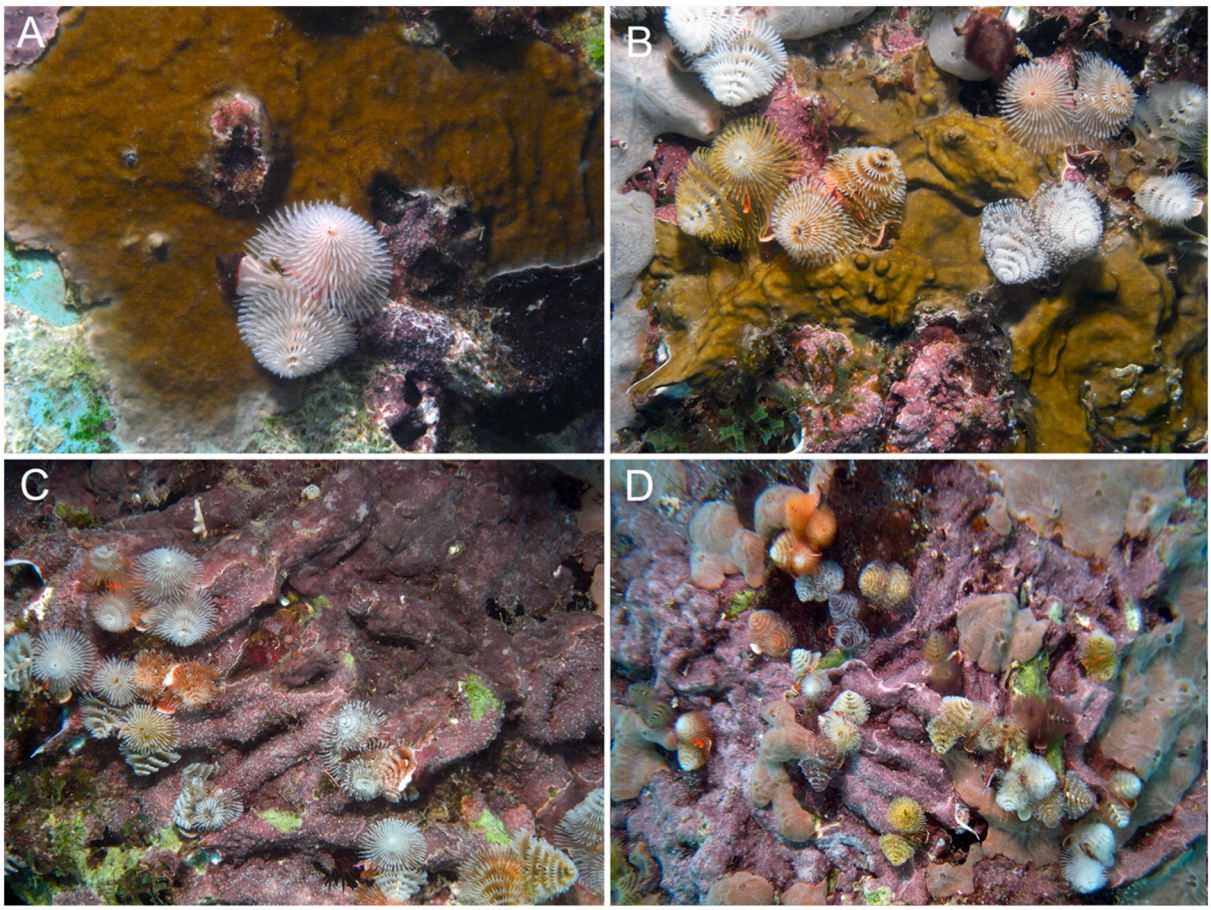


Fig. 6. *Spirobranchus giganteus* aggregation on concrete pillars near Carmabi Research Station, Curaçao (in 2014). A) *S. giganteus* next to and on top of an encrusting *Millepora* sp. B) Cluster of *S. giganteus* individuals overgrowing or adjacent to *Millepora* sp., CD) clustered *S. giganteus* individuals overgrown by crustose coralline algae. Photo credit B.W. Hoeksema.

based on AIC. Under this model, the root state was reconstructed with very high likelihood as coral-associated (Fig. S2). Stochastic character mapping inferred an average of 3.15 transitions between coral and non-coral states across the tree. Coral to non-coral and reverse transitions occurred at similar mean frequencies (1.65 and 1.50, respectively). These results suggest that substrate association has changed multiple times within the sampled *Spirobranchus* lineages, while also indicating that the deepest reconstructed state in the sampled tree is most consistent with coral association. Detailed results of the ASR analyses can be found in Supplementary Tables S2–8.

3.2. Live coral substrate projected on a cytb-based Spirobranchus phylogeny

The phylogeny of *Spirobranchus* based on the mitochondrial marker *cytochrome b* showed two clades, a small clade containing six species i.e., *S. giganteus*, *Spirobranchus richardsmithi* Pillai, 2009, *Spirobranchus gardineri* Pixell, 1913, *S. corniculatus*, *S. polycerus* and *Spirobranchus aloni* Perry et al., 2018, and a larger clade with 15 (sub)species (Fig. 2). In both clades Atlantic and Indo-West Pacific (IWP) species occurred. The large clade contains species that occur on live corals and species that are exclusively described from non-coral substrate, some of which are known to form large aggregates, such as *Spirobranchus akitsushima* Nishi et al., 2022, *Spirobranchus kraussii* (Baird, 1864), and *Spirobranchus cariniferus* (Gray, 1843) (see Smith et al., 2012; Nishi et al., 2022). The small clade on the other hand, contains almost only species that are known to be associated with live coral, since only the specimen of *S. polycerus* at Curaçao was collected from a non-coral substrate. The opercular morphology of this individual (Fig. S1) corresponds to that of the coral-associated, 7-horned *S. polycerus* and not to the gregarious 2-horned *S. polycerus augeneri* ten Hove, 1970, or intermediate morphologies (ten Hove, 1970; Marsden, 1992). In the large clade, the temperate Atlantic non-coral-associated species *Spirobranchus triqueter* (Linnaeus, 1758) and *Spirobranchus lima* (Grube, 1862) formed a sister pair, while the tropical W. Atlantic *Spirobranchus dendropoma* Mörch, 1863 was comparatively distant within the same clade. It should be noted that the taxon *S. dendropoma* for decades was included in *S. tetraceros* (synonymized by ten Hove, 1970), but more recently was recognized as full species again, with a tropical W. Atlantic distribution (e.g. Bastida-Zavala and Salazar-Vallejo, 2000; Kupriyanova et al., 2025, on basis of DNA; see Fig. 2 as well). In the small clade, the tropical W. Atlantic species *S. giganteus* and *S. polycerus* did not form a sister pair but belonged to relatively distant clades. *Galeolaria* spp. are genetically relatively remote from *Spirobranchus*, two species of this genus were selected as outgroup to stay consistent with earlier phylogenetic analyses of *Spirobranchus* (Willette et al., 2015; Simon et al., 2019; Kupriyanova et al., 2022, 2023; 2025; Nishi et al., 2022).

Phylogenetic clustering analysis showed significant results for both the parsimony-based clustering test and the patristic-distance test (Tables S9–12), indicating that coral and non-coral substrate associations are phylogenetically clustered in the *cytb* tree.

3.3. Live coral substrate projected on an ITS2-based Spirobranchus phylogeny

Fewer sequences were available for the phylogenetic analysis based

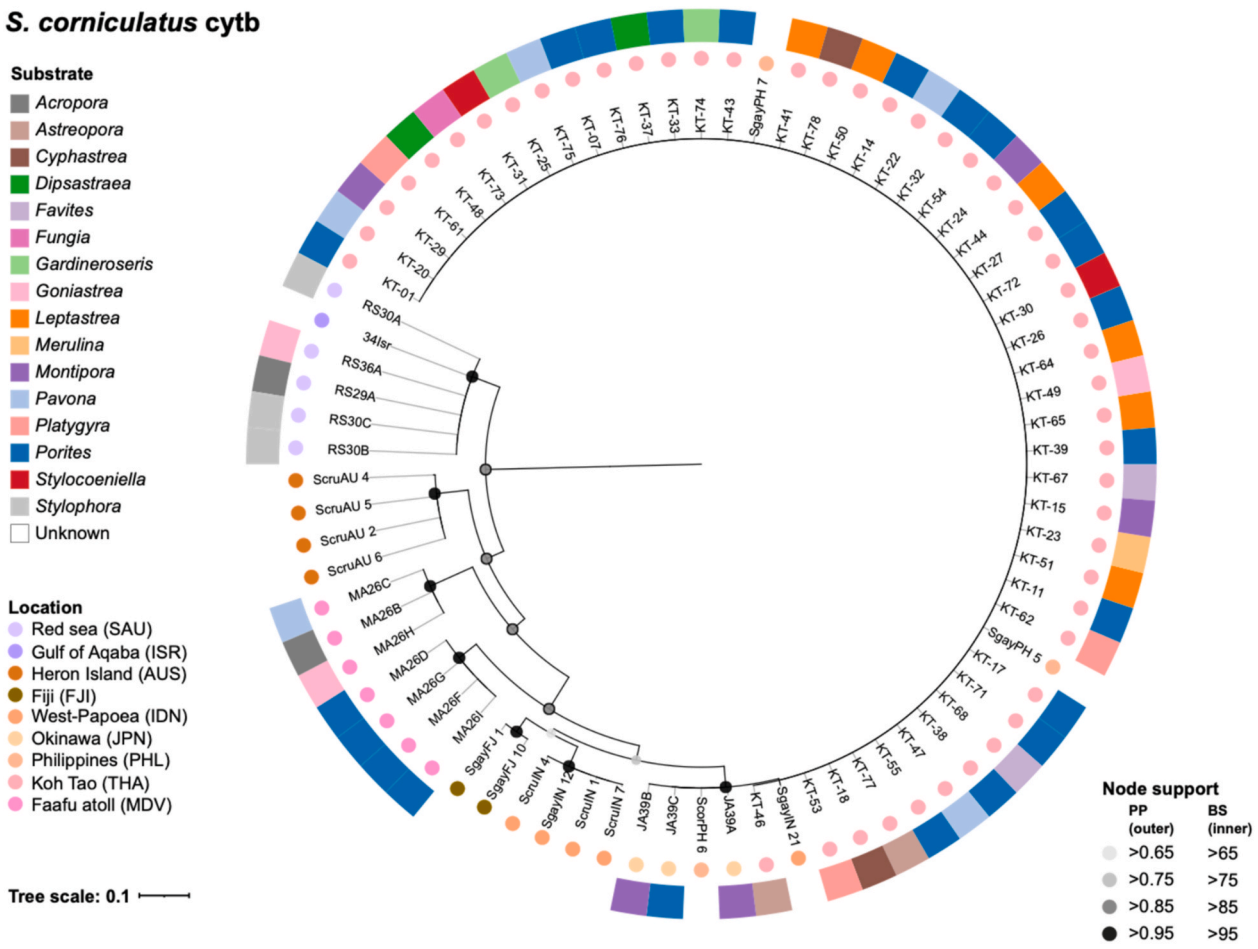


Fig. 7. Maximum likelihood phylogram of *Spirobranchus corniculatus* based on *cytochrome b*. Substrate is indicated per specimen in categories i.e., host coral genus (multiple colours) and unknown (blank). The locality the specimens are originating from is indicated with coloured circles. Supporting values are indicated with bootstrap values (BS) obtained from the Maximum likelihood analysis (inner circle) and posterior probabilities (PP) obtained from Bayesian inference (outer circle). Accession numbers and metadata of specimens can be found in Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

on ITS2 for which none of the samples of the species *S. corniculatus* of Koh Tao (Thailand) could be included. Also, fewer species of the large clade were available for this marker on GenBank. The major clades of the phylogram based on ITS2 were largely consistent with those of *cytb*, although some differences are apparent in the topology dividing the species in both clades (Fig. 3).

In the small clade, *S. polycerus* was the only absent species with respect to the *cytochrome b* phylogram, of the remaining five species especially *S. aloni* was remarkably deviant, being nested within a subclade of *S. corniculatus* and occupying a terminal position in the phylogeny, while according to *cytb* this species represents an early-diverging lineage within this clade. Furthermore, according to *cytb, S. richardsmithi* and *S. gardineri* are more closely related to *S. giganteus* than the other species in the small clade, while in the ITS2 reconstruction not *S. aloni* but *S. giganteus* forms the early-diverging lineage, being relatively equally distant from the other species in this clade.

The large clade is relatively small in the ITS2 phylogram as only for *S. akitsushima* Nishi et al., 2022 and *Spirobranchus multicornis* (Grube, 1862) sequences of both markers were available. Also, a limited number of Koh Tao samples was represented in this clade, with KT-21 and KT-28 belonging to the *S. tetraceros* (Schmarda, 1861) complex. Both are also represented in the *cytb* phylogram. The only sequence exclusively represented in the ITS2 phylogram is KT-36, which is a remarkable specimen as it was collected from a giant clam (*Tridacna squamosa* Lamarck, 1819) at Koh Tao (van der Schoot et al., 2016). Since no operculum was preserved for this specimen, identification to species level was challenging.

3.4. Live coral substrate projected on a concatenated Spirobranchus phylogeny

A subset of all samples for which sequences of both *cytochrome b* and ITS2 were available, including earlier published sequences obtained from GenBank, were used for a concatenated phylogenetic analysis (Fig. 4). The resulting phylogram is largely congruent with the ITS2 phylogram with exception of *S. corniculatus* and *S. aloni*, that are recovered as reciprocally monophyletic clades, with the *S. corniculatus* clade diverging prior to the *S. aloni* clade. Topology of both ML and BI analyses was completely congruent, on three nodes supporting values retrieved from ML analysis (BS) are low ($65 \leq BS \leq 75$) indicating slight uncertainty in the topology, supporting values obtained from BI (PP), however, are high ($PP \geq 97$) for the full topology of the phylogram.

3.5. Phylogeny and host-coral specificity of Spirobranchus spp.

The *S. giganteus* clade of the *cytochrome b* phylogram contained 81 sequences and is presented in more detail including annotation of live-coral substrate genera when available and detailed localities (Fig. 5). Most of the included specimens of *S. giganteus* were collected at Curaçao (Hoeksema et al., 2019b), two specimens were collected at St. Eustatius

(Hoeksema et al., 2022), and one sequence was obtained from GenBank which was collected at the Abrolhos archipelago, Brazil (Seixas et al., 2017; Table S1).

For *S. giganteus*, geographic sampling locality was not significantly clustered according to the parsimony-based test, whereas the patristic-distance test was significant (Tables S9–12). This suggests weak locality-level clustering, but not a strong clade-by-geography pattern in the *cytb* tree.

Limited phylogenetic divergence between the specimens from Curaçao and St. Eustatius was observed, they form a large subclade which is distinct from the specimen originating from Brazil. For 82 specimens the substrate was live coral, of these specimens the worm tube was embedded in the coral skeleton. In total, 16 different coral genera, and a minimum of 24 species (Table S1), were found in this subset to serve as a host for *S. giganteus*. The most frequently collected host genus was *Madracis* Milne Edwards & Haime, 1849 (n = 16) followed by *Porites* Link, 1807 (n = 12), *Orbicella* Dana, 1846 (n = 8) and *Millepora* Linnaeus, 1758 (n = 8). For *S. giganteus*, phylogenetic clustering tests based on specimens with known host-coral genus showed no significant clustering for this trait in the *cytb* tree (Tables S9–12). Therefore, host-genus association did not show a detectable phylogenetic structure within the sampled *S. giganteus* dataset.

Three specimens were collected from concrete pillars and were found in the vicinity of multiple other *S. giganteus* individuals, *Millepora* and crustose-coralline algae (Fig. 6). The specimen from Brazil was collected from a coral reef, specific information about its substrate is lacking however (Seixas et al., 2017).

The 78 sequences forming the *S. corniculatus* clade are divided over seven distinct subclades that correspond roughly with the geographic location the specimens were collected from (Fig. 7). All specimens from the Red Sea, Australia, Maldives and Fiji cluster each on their own separate clades. Also, the specimens from Japan (Okinawa) and one from the Philippines (ScorPH 6) form a separate lineage close to the large lineage containing all specimens from Koh Tao (Thailand) and the remaining specimens from the Philippines. Four of the five specimens from Indonesia cluster together except one (SgayIN21) which occurred on a separate lineage close to the specimens from Japan and Koh Tao. All *S. corniculatus* specimens (n = 63) were collected from live coral, belonging to 17 genera and a minimum of 20 species (Table S1). The most common host genus of all lineages together was *Porites* (n = 22) followed by *Leptastrea* Milne Edwards & Haime, 1849 (n = 6) and *Montipora* Blainville, 1830 (n = 5). The substrate of *S. corniculatus* from other studies (n = 15) was unknown.

Phylogenetic clustering analyses showed significant results for both the parsimony-based and patristic-distance tests for host-coral genus and geographic sampling locality (Tables S9–12), supporting the presence of non-random host-genus and geographic clustering among the sampled *S. corniculatus cytb* lineages.

The other species of *Spirobranchus,* i.e., *S. gardineri*, *S. polycerus*, *S. richardsmithi* and *S.* cf. *tetraceros* were present in small numbers. *S. richardsmithi* was recorded ten times, five of which in *Montipora* spp. and two times in *Porites* spp. *Spirobranchus* cf. *tetraceros* was recorded four times, three times from *Pavona* spp. (genus authority: Lamarck, 1801) and the other specimen from *Porites monticulosa* Dana, 1946. *S. gardineri* and *S. polycerus* were recorded only once in this study, respectively from *Porites* sp. in the Red Sea, and from artificial substrate at Curaçao.

3.6. Growth-form and polyp-size preferences

For 162 records the growth form of the host coral was determined and for 104 records the polyp size category was assigned (Table 1). The most frequently observed coral growth form was massive, and the most frequently occurring polyp-sizes were up to 6 mm diameter. Distinguishing between *Spirobranchus* species revealed more variety in growth form for *S. giganteus* than *S. corniculatus*. The latter was more frequently observed in host corals with small polyp sizes (0–4 mm), whereas *S. giganteus* was more frequently recorded in host species with small to intermediate polyp sizes (2–6 mm) and also more frequently in host species with large polyps (>10 mm). The less commonly recorded species *S. richardsmithi* was also predominantly observed in massive host coral species, and relatively frequently in *Montipora* spp. *Spirobranchus* cf. *tetraceros* was observed only from coral species with a non-massive growth form, three of four were *Pavona* spp.

4. Discussion

4.1. Evolutionary history of coral-association within the Serpulidae

Serpulid species known to associate with live coral are not confined to a single genus, clade, or closely related lineage. Instead, coral-associated taxa appear scattered across multiple clades (Fig. 1). The

Table 1
Frequency distribution of growth form and polyp size categories for recorded host corals. Polyp-size values were sourced from the Coral Traits Database (coraltraitsdatabase.org) and defined for each species as the midpoint between the minimum and maximum corallite width in mm.

	Total	*S. corniculatus*	*S. giganteus*	*S.* cf. *tetraceros*	*S. richardsmithi*
Growth form					
Massive	74	34	34	0	5
Branching	27	4	19	1	1
Sub massive	23	11	10	0	2
Encrusting	14	9	4	0	1
Foliose	12	4	4	3	1
Nodular	5	0	5	0	0
Plocoid	3	0	3	0	0
Plate	2	0	2	0	0
Solitary	1	1	0	0	0
Phaceloid	1	0	1	0	0
Polyp size (mm)					
0–2	33	21	8	3	1
2–4	36	13	21	0	0
4–6	18	4	14	0	0
6–8	6	0	6	0	0
8–10	4	0	4	0	0
10–12	5	1	4	0	0
12–14	1	0	1	0	0
14–16	0	0	0	0	0
16–18	0	0	0	0	0
18–20	1	0	1	0	0

only apparent exception is the subfamily Spirorbinae, for which no species have yet been documented inhabiting living coral. Whether the absence of coral association in this subfamily represents a monophyletic condition remains unresolved. This question requires further investigation, particularly because only a limited number of spirorbin species were included in the present analysis. A minority of serpulid species are currently known to be coral-associated, indicating that this sessile life strategy represents an exception rather than rule within the family Serpulidae.

The genus *Spirobranchus* stands out within its family, both in terms of the species number of coral-associates (n = 12) and number of host records. The second-most represented genus is *Hydroides*, with seven documented coral-associated species, although host information remains limited (van der Schoot and Hoeksema, 2024). Both genera fall within the subfamily Serpulinae but belong to different tribes. Another obligate coral-associated serpulid is *Pseudovermilia madracicola*ten Hove, 1989, which is not present in the molecular analysis of this study, this West Atlantic species would supposedly recover close to *Pseudovermilia occidentalis* (McIntosh, 1885)in the Filograninae, which would be consistent with the scattered occurrence of coral-association in the family. The relatively extensive *Spirobranchus* record may, however, be influenced by sampling bias, as this iconic, relatively large species is commonly observed in shallow reef habitats.

The ancestral state reconstruction, based on a representative subset of *cytb* sequences of distinct lineages as recovered in the *cytb* ML phylogenetic reconstruction, the deepest reconstructed state for the sampled extant *Spirobranchus* lineages was most likely coral-associated, while stochastic character mapping indicated multiple transitions between coral and non-coral substrate association. This suggests that shifts in substrate association have occurred repeatedly within the genus. However, this inference should be interpreted cautiously, as in the ASR tree, *S. aloni* was recovered as the earliest-diverging lineage (Fig. S2), whereas the complete *cytb* ML analysis showed a more balanced split between the two basal clades (Fig. 2). Together, these results suggest that coral association has likely originated or been lost multiple times within *Spirobranchus*. This result makes it plausible that this is also the case for Serpulidae, also given that coral association is a widespread ecological trait, which is observed among marine invertebrates belonging to eight different phyla (Stella et al., 2011). Broader, family-level conclusions, however, on the direction and timing of these transitions require more extensive taxon sampling and more stable phylogenetic resolution.

4.2. Live coral substrate in relation to Spirobranchus phylogeny

The phylogenetic analyses of *Spirobranchus* specimens based on *cytochrome b*, ITS2, and the concatenated dataset consistently recover two major basal clades, each containing a mixture of Atlantic and Indo-Pacific taxa. Within these basal clades Atlantic taxa are not closely related, except for *S. triqueter* and *S. lima* in the large clade (Fig. 2). It has to be noted that the dataset used in this study is non-exhaustive and has uneven sampling across species and geographic lineages. Nevertheless, these patterns align well with previously published phylogeny reconstructions of *Spirobranchus* (Willette et al., 2015; Nishi et al., 2022; Kupriyanova et al., 2025). As observed for the entire family Serpulidae (Fig. 1), analyses in this study show that coral association is not monophyletic within the genus *Spirobranchus* (Figs. 2–4). Notably, the smaller basal clade comprises only coral-associated species, whereas the larger basal clade includes both coral-associated species as species that live on non-coral substrates. This pattern is validated by the phylogenetic clustering analyses and supports the hypothesis that substrate association may be an inheritable trait, as has been suggested for the serpulid tubeworm *Hydroides dianthus* (Verrill, 1873) in relation to a preference for conspecific tubes as a substrate (Toonen and Pawlik, 2001). Another apparent difference between the two basal clades concerns branchial morphology, in which a distinction can be made between species with spirally arranged radioles, such as *S. giganteus*, *S. corniculatus*, *S. richardsmithi*, *S. gardineri*, and *S. polycerus,* and those that are not spirally arranged, including *S.* cf. *tetraceros, S. akitsushima*, and the *Spirobranchus kraussii* complex (ten Hove, 1970; ten Hove and Nishi, 1996; Pillai, 2009; Perry et al., 2018a; Simon et al., 2019; Nishi et al., 2022). Although a comprehensive genetic study including multiple markers and detailed morphological information is necessary for a definite conclusion, the results in this study suggest that spirally arranged radioles form a monophyletic trait.

The specimen collected from *T. squamosa* (KT-36, Fig. 3) was previously identified as *S. corniculatus* (see van der Schoot et al., 2016). In the phylogeny presented here, however, it is distant from other *S. corniculatus* samples and clusters more closely with *S.* cf. *tetraceros* and *S. multicornis*. The images of this individual, on the other hand, show radioles arranged in two spires, both with a basal median compound eye (van der Schoot et al., 2016), suggesting affinity with the *S. corniculatus* clade rather than the *S. tetraceros* clade, the latter of which is characterized by paired or irregular single eyespots (ocelli) along the aboral corners of the radioles (Bok et al., 2017). These contradictory signals raise doubt about its earlier identification and suggest that the specimen may represent an as yet unknown non-coral-associated lineage.

Although the *cytb* and ITS2 phylogenies are broadly congruent, both markers yield topological discrepancies. The most striking example is *S. aloni*, which appears as an early-diverging lineage in the *cytb* tree but is nested within a *S. corniculatus* subclade in the ITS2 topology. Such differences are consistent with known marker-specific phylogenetic behaviour and have been reported previously (Perry et al., 2018b). *Cytochrome b* has been shown to possess greater resolving power for lineage distinction than ITS2 (Kobayashi and Goto, 2021), providing a plausible explanation for the observed topological incongruence.

4.3. Phylogeny and host specificity

No clear species-level pattern of host specificity was observed among the *Spirobranchus* species for which sufficient host species data were available. Both *S. corniculatus* and *S. giganteus* were recorded from a broad range of scleractinian host corals, with no host taxon exclusively associated with either worm taxon. However, phylogenetic clustering analyses showed that host genus as a trait was significantly clustered among *S. corniculatus cytb* lineages, but not within *S. giganteus*, suggesting that host-associated structure may occur at the lineage level in *S. corniculatus* rather than at the species level. Preference for parental substrate, as observed for *S. giganteus*, was suggested to lead to sympatric genetic divergence (Marsden et al., 1990), the findings in this study, however, do not support this hypothesis. Earlier studies also reported on the large host range of *S. giganteus* (Scott, 1987; Hunte et al., 1990a; Nygaard, 2008; Hoeksema and ten Hove, 2017; Hoeksema et al., 2017, 2020), but the present account is the first one combining the host identity with a genetic confirmation of the worms.

Intra-specific lineages were detected within *S. corniculatus*, and the distribution of the morphospecies *S. cruciger* and *S. gaymardi* across these clades is consistent with the phylogenetic patterns reported by Willette et al. (2015). By contrast, and in disagreement with the conclusions of Willette et al. (2015), the exploratory clade-by-geography tests in the present study show a relationship between intra-specific lineage structure and geographic sample locality. This disagreement highlights the need for a broader, integrative framework that evaluates both morphospecies and spatial sampling to better resolve lineage diversification within the *S. corniculatus* complex. In the subset of *S. giganteus*, however, limited phylogenetic divergence was observed. One specimen from the Abrolhos Archipelago, Brazil (SW Atlantic), was genetically distant to the other specimens (Caribbean) that cluster together with less genetic variation among them. The patristic-distance analyses, however, was significant, indicating slight geographic clustering of sample locality. These findings suggest a pattern of geographic differentiation within *S. giganteus*, in which specimens show limited phylogenetic separation

within the Caribbean, whereas clearer differentiation is observed between major western Atlantic regions, including the Caribbean and the Gulf of Mexico. This result should be interpreted with caution, however, as sampling over localities was uneven.

Such a pattern is also observed in the Indo-West Pacific species *S. corniculatus*. This phylogeographic hypothesis is consistent with the long-distance dispersal capacity observed for *Spirobranchus* spp. and other serpulids (Hutchings and Kupriyanova, 2018; Brandão and Brasil, 2020) and underscores the need for more comprehensive phylogenetic studies, including both the Atlantic and the Indo-Pacific.

Although the sampling strategy aimed to include as many different host species as possible, a disproportionately large number (36.2%, Table S12) of *S. corniculatus* specimens in the Koh Tao clade was found associated with massive *Porites* spp., this is in line with earlier observations (e.g., Hoeksema et al., 2019a; Rowley et al., 2026). This can be partially explained by the common presence of multiple *Porites* species serving as host for *S. corniculatus* in the Indo-west Pacific region and by the high host preference for *Porites* species of *S. corniculatus* (Dai and Yang, 1995; Mursawal et al., 2021; Idris et al., 2022; Isdianto et al., 2024; Rowley et al., 2026). The coral genus *Madracis* harboured most host species for *S. giganteus* in the Caribbean, which is not congruent with the observed host preference for *Orbicella* spp., *Pseudodiploria strigosa* (Dana, 1846), *Porites astreoides* Lamarck, 1816, and the hydrozoan species *Millepora* spp. (Conlin, 1988; Hunte et al., 1990a; Hoeksema et al., 2019b). This is possibly explained by the analysis at genus level of the host corals i.e., the common presence of multiple species of the genus *Madracis* as host corals in the Caribbean may overrepresent this genus relatively to *Porites*, because in *Porites* only *P. astreoides*, is reported as frequent host for *S. giganteus* (Marsden et al., 1990; Hoeksema et al., 2019b).

Three specimens of *S. giganteus* were collected from a concrete pillar on which *Millepora* sp. and crustose coralline algae (CCA) were present adjacent to the tubes. It is plausible that *Spirobranchus* larvae settled first on encrusting *Millepora* corals and later expanded onto the CCA, which followed the overgrowth mechanism by secondary-hosts as described in ascidians, octocorals, sponges, and zoantharians (Hoeksema et al., 2015, 2020; García-Hernández and Hoeksema, 2017). Another possible explanation is the attraction to conspecifics, in which case chemical cues of adult worms are sufficient to induce larval settlement, as was suggested to explain the gregarious behaviour shown by *Spirobranchus* on *Porites* spp. (Montebon and Yap, 2009) and in the related serpulids *G. caespitosa* and *G. gemineoa* (see Halt et al., 2009; Watson et al., 2017).

Since *S. richardsmithi* and *S.* cf. *tetraceros* were scarcely recorded and *S. polycerus* only once, no conclusions regarding phylogeny-related host specificity can be drawn at this point. *Spirobranchus* cf. *tetraceros* has been recorded from non-coral substrate and is therefore known as a facultatively substrate-generalist species. Recent assessments have recovered multiple lineages for *S.* cf. *tetraceros*, which should therefore be considered as a species complex and lineages should be considered distinct in the context of substrate use and host specificity (Kupriyanova et al., 2022, 2025). *S. richardsmithi* has not been recorded from any other substrate than live coral and is therefore expected to be an obligate coral-associated species (van der Schoot and Hoeksema, 2024). The most observed host genera of these two species do not overlap with those of *S. corniculatus* whilst occurring in the same reefs, additionally the four host species recorded for *S.* cf. *tetraceros* are all non-massive growth-forms, this potentially indicates the presence of niche differentiation in terms of host-coral preference among coral-associated *Spirobranchus* spp. and subsequently also a relation to phylogenetic clades. Future research will have to elaborate in more detail to consolidate this hypothesis.

The *S. polycerus* specimen was collected from artificial substrate, which is the first record for this substrate-species combination, and can therefore be classified as a generalist facultatively coral-associated species (van der Schoot and Hoeksema, 2024). It is not unlikely that the specimen had settled first on a biofouling *Millepora* crust (as *S. giganteus* in Fig. 6), as this coral species is documented as a common host for *S. polycerus* (Marsden, 1992).

4.4. Host-coral characteristics

Host-coral species recorded in this study were predominantly massive with small to intermediate polyp size, which corresponds well with earlier observations (Dai and Yang, 1995; Rowley, 2008). When comparing *Spirobranchus* spp., *S. corniculatus* was more often recorded on host coral species with smaller polyps than *S. giganteus*, which was also more observed from coral species with large polyps. When considering growth forms of host corals, the frequency patterns of *S. corniculatus* and *S. giganteus* are largely comparable, yet the latter shows higher variation and was more often recorded from branching host corals. These findings suggest that *S. giganteus* is slightly less host-specific than *S. corniculatus*, particularly given that the number of reef coral species in the Indo-Pacific is tenfold that of the Caribbean (Veron, 1995; Spalding et al., 2001; DeVantier and Turak, 2017). This finding aligns well with previously reported host-specificity indices for *S. giganteus* and *S. corniculatus*, which also indicate a marginally greater host specificity for the latter species (van der Schoot and Hoeksema, 2024).

5. Conclusions

Coral association is a relatively uncommon trait within the Serpulidae, yet its presence across genetically distant species suggests that it can be readily lost and (re)gained. Based on the present study, two main clades can be distinguished within *Spirobranchus*: one in which coral-association predominates and another with alternative sessile strategies, such as inhabiting non-coral substrates or reef formation. Ancestral state reconstruction showed multiple state transitions of substrate association in the genus *Spirobranchus*, from coral to non-coral and vice versa. In addition, the basal reconstructed state was coral-associated. Together, these patterns suggest a genetic basis underlying substrate preference.

No strict host specificity was detected for any of the *Spirobranchus* species examined in this study. Both *S. corniculatus* and *S. giganteus* were found across a broad range of host corals, with *S. giganteus* appearing the more generalist of the two. For the remaining species only limited specimen–host combinations were available, leaving their degree of host specificity unresolved. Notably, in this study *S.* cf. *tetraceros* differs in both host-coral species and growth form with respect to the congeneric sympatric *S. corniculatus*. Its host specificity, however, requires further investigation.

Specimens of *S. giganteus* showed very little genetic variation, except with respect to the specimen from Brazil, suggesting a broadly distributed Caribbean population genetically distinct from other Atlantic populations. In contrast, multiple lineages were recovered within the *S. corniculatus* clade, corresponding with geographic location and host genera but not with opercular morphology, indicative for the presence of cryptic species.

CRediT authorship contribution statement

Roeland J. van der Schoot: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Conceptualization. **Dagmar Wels:** Writing – review & editing, Methodology, Investigation. **Charles H.J.M. Fransen:** Writing – review & editing, Supervision. **Harry A. ten Hove:** Writing – review & editing, Investigation. **Bert W. Hoeksema:** Writing – review & editing, Writing – original draft, Supervision.

Funding

The field research of the first author was funded by the A.M.

Buitendijk Fonds, the Jan-Joost ter Pelkwijk-Fonds and the Dutch Research Council (NWO) Doctoral Grant for Teachers Programme (nr. 023.015.036).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to the funding agencies mentioned above. We thank the staff of CARMABI and the Dive Shop (Curaçao), and the New Heaven Reef Conservation Program (Koh Tao) for their hospitality and assistance during the fieldwork. We are grateful to Yee W. Lau (Naturalis Biodiversity Center) for her help with the lab work. We want to thank Ivison C. Brandão (University of São Paulo) for supplying the *S. polycerus cytochrome b* sequence. Lastly, we want to thank two anonymous reviewers for their constructive comments, which helped us to improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2026.10.003.

Data availability

Data are shared as electronic supplementary material

References

Alamaru, A., Hoeksema, B.W., van der Meij, S.E.T, Huchon, D., 2017. Molecular diversity of benthic ctenophores (Coeloplanidae). Sci. Rep. 7, 6365. https://doi.org/10.1038/s41598-017-06505-4.

Bastida-Zavala, J.R., Salazar-Vallejo, S.I., 2000. Serpúlidos (Polychaeta: Serpulidae) del Caribe noroccidental con claves para la región del Gran Caribe: *salmacina, Ficopomatus, Pomatoceros, Pomatostegus, Protula, Pseudovermilia, Spirobranchus* y *Vermiliopsis*. Rev. Biol. Trop. 48, 807–840.

Bok, M.J., Porter, M.L., ten Hove, H.A., Smith, R., Nilsson, D., 2017. Radiolar eyes of serpulid worms (Annelida, Serpulidae): structures, function, and phototransduction. Biol. Bull. 233, 39–57. https://doi.org/10.1086/694735.

Boore, J.L., Brown, W.L., 2000. Mitochondrial genomes of *Galathealinum, Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17, 87–106. https://doi.org/10.1093/oxfordjournals.molbev.a026241.

Brandão, I.C., Brasil, A.C.D.S., 2020. On a new species of *Spirobranchus* (Annelida: Serpulidae) and considerations on the genus along the Brazilian coast. Pap. Avulsos Zool. (Sao Paulo) 60, e20206051. https://doi.org/10.11606/1807-0205/2020.60.51.

Britayev, T.A., Antokhina, T.I., Deart, Y.V., Hoeksema, B.W., Hoang, D.T., Zvonareva, S. S., 2025. The first record of symbiosis between a new species of scaleworm (Polychaeta, Polynoidae) with fungiid corals (Scleractinia, Fungiidae). Symbiosis 97, 236–249. https://doi.org/10.1007/s13199-025-01093-8.

Burnette, A.B., Struck, T.H., Halanych, K.M., 2005. Holopelagic *Poeobius meseres* ("Poeobiidae," Annelida) is derived from benthic flabelligerid worms. Biol. Bull. 208, 213–220. https://doi.org/10.2307/3593153.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Chan, J.Y., Lee, S.S., Rahim, S.Z.Z., Teo, S.L., 2014. Settlement inducers for larvae of the tropical fouling serpulid, *Spirobranchus kraussii* (Baird, 1865) (Polychaeta: Annelida). Int. Biodeterior. Biodegrad. 94, 192–199. https://doi.org/10.1016/j.ibiod.2014.07.017.

Conlin, B.E., 1988. Aspects of Habitat Selection by a Tropical Serpulid Polychaete *Spirobranchus giganteus* (Pallas). MSc, McGill University, Canada.

Dai, C.F., Yang, H.P., 1995. Distribution of *Spirobranchus giganteus corniculatus* (Hove) on the coral reefs of southern Taiwan. Zool. Stud. 34, 117–125.

DeVantier, L., Turak, E., 2017. Species richness and relative abundance of reef-building corals in the Indo-West Pacific. Diversity 9, 25. https://doi.org/10.3390/d9030025.

Fisher, R., O'Leary, R.A., Low-Choy, S., Mengersen, K., Knowlton, N., Brainard, R.E., et al., 2015. Species richness on coral reefs and the pursuit of convergent global estimates. Curr. Biol. 25, 500–505. https://doi.org/10.1016/j.cub.2014.12.022.

Floros, C., Samways, M., Armstrong, B., 2005. Polychaete (*Spirobranchus giganteus*) loading on South African corals. Aquat. Conserv. Mar. Freshw. Ecosyst. 15, 289–298. https://doi.org/10.1002/aqc.666.

García-Hernández, J.E., Hoeksema, B.W., 2017. Sponges as secondary hosts for Christmas tree worms at Curaçao. Coral Reefs 36, 1243. https://doi.org/10.1007/s00338-017-1617-2.

Giangrande, A., Gambi, M.C., Gravina, M.F., 2020. Polychaetes as habitat former: structure and function. In: Rossi, S., Bramanti, L. (Eds.), Perspectives on the Marine Animal Forests of the World. Springer, Cham, Switzerland, pp. 219–237. https://doi.org/10.1007/978-3-030-57054-5_8.

Gittenberger, A., Gittenberger, E., 2011. Cryptic, adaptive radiation of endoparasitic snails: sibling species of *Leptoconchus* (Gastropoda: Coralliophilidae) in corals. Org. Divers. Evol. 11, 21–41. https://doi.org/10.1007/s13127-011-0039-1.

Gosselin, L.A., Gallego, R., Peters-Didier, J., Sewell, M.A., 2019. Field evidence of interpopulation variation in oocyte size of a marine invertebrate under contrasting temperature and food availability. Mar. Ecol. Prog. Ser. 619, 69–84. https://doi.org/10.3354/meps12959.

Gosselin, L.A., Sewell, M.A., 2013. Reproduction, larval development and settlement of the intertidal serpulid polychaete *Spirobranchus cariniferus*. J. Mar. Biol. Assoc. U. K. 93, 1249–1256. https://doi.org/10.1017/S0025315412001701.

Goulet, T.L., Goulet, D., 2021. Climate change leads to a reduction in symbiotic derived cnidarian biodiversity on coral reefs. Front. Ecol. Evol. 9, 636279. https://doi.org/10.3389/fevo.2021.636279.

Halt, M.N., Kupriyanova, E.K., Cooper, S.J.B., Rouse, G.W., 2009. Naming species with no morphological indicators: species status of *Galeolaria caespitosa* (Annelida : Serpulidae) inferred from nuclear and mitochondrial gene sequences and morphology. Invertebr. Systemat. 23, 205–222. https://doi.org/10.1071/IS09003.

Hoeksema, B.W., 2017. The hidden biodiversity of tropical coral reefs. Biodiversity 18, 8–12. https://doi.org/10.1080/14888386.2017.1307787.

Hoeksema, B.W., Cairns, S., 2025. World list of Scleractinia. Available from: https://www.marinespecies.org/scleractiniaatVLIZ. (Accessed 18 June 2025).

Hoeksema, B.W., García-Hernández, J.E., van Moorsel, G.W.N.M., Olthof, G., ten Hove, H.A., 2020. Extension of the recorded host range of Caribbean Christmas tree worms (*Spirobranchus* spp.) with two scleractinians, a zoantharian, and an ascidian. Diversity 12, 115. https://doi.org/10.3390/d12030115.

Hoeksema, B.W., Lau, Y.W., ten Hove, H.A., 2015. Octocorals as secondary hosts for Christmas tree worms off Curaçao. Bull. Mar. Sci. 91, 489–490.

Hoeksema, B.W., ten Hove, H.A., 2017. The invasive sun coral *Tubastraea coccinea* hosting a native Christmas tree worm at Curaçao. Dutch Caribbean. Mar. Biodivers. 47, 59–65. https://doi.org/10.1007/s12526-016-0472-7.

Hoeksema, B.W., van Beusekom, M., ten Hove, H.A., Ivanenko, V.N., van der Meij, S.E.T., van Moorsel, G.W.N.M., 2017. *Helioseris cucullata* as a host coral at St. Eustatius, Dutch Caribbean. Mar. Biodivers. 47, 71–78. https://doi.org/10.1007/s12526-016-0599-6.

Hoeksema, B.W., van der Loos, L.M., van Moorsel, G.W.N.M., 2022. Coral diversity matches marine park zonation but not economic value of coral reef sites at St. Eustatius, eastern Caribbean. J. Environ. Manag. 320, 115829. https://doi.org/10.1016/j.jenvman.2022.115829.

Hoeksema, B.W., van der Schoot, R.J., Wels, D., Scott, C.M., ten Hove, H.A., 2019a. Filamentous turf algae on tube worms intensify damage in massive *Porites* corals. Ecology 100, e02668. https://doi.org/10.1002/ecy.2668.

Hoeksema, B.W., Wels, D., van der Schoot, R.J., ten Hove, H.A., 2019b. Coral injuries caused by *Spirobranchus* opercula with and without epibiotic turf algae at Curaçao. Mar. Biol. 166, 60. https://doi.org/10.1007/s00227-019-3504-6.

Hu, J., Zhang, Y., Yiu, S.K.F., Xie, J.Y., Qiu, J.W., 2020. A new species of predatory nudibranch (Gastropoda: Trinchesiidae) of the scleractinian coral *Goniopora*. Zool. Stud. 59, e62. https://doi.org/10.6620/ZS.2020.59-62.

Humann, P., Deloach, N., 2013. Reef Coral Identification: Florida, Caribbean. Bahamas, third ed. New World Publications, Jacksonville, Florida.

Hunte, W., Conlin, B.E., Marsden, J.R., 1990a. Habitat selection in the tropical polychaete *Spirobranchus giganteus*: I. Distribution on corals. Mar. Biol. 104, 87–92. https://doi.org/10.1007/BF01313161.

Hunte, W., Marsden, J.R., Conlin, B.E., 1990b. Habitat selection in the tropical polychaetes *Spirobranchus giganteus* II. Effects of coral species on body size and body proportions. Mar. Biol. 104, 101–107. https://doi.org/10.1007/BF01313163.

Hutchings, P., Kupriyanova, E., 2018. Cosmopolitan polychaetes – fact or fiction? Personal and historical perspectives. Invertebr. Systemat. 32, 1–9. https://doi.org/10.1071/IS17035.

Idris, I., Mohd-Salleh, N.A., Ahmad Fadzil, N.D.N., 2022. Host preferences and colouration of Christmas tree worms, *Spirobranchus corniculatus* (Grube, 1862) from Bidong Island, South China Sea. In: Chuan, O.M., Martin, M.B., Nurulnadia, M.Y., Afzan Azmi, W. (Eds.), Bidong Island. Geography of the Physical Environment. Springer, Cham, Switzerland, pp. 177–187. https://doi.org/10.1007/978-3-030-91924-5_15.

Isdianto, A., Amanda, S.S., Yamindago, A., Dewi, C.S.U., Aliviyanti, D., Luthfi, O.M., et al., 2024. Water quality impact christmas tree worms (*Spirobranchus* spp.) distribution and community structure on hard corals at Sempu Island Nature Reserve, Malang, Indonesia. J. Ecol. Eng. 25, 175–186. https://doi.org/10.12911/22998993/186161.

Jia, Z., Tian, P., Wang, W., Cao, B., Xu, Z., Xiao, J., et al., 2023. First record of corallivorous nudibranch *Pinufius* (Gastropoda: Nudibranchia) in the South China Sea: a suspected new species of *Pinufius*. Diversity 15, 226. https://doi.org/10.3390/d15020226.

Kobayashi, G., Goto, R., 2021. Molecular phylogenetic assessment of *Spirobranchus kraussii*-complex (Annelida: Serpulidae) from the Japanese Archipelago. PeerJ 9, e11746. https://doi.org/10.7717/peerj.11746.

Kolbasov, G.A., Tsao, Y.F., Chan, B.K.K., 2025. Unveiling a hitherto monotypic crustacean coral endoparasite: diversity and morphology of the genus *Zibrowia* Grygier, 1995 (Thecostraca: Ascothoracida) through integrative taxonomy. Mar. Biodivers. 55, 41. https://doi.org/10.1007/s12526-025-01516-1.

Kupriyanova, E.K., Daffe, G., Pazoki, S., Al-Kandari, M., 2025. Not so Lessepsian migrants of the *Spirobranchus tetraceros* complex (Serpulidae, Annelida). Aquat. Invasions 20, 89–100. https://doi.org/10.3391/ai.2025.20.1.136400.

Kupriyanova, E.K., Flaxman, B., Burghardt, I., 2022. A puzzle no more: the identity of *Spirobranchus tetraceros* (Schmarda, 1861) (Annelida, Serpulidae) is revealed. Record Aust. Mus. 74, 201–214. https://doi.org/10.3853/j.2201-4349.74.2022.1820.

Kupriyanova, E.K., ten Hove, H.A., Rouse, G.W., 2023. Phylogeny of Serpulidae (Annelida, Polychaeta) inferred from morphology and DNA sequences, with a new classification. Diversity 15, 398. https://doi.org/10.3390/d15030398.

Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30, 3276–3278. https://doi.org/10.1093/bioinformatics/btu531.

Letunic, I., Bork, P., 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. 52, W78–W82. https://doi.org/10.1093/nar/gkae268.

Wong, T.K., Ly-Trong, N., Ren, H., Demotte, P., Baños, H., Roger, A.J., et al., 2026. IQ-TREE 3: phylogenomic inference software using complex evolutionary models. Mol. Biol. Evol. 43, msag117. https://doi.org/10.1093/molbev/msag117.

Maggioni, D., Arrigoni, R., Seveso, D., Galli, P., Berumen, M.L., Denis, V., et al., 2022. Evolution and biogeography of the *Zanclea*-Scleractinia symbiosis. Coral Reefs 41, 779–795. https://doi.org/10.1007/s00338-020-02010-9.

Marsden, J.R., 1987. Coral preference behaviour by planktotrophic larvae of *Spirobranchus giganteus corniculatus* (Serpulidae: Polychaeta). Coral Reefs 6, 71–74. https://doi.org/10.1007/BF00301376.

Marsden, J.R., 1992. Reproductive isolation in two forms of the serpulid polychaete, *Spirobranchus polycerus* (Schmarda) in Barbados. Bull. Mar. Sci. 51, 14–18.

Marsden, J.R., Conlin, B.E., Hunte, W., 1990. Habitat selection in the tropical polychaete *Spirobranchus giganteus* II. Larval preferences for corals. Mar. Biol. 104, 93–99. https://doi.org/10.1007/BF01313162.

Marsden, J.R., Meeuwig, J., 1990. Preferences of planktotrophic larvae of the tropical serpulid *Spirobranchus giganteus* (Pallas) for exudates of corals from a Barbados reef. J. Exp. Mar. Biol. Ecol. 137, 95–104. https://doi.org/10.1016/0022-0981(90)90063-I.

Martin, D., Britayev, T.A., 1998. Symbiotic polychaetes: review of known species. Oceanogr. Mar. Biol. Annu. Rev. 35, 217–340.

Martin, D., Britayev, T.A., 2018. Symbiotic polychaetes revisited: an update of the known species and relationships (1998–2017). Oceanogr. Mar. Biol. Annu. Rev. 56, 371–447.

Mehrotra, R., Arnold, S., Wang, A., Chavanich, S., Hoeksema, B.W., Caballer Gutierrez, M., 2020. A new species of coral-feeding nudibranch (Mollusca: Gastropoda) from the Gulf of Thailand. Mar. Biodivers 50, 36. https://doi.org/10.1007/s12526-020-01050-2.

Mehrotra, R., Caballer, M., Kaullysing, D., Jualaong, S., Hoeksema, B.W., 2024. Parasites or predators? Gastropod ectoparasites and their scleractinian host corals at Koh Tao, Gulf of Thailand, with the description of a new species. Symbiosis 92, 209–230. https://doi.org/10.1007/s13199-023-00967-z.

Molodtsova, T.N., Britayev, T.A., Martin, D., 2016. Cnidarians and their polychaete symbionts. In: Goffredo, S., Dubinsky, Z. (Eds.), The Cnidaria, Past, Present and Future. Springer, Cham, Switzerland, pp. 387–413. https://doi.org/10.1007/978-3-319-31305-4_25.

Montano, S., 2020. The extraordinary importance of coral-associated fauna. Diversity 12, 357. https://doi.org/10.3390/d12090357.

Montano, S., 2022. Diversity of coral-associated fauna: an urgent call for research. Diversity 14, 765. https://doi.org/10.3390/d14090765.

Montano, S., Maggioni, D., 2018. Camouflage of sea spiders (Arthropoda, Pycnogonida) inhabiting *Pavona varians*. Coral Reefs 37, 153. https://doi.org/10.1007/s00338-017-1642-1.

Montebon, A.R.F., Yap, H.T., 2009. Abundance, density, and size structure of *Spirobranchus gaymardi* (Polychaeta, Serpulidae) in Philippine coral reefs. Bull. Mar. Sci. 84, 93–108.

Mursawal, A., Sarong, M.A., Irham, M., Hermi, R., 2021. The study of *Spirobranchus* (christmas worms) based on substrate in Lamteung waters of Pulo Aceh, Aceh Besar District. IOP Conf. Ser. Earth Environ. Sci. 674, 012070.

Nishi, E., 1996. Serpulid polychaetes associated with living and dead coral at Okinawa Island, Southwest Japan. Publ. Seto Mar. Biol. Lab. 37, 305–318. https://doi.org/10.5134/176262.

Nishi, E., Abe, H., Tanaka, K., Jimi, N., Kupriyanova, E.K., 2022. A new species of the *Spirobranchus kraussii* complex, *S. akitsushima* (Annelida, Polychaeta, Serpulidae), from the rocky intertidal zone of Japan. ZooKeys 1100, 1–28. https://doi.org/10.3897/zookeys.1100.79569.

Nygaard, L., 2008. Size distribution of *Spirobanchus giganteus* in Bonaire: is there a benefit of recruitment to live coral? Physis 3, 25–30.

Oliveira, R.R.M., Nunes, G.L., de Lima, T.G.L., Oliveira, G., Alves, R., 2018. PIPEBAR and OverlapPER: tools for a fast and accurate DNA barcoding analysis and paired-end assembly. BMC Bioinf. 19, 297. https://doi.org/10.1186/s12859-018-2307-y.

Perry, O., Bronstein, O., Simon-Blecher, N., Atkins, A., Kupriyanova, E., ten Hove, H., et al., 2018a. On the genus *Spirobranchus* (Annelida, Serpulidae) from the northern Red Sea, and a description of a new species. Invertebr. Systemat. 32, 605–626. https://doi.org/10.1071/IS17061.

Perry, O., Sapir, Y., Perry, G., ten Hove, H., Fine, M., 2018b. Substrate selection of Christmas tree worms (*Spirobranchus* spp.) in the Gulf of Eilat, Red Sea. J. Mar. Biol. Assoc. U. K. 98, 791–799. https://doi.org/10.1017/S0025315416002022.

Pillai, T.G., 2009. Descriptions of new serpulid polychaetes from the Kimberleys of Australia and discussion of Australian and Indo-West Pacific species of *Spirobranchus* and superficially similar taxa. Record Aust. Mus. 61, 93–199. https://doi.org/10.3853/j.0067-1975.61.2009.1489.

R Core Team, 2026. R: a language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Rauch, C., Hoeksema, B.W., Hermanto, B., Fransen, C.H.J.M., 2019. Shrimps of the genus *Periclimenes* (Crustacea, Decapoda, Palaemonidae) associated with mushroom corals (Scleractinia, Fungiidae): linking DNA barcodes to morphology. Contrib. Zool. 88, 201–235. https://doi.org/10.1163/18759866-20191357.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., et al., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.

Rowley, S.J., 2008. Morphological Diversity and Abundance in the tube-dwelling Polychaete *Spirobranchus* giganteus (Pallas, 1766) in the Indo-Pacific: Adaptations to its Coral Host? Bachelor's Thesis. University of Plymouth, England. https://doi.org/10.13140/RG.2.1.3277.3609.

Rowley, S.J., Melbourne-Thomas, J., Kornel, R., Attrill, M.J., 2026. Drivers of *Spirobranchus corniculatus* (Grube, 1862) distribution on coral reefs in SE Sulawesi, Indonesia. Mar. Biol. 173, 96. https://doi.org/10.1007/s00227-026-04834-5.

Scott, P.J.B., 1987. Associations between corals and macro-infaunal invertebrates in Jamaica, with a list of Caribbean and Atlantic coral associates. Bull. Mar. Sci. 40, 271–286.

Seixas, V.C., de Moraes Russo, C.A., Paiva, P.C., 2017. Mitochondrial genome of the Christmas tree worm *Spirobranchus giganteus* (Annelida: Serpulidae) reveals a high substitution rate among annelids. Gene 605, 43–53. https://doi.org/10.1016/j.gene.2016.12.024.

Simon, C.A., van Niekerk, H.H., Burghardt, I., ten Hove, H.A., Kupriyanova, E.K., 2019. Not out of Africa: *spirobranchus kraussii* (Baird, 1865) is not a global fouling and invasive serpulid of Indo-Pacific origin. Aquat. Invasions 14, 221–249. https://doi.org/10.3391/ai.2019.14.2.05.

Smith, A.M., Henderson, Z.E., Kennedy, M., King, T.M., Spencer, H.G., 2012. Reef formation versus solitariness in two New Zealand serpulids does not involve cryptic species. Aquat. Biol. 16, 97–103. https://doi.org/10.3354/ab00444.

Spalding, M., Ravilious, C., Green, E.P., 2001. World Atlas of Coral Reefs. Univ. of California Press, Berkeley.

Stella, J.S., Pratchett, M.S., Hutchings, P.A., Jones, G.P., 2011. Coral-associated invertebrates: diversity, ecological importance and vulnerability to disturbance. Oceanogr. Mar. Biol. Annu. Rev. 49, 43–104.

ten Hove, H.A., 1970. Serpulinae (Polychaeta) from the Caribbean: I–the genus *Spirobranchus*. Stud. Fauna Curaçao Caribb. Is. 32, 1–57.

ten Hove, H.A., Nishi, E., 1996. A redescription of the Indo-West Pacific *Spirobranchus corrugatus* Straughan, 1967 (Serpulidae, Polychaeta), and an alternative hypothesis on the nature of a group of Middle Miocene microfossils from Poland. Beaufortia 46, 83–96.

ten Hove, H.A., van den Hurk, P., 1993. A review of recent and fossil serpulid 'reefs'; actuopalaeontology and 'Upper Malm' serpulid limestones in NW Germany. Geol. Mijnbouw 72, 23–67.

Toonen, R.J., Pawlik, J.R., 2001. Foundations of gregariousness: a dispersal polymorphism among the planktonic larvae of a marine invertebrate. Evolution 55, 2439–2454. https://doi.org/10.1111/j.0014-3820.2001.tb00759.x.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., et al., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115. https://doi.org/10.1093/nar/gks596.

van der Schoot, R.J., Hoeksema, B.W., 2022. Abundance of coral-associated fauna in relation to depth and eutrophication along the leeward side of Curaçao, southern Caribbean. Mar. Environ. Res. 181, 105738. https://doi.org/10.1016/j.marenvres.2022.105738.

van der Schoot, R.J., Hoeksema, B.W., 2024. Host specificity of coral-associated fauna and its relevance for coral reef biodiversity. Int. J. Parasitol. 54, 65–88. https://doi.org/10.1016/j.ijpara.2023.09.002.

van der Schoot, R.J., Hoeksema, B.W., 2025. Coral-associated invertebrates as indicators for reef health in the Caribbean. Ecol. Indic. 178, 114015. https://doi.org/10.1016/j.ecolind.2025.114015.

van der Schoot, R.J., Scott, C.M., ten Hove, H.A., Hoeksema, B.W., 2016. Christmas tree worms as epibionts of giant clams at Koh Tao, Gulf of Thailand. Mar. Biodivers 46, 751–752. https://doi.org/10.1007/s12526-015-0439-0.

Veron, J.E.N., 1995. Corals in Space and Time: the Biogeography and Evolution of the Scleractinia. Cornell Univ. Press.

Veron, J.E.N., Stafford-Smith, M.G., Turak, E., DeVantier, L.M., 2016. Corals of the World, version 0.01. https://www.coralsoftheworld.org/page/home/?version=0.01. (Accessed 18 June 2025).

Watson, M.G., Scardino, A.J., Zalizniak, L., Shimeta, J., 2017. A larval-settlement assay method for the gregarious serpulid polychaete, *Galeolaria caespitosa*. J. Exp. Mar. Biol. Ecol. 496, 49–55. https://doi.org/10.1016/j.jembe.2017.08.003.

Willette, D.A., Iñiguez, A.R., Kupriyanova, E.K., Starger, C.J., Varman, T., Toha, A.H., et al., 2015. Christmas tree worms of Indo-Pacific coral reefs: untangling the

Spirobranchus corniculatus (Grube, 1862) complex. Coral Reefs 34, 899–904. https://doi.org/10.1007/s00338-015-1294-y.

Wolf, R.P., 2024. Aggregative versus solitary settlement in *Spirobranchus cariniferus* Gray, 1843 (Serpulinae). What is the trade-off? Mar. Environ. Res. 200, 106670. https://doi.org/10.1016/j.marenvres.2024.106670.

Zimmermann, L., Stephens, A., Nam, S.Z., Rau, D., Kübler, J., Lozajic, M., et al., 2018. Completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. J. Mol. Biol. 430, 2237–2243. https://doi.org/10.1016/j.jmb.2017.12.007.