Taxonomy, barcoding and phylogeny of *Hydroides* (Serpulidae, Annelida), the largest genus of notorious fouling and invading calcareous tubeworms

Yanan Sun

Department of Biological Sciences

Faculty of Science and Engineering

Macquarie University

For the degree of Doctor of Philosophy in Biological Sciences

Supervisors

Assoc. Prof. Jane Williamson, Dr. Elena Kupriyanova, Dr. Pat Hutchings

Table of Contents

SUMMARY

Hydroides Gunnerus, 1768 is the most speciose genus of the calcareous tubeworm family Serpulidae (Polychaeta, Annelida). It is one of the most economically important group of marine invertebrates as it includes notorious foulers and common bioinvaders, yet the biodiversity of the genus is poorly known. Molecular tools, especially cytochrome *c* oxidase subunit I (COI) barcoding that have proven useful for species delimitation and discovery in eukaryotes, have not been widely used in *Hydroides* due to the high amplification failure rate of the COI gene. The aim of this study was to revise this genus using both morphological and molecular approaches and to provide reliable identification tools for biosecurity purposes.

The first detailed regional morphological taxonomic revision of *Hydroides* of Australia was conducted, based both on collections from Australian museums and newly collected material from New South Wales, Victoria, Queensland, Northern Territory, and Western Australia. As a result, 25 species are currently considered valid in Australia, including three newly described species. Furthermore, based on collections of 21 museums in 15 countries, taxonomic identification keys accompanied by diagnoses and original high-quality photographs were developed for 96 species word-wide.

To develop molecular identification tools for *Hydroides*, the entire mitochondrial genomes of eight *Hydroides* species were sequenced by next-generation sequencing and the resultant reference sequences were used to design new taxon-specific COI primers. Analyses of mitochondrial genomes of *Hydroides* showed a substantial rearrangement in gene order and numerous rarely reported large intergenic regions when compared with genomes of other known polychaetes. Using the newly designed primers, a COI barcoding database including 45 species of *Hydroides* was established and further used to explore cryptic speciation and invasion pathways in *Hydroides*. In particular, COI sequence data revealed the existence of two cryptic species in the common global invader *Hydroides dianthus* and suggested that the native range of *H. dianthus sensu stricto* is the Mediterranean rather than the Atlantic coast of the USA as historically assumed.

In addition to COI barcoding, another four gene markers (18S, 28S, ITS, cytochrome *b*) were also used to explore the taxonomic status and phylogenetic relationship of *Hydroides*. Combined data of three gene markers (18S, ITS, cytochrome *b*) and morphological data were used to resolve the taxonomic status of another widely distributed species, *Hydroides brachyacantha*. The study showed that *H. brachyacantha* is a complex of species with regionally restricted distribution ranges. A new Australian species *H. amri* and its cryptic lineage *H. nikae* were separated from the complex. A large-scale study of phylogenetic

relationships of *Hydroides* world-wide, based on 45 species using the integrated data of all five genetic markers, indicated closer phylogenetic relationships among species that occur in close biogeographic regions.

While the current study resolved the taxonomic status of most morphologically distinct *Hydroides* species, it also revealed the presence of a large number of cryptic species in the genus, which casts doubts on the status of other widespread species of *Hydroides*. Further molecular studies of widely dispersed species are needed to fully understand whether such wide distributions are caused by forces such as anthropogenic transport or are an indication of unresolved species complexes.

DECLARATION

I declare that the work in this thesis entitled “Taxonomy, barcoding and phylogeny of *Hydroides* (Serpulidae, Annelida), the largest genus of notorious fouling and invading calcareous tubeworms” is an original piece of research and it is entirely my own work. I also certify that the work has not been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and during the preparation of the thesis have been appropriately acknowledged.

Yanan Sun

43101054

December 2016

CHAPTER DECLARATIONS

Chapter 1: General Introduction

Written by myself with guidance and corrections from my supervisors, Elena Kupriyanova, Pat Hutchings, and Jane Williamson.

Chapter 2: Revision of the genus *Hydroides* (Annelida: Serpulidae) from Australia

Observation and measurement of all specimens were performed by myself. Photographing of specimens and colour photo plates editing were performed by Eunice Wong. Making maps and write-up were performed by myself with the guidance and correction from all the co-authors.

Chapter 3: A world-wide synopsis of the genus *Hydroides*

Observation of all specimens were performed by myself. Photographing of specimens were performed by Eunice Wong. Colour photo plates were made by myself. Write-up was performed by myself with guidance and corrections from Elena Kupriyanova, Pat Hutchings, Jane Williamson, and Shane Ahyong.

Chapter 4: Is *Hydroides brachyacantha* (Serpulidae: Annelida) a widespread species?

Specimens were obtained from the field trips of Elena Kupriyanova, Eunice Wong, and myself, as well as donations from our oversea collaborators. Molecular work was performed by Eunice Wong and myself. Data analyses were performed by myself. Write-up was performed by myself with the guidance and correction from all co-authors.

Chapter 5: A global invader or a complex of regionally distributed species? Clarifying the status of an invasive calcareous tubeworm *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) using DNA barcoding

Specimens were donated by our overseas collaborators. Molecular experiments were performed by Eunice Wong and myself. Data analyses were performed by myself. Write-up was performed by myself with the guidance and correction from all co-authors.

Chapter 6: Another exception of conserved gene order in Annelida: Mitogenomics reveals extensive gene rearrangement in the tubeworm genus *Hydroides* (Serpulidae)

Specimens were obtained from the field trips of Elena Kupriyanova, Eunice Wong, Pat Hutchings, Yanjie Zhang and myself. Genome sequencing were performed by Australian Genome Research Facility (Melbourne, Australia) and Beijing Genomics Institute (Shenzhen, China). Genome assembling and subsequent analyses were performed by myself. Write-up was performed by myself with guidance and corrections from my supervisors, Elena Kupriyanova, Pat Hutchings, and Jane Williamson.

Chapter 7: COI barcoding of *Hydroides*

Specimens were obtained from the field trips of Elena Kupriyanova, Eunice Wong, Pat Hutchings and myself, as well as donations from our overseas collaborators. Molecular experiments were performed by Eunice Wong and myself. Data analyses were performed by myself. Write-up was performed by myself with guidance and corrections from Elena Kupriyanova, Pat Hutchings, Jane Williamson, and Shane Ahyong.

Chapter 8: Phylogenetics and biogeography of the globally important calcareous tubeworm genus *Hydroides* Gunnerus, 1768 (Serpulidae, Polychaeta) inferred with multiple gene markers

Specimens were obtained from the field trips of Elena Kupriyanova, Eunice Wong, Pat Hutchings and myself, as well as donations from our oversea collaborators. Molecular experiments were performed by Eunice Wong and myself. Data analyses were performed by myself. Write-up was performed by myself with guidance and corrections from Elena Kupriyanova, Pat Hutchings, Jane Williamson, and Shane Ahyong

Chapter 9: General summary and conclusions

Writing up was entirely by myself with guidance and corrections from my supervisors Elena Kupriyanova, Pat Hutchings, Jane Williamson, and Shane Ahyong.

ACKNOWLEDGEMENTS

I would like to address my appreciation to many people who supported and helped me in many different ways. Without their help, this thesis would not have been possible.

I am deeply thankful to my supervisors Elena Kupriyanova, Jane Williamson and Pat Hutchings. Elena, you are an excellent mentor and a great friend, thanks for your patient guidance and encouragement during the three and a half years, for bringing me towards diving. Jane, thank you for taking me on as your student and your support whenever required. I will miss the field trip in Heron Island. Pat, you and Lena introduced me to the network of world polychaete scientists, which is invaluable for my further research.

A lot of thanks go to Eunice Wong for helping me with lab works and field trips. Without your help, I would not have finished the whole thesis on time. Good luck for your own PhD!

I am grateful to Harry ten Hove who hosted me during my visit to Naturalis Biodiversity Center and shared with me your incredible knowledge on tubeworms, to Chris Glasby who hosted me during our sampling trip in Darwin, and to João Miguel de Matos Nogueira, Orlemir Carrerette, Felipe Ramon De Chiara, and Carlos Diego who hosted me during my sampling in Brazil. Many thanks are due to the members of the Marine Invertebrate group and the Australian Centre for Wildlife Genomics group in Australian Museum. I am grateful to Stephen Keable, Alex Hegedus, and Scott Ginn for helping me with specimen deposition and registration, to Andrew King, Don Colgan, and Shane Ahyong who kindly sharing their experiments on research.

I also wish to thank all of our collaborators and colleagues Anja Schulze, Aylin Ulman, Eijiroh Nishi, Elena Lisitskaya, Erica Keppel, Giuseppe Suaria, James Carlton, Jixing Sui, Joachim Langeneck, María Ana Tovar-Hernández, Maria Capa, Melih Ertan Çinar, Priti Kubal, Tulio F. Villalobos-Guerrero, and Xuwen Wu who generously donated specimens from all over the world outside Australia.

To my families and my friends back in China, I cannot put into words my gratitude for your support while I have been overseas. Without your unconditional emotional support, I would never be able to get through my PhD. Thank you my new friends in Australia who helped me adapting to the new life quickly.

Finally, I appreciate the financial support of all the organisations that funded my research: Macquarie University, Australian Biological Resources Study (ABRS), and Australian Museum Research Institute.

Chapter 1

General Introduction

Author:

Yanan Sun

Serpulid polychaetes (Family Serpulidae Rafinesque, 1815), commonly known as "calcareous tubeworms", are a unique and highly specialized group of marine segmented worms. These polychaetes have adapted to inhabiting self-secreted calcareous tubes attached to a wide range of hard substrates from the intertidal to abyssal zone (ten Hove and Kupriyanova, 2009). Serpulids can be recognized by their calcareous tube, colourful radiolar crown and, in most cases, a well-differentiated tube plug called the operculum. With 98 nominal species (as of 2013, WoRMS, 2013), *Hydroides* Gunnerus, 1768 is not only the most speciose genus in the family Serpulidae, but also one of the most ecologically and economically important group of marine invertebrates that includes a number of species known as notorious foulers and bioinvaders (Lewis et al., 2006; Link et al., 2009; Michio and Yamanishi, 2010; Tovar-Hernández et al., 2009).

Like other serpulids, the body of *Hydroides* spp. is divided into three regions: radiolar crown, thorax, and abdomen. The radiolar crown is comprised of radioles with a double row of ciliated pinnules. The most dorsal radiole is transformed into the opercular peduncle and distal operculum. The operculum of *Hydroides* is distinctive and forms a complex two-tiered structure that consists of a basal funnel and a distal crown made of chitinous spines called a verticil. These structures present useful characters for species identification of *Hydroides*. The thorax is covered by the thoracic membranes that surround the base of the radiolar crown forming the collar. The border between the thorax and the abdomen is marked by chaetal inversion, the situation when hair-like chaetae are positioned dorsally and comb-shaped uncini are located ventrally in the thorax and their positions are reversed in the abdomen. Excluding several species reported from temperate and cold waters, species of *Hydroides* are distributed mainly in shallow shelves in tropical and subtropical areas (Bastida-Zavala and ten Hove, 2003; Ben-Eliahu and ten Hove, 1992; ten Hove and Kupriyanova, 2009).

Species of *Hydroides* exhibit similar life histories. Reproduction in *Hydroides* is sexual; the sexes are separate and external fertilization is followed by a typical planktotrophic larval development (reviewed by Kupriyanova et al., 2001). Planktotrophic larvae can last from two days to weeks, depending on food type and availability, salinity and temperature (Qiu and Qian, 1997; Toonen and Pawlik, 2001). Larval settlement is induced by both bacterial and chemical cues, and metamorphosis is generally completed in four to five days (Bryan et al., 1998; Beckmann et al., 1999; Qian, 1999; Hadfield and Paul, 2001; Hadfield, 2011). Several species of *Hydroides,* such as *Hydroides dianthus* (Verrill, 1873), *Hydroides ezoensis dirampha* Mörch, 1863, and *Hydroides elegans* (Haswell, 1883), settle gregariously (Scheltema et al., 1987; Okamoto et al., 1998; Hadfield and Paul, 2001; Toonen and Pawlik, 2001). These gregarious species can form dense colonies of tenaciously adherent calcareous tubes on underwater structures such as aquaculture nets, seawater intake pipes (Qiu and Qian, 1997), ship hulls and buoys (Wang and Huang, 1993), as well as on commercial molluscs (Arakawa, 1971). These fouling communities can have a negative impact on local community structure, such as modification of ecosystem dynamics and species assemblages through competition for space and food. Fouling by gregarious serpulids can also interfere with human activities, such as decreasing ship speed, blocking water pipes, increasing the weight and drag of buoys, and damaging aquaculture infrastructure (Arakawa, 1971; Ben-Eliahu and ten Hove, 1992; Wang and Huang, 1993; Chandra Mohan and Aruna, 1994). These impacts lead to high economic costs associated with removal of tubes from artificial structures, as well as reduced prices for fouled molluscs sold for human consumption.

Species of *Hydroides* are considered relatively ubitiquous, and are easily dispersed *via* human related vectors (e.g. shipping, agriculture, aquaculture). Species of *Hydroides* can be transported across oceans on ship's hulls or as planktotrophic larvae in ballast water, leading to cosmopolitan bioinvasions (Thorp et al., 1987; Zibrowius, 1991, 2002; Lewis et al., 2006; Link et al., 2009). Eight of the 100 "worst invasives" of the Mediterranean Sea list compiled by Streftaris and Zenetos (2006) are *Hydroides*. Among them, at least four species, *H. elegans*, *H. dirampha*, *H. dianthus* and *H. brachyacantha* Rioja, 1941, have been widely reported from tropical and subtropical regions.

Correct identification is critical in understanding the risk and potential invasion of particular species. Species of *Hydroides* are distinguished by their distinctive and varied structure of the operculum, especially of the verticil spines. Although numerous regional investigations and revisions have been published in the last 240 years, the large number of descriptive works with inconsistent terminology makes it difficult to distinguish species based only on their original descriptions (ten Hove, 1990). Many taxonomic problems exist due to the plasticity of some characteristics, incomplete descriptions in the historical literature, and confused identifications that are indicated by a complex list of synonyms (Bastida-Zavala and ten Hove, 2002). Thus, a systematic taxonomic revision of the whole genus is required.

In addition to the confusion surrounding identification, significant ontogenetic variability accompanied by the variability observed in regenerating opercula makes morphologically-based species delimitation problematic and difficult to resolve without molecular identification tools. COI barcoding, which uses a locus of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, has been proven effective in correctly identifying diverse groups of polychaetes at the species level (Barroso et al., 2010; Carr et al., 2011; Nygren and Pleijel, 2010; Lobo et al., 2013). However, from a barcoding point of view, serpulid polychaetes in general and *Hydroides* in particular, are one of the most problematic polychaete groups. All earlier attempts to amplify and sequence COI in serpulids using universal primers (Folmer et al., 1994) or to develop taxon-specific primers have failed (Elena

Kupriyanova, pers. comm.; Toril Loennechen Moen, pers. comm., see Sun et al., 2012). The only successful amplification and taxon-specific primer design for the COI fragment in *Hydroides* was achieved by Sun et al. (2012). Nevertheless, as already noted by Sun et al. (2012), the amplification failure of the first taxon-specific primer set on a number of *Hydroides* species indicated a requirement for new barcoding primers for the genus.

Although the most important fouling species, such as *H. elegans* and *H. dianthus*, have been intensively studied in terms of their reproduction, larval development, growth and settlement (Kupriyanova et al., 2001; Qian et al., 2007; Shikuma et al., 2016), little is known about most species beyond their basic taxonomic description (Bastida-Zavala and ten Hove, 2002). For instance, the phylogeny and phylogeography of *Hydroides*, which indicate how species of *Hydroides* evolved and dispersed, are still unclear. Kupriyanova et al. (2008) performed phylogenetic analyses of the *Serpula–Crucigera–Hydroides* clade that included eight species of *Hydroides*, but only five species were reasonably supported, suggesting that phylogenetic relationships needed further corroboration. Clearly, an up-to-date phylogenetic and phylogeographic study of the entire genus of *Hydroides* is required to understand the phylogenetic relationships and biogeographic patterns shaped by evolutionary history and anthropogenic transport of these species.

The main objective of this thesis was to conduct the first world-wide revision of *Hydroides* using both morphological and molecular data to provide effective tools for species identification and assessment of cryptic biodiversity within this group. Australasia is recognised as a region with high biodiversity and as a major area of invasive species reception (Carlton, 1987) and invasive species constantly arrive, yet the biodiversity of Australian *Hydroides* is poorly known due to the insufficient taxonomic effort. Thus, the revision will initially focus on defining Australian species. A taxonomic revision using standard terminology was performed to help resolve confused identification caused by incomplete descriptions in literature and lack of comparisons with type specimens. To provide reference sequences for the design of new taxon-specific COI barcoding primers, the whole mitochondrial genomes of several species of *Hydroides* were sequenced by next generation sequencing. Given the rich phylogenetic information, these mitochondrial genomes not only provided reference sequences needed to design primers to successfully sequence CO1 in *Hydroides*, but also provided additional molecular characters for phylogenetic analyses. Molecular markers that have been widely used for species identification and phylogenetic analyses of polychaetes, such as 18S, 28S rDNA, cytochrome b (Halanych and Janosik, 2006), together with the COI barcoding gene, were utilized to provide valuable resources to the wider community for species identification and genetic diversity within *Hydroides*, as well as to investigate the phylogenetic relationships and biogeographic patterns within *Hydroides*.

The following data chapters were compiled to achieve these objectives:

Chapter 2 outlines the first morphology-based revision of species of *Hydroides* in Australia based on all specimens of *Hydroides* in Australia both historical and newly collected. This revision includes a comprehensive species list, a detailed fully-illustrated taxonomic description and distribution pattern of each species, as well as a key to all species revised. This contributes to the documentation of Australia's native marine biodiversity and facilitates identification of invasive species in Australian waters. This section has been published in *Zootaxa* (Sun et al., 2015).

Chapter 3 provides the most comprehensive information source for quick and easy morphology-based species identification based on available specimens of all described species of *Hydroides* collected or loaned from 21 museums globally. It constitutes the first morphological synopsis of all nominal 98 species of *Hydroides* by providing a diagnosis for each species accompanied by an original high-quality photograph of the operculum, as well as a key to 98 species. This section will become a part of a proposed book entitled "*Hydroides* of the world".

Chapter 4 integrates morphological examination and molecular analyses to investigate the status of the questionable worldwide distributed species complex *H. brachyacantha*. It provides molecular support for the morphology-based separation of *H. amri* Sun et al., 2015 from *H. brachyacantha sensu stricto* and the presence of cryptic species *H. nikae* Sun et al., 2016. This section has been published in *Invertebrate Systematics* (Sun et al., 2016).

Chapter 5 assesses the utility of barcoding gene cytochrome *c* oxidase subunit I (COI) in exploring the taxonomic status of the common fouling invasive species *Hydroides dianthus* (Verrill, 1873). It also investigates whether widespread populations of *H. dianthus* comprise a single species or a species complex, and provides insight into the sources of invasion. It also assesses the application of COI barcoding in differentiating between potential cryptic species within *Hydroides*. This chapter has been accepted for publication in *Marine Biology*.

Chapter 6 describes the mitochondrial genomes of eight *Hydroides*, and discusses the unusual gene rearrangements and presence of numerous noncoding regions observed in the genus. It also includes a phylogenetic analysis based on mitochondrial protein-coding genes to investigate the phylogenetic position of Serpulidae within Annelida. This chapter provides reference sequences for the new taxon-specific COI barcoding primer design in Chapter 7. This chapter has been formatted as a manuscript to be submitted to *Molecular Phylogenetics and Evolution*.

Chapter 7 provides new taxon-specific COI barcoding primer sets for *Hydroides* designed according to the whole COI sequences from mitochondrial genomes. The new primer sets are tested on all specimens available for this thesis. This section provides DNA barcoding data for *Hydroides* for the first time, which can be used as a valuable source for fast DNA identification of *Hydroides* species. COI barcoding data from this section will be submitted to the Barcode of Life Data Systems (BOLD).

Chapter 8 provides a well-supported, comprehensive phylogeny for the genus *Hydroides* derived from phylogenetic analyses of a large multi-locus dataset of five markers (three nuclear and two mitochondrial) from 46 *Hydroides* morphospecies sampled worldwide. The results provide support for the morphologically-based species identification, including elucidation of potential cryptic species. Divergence times of the major groups of *Hydroides* and the historical biogeographic patterns are reconstructed in this chapter to estimate the evolution and dispersal history of *Hydroides*. This section has been formatted as a manuscript to be submitted to *Molecular Phylogenetics and Evolution*.

Chapter 9 provides a synthesis on the status of genus *Hydroides*, including taxonomy and phylogeny of the genus. It also collates the integrative tools used here for species identification, and their broader utilization in distinguishing natives from exotics.

References

Arakawa, K.Y. (1971). Notes on a serious damage to cultured oyster crops in Hiroshima caused by unique and unprecedented outbreak of a serpulid worm, *Hydroides norvegica* (Gunnerus) in 1969. *Venus* **30**, 75–82.

Bastida-Zavala, J.R., and ten Hove, H.A. (2002). Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the Western Atlantic Region. *Beaufortia* **52**, 103–178.

Bastida-Zavala, J.R., and ten Hove, H.A. (2003). Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the Eastern Pacific Region and Hawaii. *Beaufortia* **53**, 67–110.

Barroso, R., Klautau, M., Solé-Cava, A.M., and Paiva, P.C. (2010). *Eurythoe complanata* (Polychaeta: Amphinomidae), the ‘cosmopolitan’ fireworm, consists of at least three cryptic species. *Marine Biology* **157**, 69–80.

Beckmann, M., Harder, T., and Qian, P.Y. (1999). Induction of larval attachment and metamorphosis in the serpulid polychaetes *Hydroides elegans* by dissolved free amino acids: mode of action in laboratory bioassays. *Marine Ecology Progress Series* **190**, 181–190.

Ben-Eliahu, M.N., and ten Hove, H.A. (1992). Serpulids, Annelida: Polychaeta, along the Mediterranean coast of Israel—new population build-ups of Lessepsian migrants. *Israel Journal of Zoology* **38**, 35–53.

Bryan, P.J., Kreider, J.L., and Qian, P.Y. (1998). Settlement of the serpulid polychaetes *Hydroides elegans* (Haswell) on the arborescent bryozoans *Bugula neritina* (L.): evidence of a chemically mediated relationship. *Journal of Experimental Marine Biology and Ecology* **220**, 171–190.

Carlton, J.T. (1987). Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science* **41**, 452–465.

Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T.A., and Hebert, P.H.N. (2011). A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* **6**, e22232.

Chandra Mohan, P., and Aruna, C.H. (1994). The biology of serpulid worms in relation to biofouling control. In: Thompson, M.F., Nagabhushanam, R., Sarojini, R. (Eds.), *Recent Developments in Biofouling control*. Oxford and IBH. Pub1 Co, New Delhi, pp. 60–64.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Gunnerus, J.E. (1768). Om nogle Norske Coraller. *Det Kongelige Norske Videnskabernes Selskabs Skrifter* **4**, 38–73.

Hadfield, M.G. (2011). Biofilms and marine invertebrate larvae: What bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science* **3**, 453–470.

Hadfield, M.G., and Paul, V.J. (2001). Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae. In: McClintock, J.B., Baker, B.J. (Eds.), *Marine chemical ecology*. CRC Press, Boca Raton, pp. 431–461.

Halanych, K.M., and Janosik, A.M. (2006). A review of molecular markers used for Annelid phylogenetics. *Integrative and Comparative Biology* **46**, 533–543.

Haswell, W.A. (1883). On some new Australian tubicolous annelids. *Proceedings of the Linnean Society of New South Wales* **7**, 633–638.

Hove, H.A. ten (1990). Description of *Hydroides bulbosus* sp. nov. (Polychaeta, Serpulidae), from the Iranian Gulf, with a terminology for opercula of *Hydroides*. *Institute of Taxonomic Zoology (Zoological Museum) University of Amsterdam* **41**, 115–120.

Hove, H.A. ten, and Kupriyanova, E.K. (2009). Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs. *Zootaxa* **2036**, 1–126.

Kupriyanova, E.K., Nishi, E., ten Hove, H.A., and Rzhavsky, A.V. (2001). Life-history patterns in Serpulimorph polychaetes: ecological and evolutionary perspectives. *Oceanography and Marine Biology: an Annual Review* **39**, 1–101.

Kupriyanova, E.K., Bastida-Zavala, R., Halt, M.N., Lee, M.S.Y., and Rouse, G.W. (2008). Phylogeny of the *Serpula–Crucigera–Hydroides* clade (Serpulidae: Annelida) using molecular and morphological data: implications for operculum evolution. *Invertebrate Systematics* **22**, 425–437.

Lewis, J.A. (2006). Establishment of the Caribbean serpulid tubeworm *Hydroides sanctaecrucis* Krøyer [in] Mörch, 1863, in northern Australia. *Biological Invasions* **8**, 665–671.

Link, H., Nishi, E., Tanaka, K., Bastida-Zavala, R., Kupriyanova, E.K., and Yamakita, T. (2009). *Hydroides dianthus* (Polychaeta: Serpulidae), and alien species introduced into Tokyo Bay, Japan. *Marine Biodiversity Records* **2**, 1–5.

Lobo, J., Costa, P.M., Teixeira, M.A.L., Ferreira, M.S.G., Costa, M.H., and Costa, F.O. (2013). Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecology* **13**, 34.

Michio, O., and Yamanishi, R. (2010). Distribution of the alien species *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) in Osaka Bay, Japan, with comments on the factors limiting its invasion. *Plankton and Benthos Research* **5**, 62–68.

Mörch, O.A.L. (1863). Revisio critica Serpulidarum. *Et Bidrag til Rørormenes Naturhistorie. Naturhistorisk Tidsskrift, København, Ser. 3* **1**, 347–470.

Nygren, A., and Pleijel, F. (2011). From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. *Molecular Phylogenetics and Evolution* **58**, 132–141.

Okamoto, K., Watanabe, A., Sakata, K., and Watanabe, N. (1998). Chemical signals involved in larval metamorphosis in *Hydroides ezoensis* (Serpulidae; Polychaeta). Part I: induction of larval metamorphosis by extract of adult tube clumps. *Journal of Marine Biotechnology* **6**, 7–10.

Qian, P.Y. (1999). Larval settlement of polychaetes. *Hydrobiologia* **402**, 239–253.

Qian, P.Y., Lau, S.C.K., Dahm, H.U., Dobretsov, S., and Harder, T. (2007). Marine biofilms as mediators of colonization by marine macroorganisms: implications for antifouling and aquaculture. *Marine Biotechnology* **9**, 399–410.

Qiu, J.W., and Qian, P.Y. (1997). Combined effects of salinity, temperature and food on early development of the polychaete *Hydroides elegans*. *Marine Ecology Progress Series* **152**, 79–88.

Rafinesque, C.S. (1815). *Analyse de la Nature ou Tableau de l'univers et des corps organisés*.Aux dépens de l'auteur, Palerme, 224 pp.

Rioja, E. (1941). Estudios Anelidologicos. III. Datos para el conocimiento de la fauna de poliquetos de las costas del pacifico de Mexico. *Anales del Instituto de Biologia, Mexico* **12**, 669–746.

Scheltema, R.S., Williams, I.P., Shaw, M.A., and Loudon, C. (1981). Gregarious settlement by the larvae of *Hydroides dianthus* (Polychaeta: Serpulidae). *Marine Ecology Progress Series* **5**, 69–74.

Shikuma, N.J., Antoshechkin, I., Medeiros, J.M., Pilhofer, M., and Newman, D.K. (2016). Stepwise metamorphosis of the tubeworm *Hydroides elegans* is mediated by a bacterial inducer and MAPK signalling. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 10097–10102.

Streftaris, N., and Zenetos, A. (2006). Alien marine species in the Mediterranean – the 100 ‘worst invasives’ and their impact. *Mediterranean Marine Science* **7**, 87–118.

Sun, Y., Kupriyanova, E.K., and Qiu, J.W. (2012). COI barcoding of *Hydroides*: a road from impossible to difficult. *Invertebrate Systematics* **26**, 539–547.

Sun, Y., Wong, E., Hove, H.A. ten, Hutchings, P.A., Williamson, J.E., and Kupriyanova, E.K. (2015). Revision of the genus *Hydroides* (Annelida: Serpulidae) from Australia. *Zootaxa* **4009**, 1–99.

Sun, Y., Wong, E., Tovar-Hernández, M.A., Williamson, J.E., and Kupriyanova, E.K. (2016). Is *Hydroides brachyacantha* (Serpulidae: Annelida) a widespread species? *Invertebrate Systematics* **30**, 41–59.

Verrill, A.E. (1873). VIII. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. *Report of the United States Commission of Fish and Fisheries* 757 pp.

Wang, J., and Huang, Z. (1993). Fouling polychaetes of Hong Kong and adjacent waters. *Asian Marine Biology* **10**, 1–12.

Thorp, C.H., Pyne, S., and West, S.A. (1987). *Hydroides ezoensis* Okuda, a fouling serpulid new to British coastal waters. *Journal of Natural History* **21**, 863–877.

Toonen, R.J., and Pawlik, J.R. (2001). Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). I. Gregarious and nongregarious settlement. *Marine Ecology Progress Series* **244**, 103–114.

Tovar-Hernández, M.A., Méndez, N., and Villalobos-Guerrero, T.F. (2009). Fouling polychaete worms from the Southern Gulf of California: Sabellidae and Serpulidae. *Systematics and Biodiversity* **7**, 319–336.

Zibrowius, H. (1991). Ongoing modification of the Mediterranean marine fauna and flora by the establishment of exotic species. *Mésogée* **51**, 83–107.

Zibrowius, H. (2002). Assessing scale and impact of ship-transported alien fauna in the Mediterranean? In: Briand, F. (Ed.), *CIESM Workshop Monograph no. 20, Istanbul, 6–9 November 2002. Alien marine organisms introduced by ships in the Mediterranean and Black Sea*. CIESM, Monaco, pp. 63–68.

Chapter 2 (pp. 11-146) of this thesis have been removed as they contain published material under copyright. Removed contents published as:

Sun, Y., Wong, E., Hove, H., Hutchings, P., Williamson, J., & Kupriyanova, E. (2015). Revision of the genus Hydroides (Annelida: Serpulidae) from Australia. *Zootaxa*, 4009(1), pp. 1–99.
http://dx.doi.org/10.11646/zootaxa.4009.1.1

Chapter 3

A world-wide synopsis of the genus *Hydroides*

Author:

Yanan Sun

Abstract

As the most species-rich and economically important serpulid genus, *Hydroides* includes many species that have been investigated and described worldwide during the last 200 years. However, all taxonomic reviews to date are based on regional faunas. Many taxonomic problems exist due to incomplete descriptions in literature published prior to the standardization of terminology and methods for serpulid taxonomy, misidentifications in literature, and lack of comparison among specimens on the global scale. In addition, a ruling by the International Code of Zoological Nomenclature (ICZN) determined that the gender of genus *Hydroides* should revert to the original feminine, in contrary to the masculine usage of the name since 1992. This gender change of the genus name has been completely overlooked by serpulid taxonomists in the last 16 years. Thus, updates to the species names, a key to all species, and diagnoses using standard terminology are needed. Here, an updated and corrected checklist of all 98 valid species of *Hydroides* is presented. A comprehensive taxonomic key to the 98 recognized species of *Hydroides*, a diagnosis and figures for each species, and a map showing type localities of all the species are provided in this chapter.

Introduction

Among polychaetes, the family Serpulidae Rafinesque, 1815 is a uniquely specialized group of marine segmented worms that inhabits a self-secreted calcareous tube attached to a wide range of hard substrates, from the intertidal to abyssal zone (ten Hove and Kupriyanova, 2009). Serpulids are easily recognized by their calcareous tube, colourful radiolar crown and, in most cases, a well-differentiated tube plug called the operculum. Within the Serpulidae, *Hydroides* Gunnerus, 1768 is the most speciose genus with 98 nominal species (WoRMS, 2016). Most species of *Hydroides* occur in shallow-water tropical and subtropical regions world-wide, although a few species are found in temperate and cold waters. Unlike other serpulid genera, *Hydroides* has a complex two-tiered operculum that consists of a basal funnel and a distal crown made of chitinous spines called the verticil.

The genus *Hydroides* was erected by Gunnerus (1768), with *H. norvegica* as the type species. Philippi (1844) established *Eupomatus* for those species that have verticil spines without lateral spinules, with *E. uncinatus* as the type species. Thus, the name *Hydroides* was maintained for species that have lateral spinules. Later, the subgenera *Eucarphus*, *Polyphragma*, *Glassopsis*, *Schizocraspedon*, and *Olgaharmania* were erected to separate species according to the presence of lateral spinules, the symmetrical or asymmetrical arrangement of verticil spines, the presence of bifurcated verticil spines, and the presence of bulbous spines. However, since their generic status has not been justified by synapomorphies, the names *Eucarphus*, *Glossopsis*, *Olgaharmania*, *Polyphragma* and *Schizocraspedon* were not used extensively, and the genus *Eupomatus* was synonymised with *Hydroides* (Pillai, 1972).

As a result of regional taxonomic reviews, the number of described species of *Hydroides* has increased almost linearly during the past 100 years (See Fig. 1 in Bastida-Zavala and ten Hove, 2002). Among those species, 26 species were reviewed from the west coast of the Atlantic Ocean (Rioja, 1941b; Bastida-Zavala and ten Hove, 2002); 20 species were reported from the east coast of the Atlantic Ocean and Mediterranean (Zibrowius, 1968, 1971, 1972a, 1973a, b); seven species were described from the coast of the Red Sea and the Gulf of Iran (ten Hove, 1970, 1990; Ben-Eliahu, 1972); 14 species were reported from India and Sri Lanka (Hartman, 1959; Pillai, 1960, 1961, 1971); 12 species were reported from Japan (Imajima, 1976a, b, 1978; Uchida, 1978; Imajima and ten Hove, 1989); 32 species were reported from the coast of China, including the South China Sea (Zibrowius, 1972b; Chen and Wu 1978, 1980; Wu and Chen, 1981; Fiege and Sun, 1999; Sun and Yang, 2000; Sun et al., 2012; Sun and Yang, 2014); 15 species were reported from the tropical West Pacific (Zibrowius, 1972b, Imajima, 1982; Imajima and ten Hove ,1984, 1986); 27 species were revised from the coast of Australia (Dew, 1959; Straughan, 1967a, b; Pillai, 2009; Sun et al., 2015; Kupriyanova et al., 2015); and 22 species were reported from the East Pacific and Hawaii (Rioja, 1941a; Bailey-Brock, 1991; Bastida-Zavala and ten Hove, 2003; Bastida-Zavala, 2008; Tovar-Hernández et al., 2016).

Species delimitation in *Hydroides* relies heavily on the distinctive and varied structure of the operculum, especially of the verticil spines. A standardized terminology for the *Hydroides* operculum was first proposed by ten Hove (1990) and then updated by Bastida-Zavala and ten Hove (2002). Most descriptions before that, however, are incomplete, combining a variety of terms used by different authors for the same characters. This is a substantial issue as this includes original descriptions of more than 50 species. Moreover, given the plasticity of some characters, such as variable numbers of lateral spinules on verticil spines in *H. elegans*, *H. norvegica*, and *H. capensis*, distinguishing species of *Hydroides* is inherently difficult. These confused identifications are indicated by the complex lists of synonyms (Bastida-Zavala and ten Hove, 2002; WoRMS, 2016). Recent applications of molecular techniques not only provide powerful tools to distinguish morphological similar species within *Hydroides* (Sun et al., 2016; Tovar-Hernández et al., 2016), but has also further revealed hidden diversity within the genus (Sun et al., 2016).

The aim of this chapter is to present a worldwide revision of all species of *Hydroides*, to give a complete taxonomic key to all species of *Hydroides*, and to provide a diagnosis for each species accompanied by an original high-quality photograph of the operculum. This chapter provides the most comprehensive information for easy and quick morphology-based species identification in the genus.

Material and methods

The present chapter is based on historical collections deposited in the following institutions: AM – Australian Museum, Sydney, Australia; AMNH – American Museum of Natural History, New York, USA; BMNH – British Museum (Natural History), nowadays Natural History Museum, London, UK; BPBM – Bernice Pauahi Bishop Museum, Hawaii, USA; ECOSUR – El Colegio de la Frontera Sur, Chetumal, Mexico (reference collection); HUJ – Hebrew University of Jerusalem, Section of Invertebrates of the National Natural History Collections; MAGNT – Museum and Art Gallery of Northern Territory, Darwin, Australia; MBMCAS – Marine Biological Museum of the Chinese Academy of Sciences, Qingdao, China; MNHN – Muséum National d'Histoire Naturelle, Paris, France; MNHW – Museum of Natural History, Wroclaw University, Poland; MRAC – Musée Royal de l'Afrique Centrale, Tervuren, Belgium; NHMW – Natural History Museum Wien, Austria; NSMT – National Science Museum, Tokyo, Japan; SAM – South Australian Museum, Adelaide, Australia; USNM –Smithsonian National Museum of Natural History (NMNH), Washington, formerly United States National Museum; YPM – Yale Peabody Museum, New Haven, Connecticut, USA; ZINRAS – Zoological Institute Academy of Sciences, St. Petersburg, Russia; ZMA – Zoological Museum of Amsterdam, nowadays incorporated in the Naturalis Biodiversity Centre, Leiden, the Netherlands; ZMB – Zoologisches Museum der Humboldt-Universität, Berlin, Germany; ZMC – Zoological Museum, Copenhagen, Denmark; ZMH –Zoologisches Institut und Zoologisches Museum, Hamburg, Germany.

Type specimens of each species were observed (if possible) under a Leica MZ 16 dissecting microscope. For species where type material was unavailable, specimens collected from type localities, or as close to type localities as possible, were examined. Observed morphological characteristics were compared with their original description. Selected specimens (type specimens if possible) were photographed with a SpotFlex 15.2 camera mounted on a Leica MZ16 stereomicroscope at the AM. Helicon Focus 5.3 Pro software was used to create a sharply focused photomontage.

All original literature of each species of *Hydroides* was examined. Correct endings were given to each species according to the gender agreement under the requirements and exceptions of the Code article 31 (ICZN 1999), as well as professional comments from Geoff Read and Harry ten Hove. Short diagnoses were given to imply the most useful characteristics for species identification. Terminologies used in the key and diagnoses followed those of Bastida-Zavala and ten Hove (2002) and ten Hove and Kupriyanova (2009). For species that could not be separated by morphology, the names were listed together in the key. The type locality (Map 1) and institute where holotype specimens deposited were given for each species.

Map 1. Type localities of species of *Hydroides*. Numbers on the map correspond to the numbers assigned to each species in the Taxonomic account.

Key to the species of *Hydroides*

1a. Opercular funnel absent. Verticil with 16-19 straight spines similar in size, with pointed tips…………………..........……………………………….. *Hydroides bannerorum* (Fig. 1A)

1b Opercular funnel present…………………...2

2a Spines of opercular verticil all similar in shape, spine size may change gradually..............3

2b Spines of opercular verticil dissimilar, one or more dorsal spines different…...…………59

3a Spines of opercular verticil all similar in size and shape…………………..…………..….4

3b Spines of opercular verticil all similar in shape, but with one or more dorsal spines significant larger in size, or becoming gradually smaller from dorsal to ventral side.............54

4a. Verticil spines very short, similar in size to tips of funnel........ *Hydroides plateni* (Fig. 1B)

4b Verticil spines significantly larger than tips of funnel radii………………………………...5

5a Spines of both verticil and funnel radii elongated and bifurcated at mid-length.. *Hydroides furcifera* (Fig. 1C)

5b Spines of verticil and funnel radii not bifurcated at mid-length…………………..……….6

6a Verticil spines rhomboid or triangular ……………………………….……………….....7

6b Verticil spines elongated…………………………………………………………...………...10

7a Verticil with 7-8 spines ……………………………….…… *Hydroides uniformis* (Fig. 1D)

7b Verticil with 5-6 spines……………………………………………….……….…………8

8a Verticil spines with distal knob………………………...*Hydroides kimberleyensis* (Fig. 1E)

8b Verticil spines distally pointed, but without distal knob……………………………………9

9a Verticil spines rhomboid in cross-section, collar chaetae with two conical teeth only…………………………………………………...…... *Hydroides rhombobula* (Fig. 1F)

9b Verticil spines triangular, collar chaetae with two large teeth and 2 to 3 small teeth……………………………………………………… *Hydroides xishaensis* (Fig. 1G)

10a Verticil spines without external, lateral spinules, or mid-internal spinules, basal spinule may be present……………...…………………………………………………...11

10b Verticil spines with external, lateral spinules, or mid-internal spinules, basal spinule may be present …………………………….……………………………...……….....32

11a Verticil with simple blunt, round or globular tips ……………………………….....12

11b Verticil tips with laterally positioned or radially positioned projections………………...14

11c Verticil spines with pointed tips…………………………………………………….....18

12a Verticil with blunt tips…………………………………..….. *Hydroides arnoldi* (Fig. 1H)

12b Verticil with slightly swollen tips ….……………………….………………...………....13

13a Verticil composed of 12-16 spines with distinctly globular tips……………………………
………..…………………………………………………………*Hydroides microtis* (Fig. 1I)

13b Verticil composed of 7-9 uniform spines with dilated or blunt tips……………………..
…………………………………….…………….………*Hydroides novaepommeraniae* (Fig. 1J)

13c Verticil composed of 7-9 uniform spines with globular tips………………………………..
…..…………………………………..………………..... *Hydroides bandaensis* (Fig. 1K)

14a Verticil tips with laterally positioned projections (tips T-shaped or anchor shaped)…….15

14b Verticil tips with radially positioned projections……………………..……................16

15a Basal internal spinules absent, funnel radii with T-shaped tips..…*Hydroides qiui* (Fig. 1L)

15b Basal internal spinules present; funnel radii with pointed tip………………………………
……………………………………………………………*Hydroides dirampha* (Fig. 1M)

16a Verticil tips with terminal dark hooks pointing inwards and a long whip-like filaments pointing outwards…………………………...……………. *Hydroides bisecta* (Fig. 1N)

16b Radially positioned projections of verticil spine tips otherwise ……………..……........17

17a Pointed spine tips with external distal spinule................*Hydroides recurvispina* (Fig. 1O)

17b Pick-axe modified tips………………………………………*Hydroides dolabrus* (Fig. 1P)

17c Triangular modifications of tips radii............................. *Hydroides panamensis* (Fig. 1Q)

18a Tips of verticil spines curved into hooks pointing in clockwise direction 19

18b Tips of verticil spines not pointing in clockwise direction ....……………..……23

19a Basal internal spinules long, reaching verticil centre, verticil dark-coloured……………… ………………………..…...............................………………… *Hydroides fusca* (Fig. 1R)

19b Basal internal spinules short, verticil light-coloured……............................…………20

20a Funnel with pointed radii……………………………...….... *Hydroides trompi* (Fig. 1S)

20b Funnel with T-shaped radii……………………………….......... *Hydroides gairacensis*

23a Verticil spines and their tips straight, not curved or slightly curved outwards................24

23b Verticil spines or their tips only curved inwards………...............………………..…..27

24a Verticil larger than funnel……………………...…………………………….……..…25

24b Verticil smaller than funnel………………………….………………………….……….26

25a Verticil with 7-20 spines, each spine with a basal spinule..... *Hydroides fusicola* (Fig. 1T)

25b Verticil with 14 spines without basal spinule ..………………..…. *Hydroides huanghaiensis*

26a Verticil with 11-15 spines curving outwards, each spine with inner basal knob………… ……………………………………………….........…... *Hydroides protulicola* (Fig. 2A)

26bVerticil with 7-10 spines, curving outwards or straight, no internal spinules……………… …………………………………………….…..……………… *Hydroides humilis* (Fig. 2B)

26c Verticil with 8-9 straight spines, no internal spinules, long base of funnel………………... ..…………………………….………………………......... *Hydroides longistylaris* (Fig. 2C)

27a Basal part of verticil spines flattened into wings……………………………...…...….28

27b Basal part of verticil spines not flattened into wings..………………...…………………...…31

28a Funnel with 45-61 of radii ending in very sharp elongated tips…………………………… ....*Hydroides spongicola* (Fig. 2D)

28b Funnel with 26-38 triangular radii…………………………………........................….29

29a Internal basal spinule short, never crossing mid-point of central disk…........................…30

29b Internal basal spinule long, crossing mid-point of central disk……………………………. ..………………………………..……………………..…... *Hydroides alatalateralis* (Fig. 2E)

30a Wings occupying less than half of the spine length………...*Hydroides floridana* (Fig. 2F)

30b Wings occupying more than half of the spine length......... *Hydroides elegantula* (Fig. 2G)

31a Funnel radii tips elongated pointed with an additional internal spinule each………….. .... *Hydroides pseudouncinata* (Fig. 2H)

31b Funnel radii tips elongated pointed without an additional internal spinule each………… ....*Hydroides gracilis* (Fig. 2I) (*H. basispinosa*)

32a Verticil spines with internal spinules only...33

32b Verticil spines with external and/or lateral spinules, internal spinule maybe present…..35

33a Verticil spines distally pointed, each spine with a row of 6-8 internal spinules………… …….................……………………………...……………….... *Hydroides ezoensis* (Fig. 2J)

33b Verticil spines distally blunt, one large mid-level internal spinule present……………......34

34a Each spine with a basal small internal spinule and a mid-level one pointing down……… ………………………………………………………….………….... *Hydroides augeneri* (Fig. 2K)

34b Each spine with large conspicuous internal spinules pointing slightly upwards………….. ……………………………………………………………....….. *Hydroides sinensis* (Fig. 2L)

35a Verticil spines with both external and lateral spinules..36

35b Verticil spines with external or lateral spinules..37

36a Verticil spines with groups of 2-4 yellow small external spinules halfway and 3-5 pairs of black lateral spinules in basal-middle position...........................*Hydroides lambecki* (Fig. 2M)

36b. Verticil spines with groups of yellow 5-13 tiny external spinules halfway; 4-5 pairs of black lateral spinules in basal-middle position.....................*Hydroides mongeslopezi* (Fig. 2N)

37a Spines of verticil with external spinules only...38

37b Spines of verticil with lateral spinules, external spinule absent......................................39

38a Verticil spines with simple pointed tips. Each verticil spine with a single pointed external spinule at mid-length..*Hydroides sanctaecrucis* (Fig. 2O)

38b Verticil spines with radially positioned modified tips. Each verticil spine with a single blunt external spinule at mid-length..*Hydroides calopoma* (Fig. 2P)

39a Verticil spines with one pair of lateral spinules..40

39b Verticil spines with more than one pair of lateral spinules..47

40a Verticil spines with a pair of outwardly curved lateral spinules and an internal spinule at mid-length..*Hydroides tambalagamensis* (Fig. 2Q)

40b Lateral spinules of verticil spines not outwardly curved, no internal spinule at mid-length...41

41a Verticil with central tooth, spines with tips curved inwards, each with a pair of straight lateral spinules at about mid-length (exceptionally with additional lateral spinule) and an internal basal spinule.. *Hydroides nodosa* (Fig. 2R)

41b Verticil without central tooth...42

42a Funnel with rounded tips..43

42b Funnel with pointed tips...45

43a Short flattened incurved spines, no internal basal spinule...*Hydroides stoichadon* (Fig. 2S)

43b Slender long incurved spines, one sharply pointed inner basal spinule……………........44

44a Funnel tips simple rounded...*Hydroides bispinosa* (Fig. 2T)

44b Funnel tips rounded, separated by a constriction from two lateral processes……………… ....*Hydroides homoceros* (Fig. 3A)

45a Verticil with straight spines ending in blunt tips................*Hydroides mucronata* (Fig. 3B)

45b Verticil with spines curving inwards ending in pointed tips..46

46a Verticil spines curving inwards without a pronounced distal knob……*Hydroides crucigera* (Fig. 3C)

46b Verticil spines abruptly curving inwards, with a pronounced distal knob…………………………………………………………………………………………… *Hydroides parva* (Fig. 3D)

47a Verticil spines with up to 20 pairs of lateral spinules….. *Hydroides ochotereana* (Fig. 3E)

47b Verticil spines with 2-6 pairs of lateral spinules..48

48a Central tooth absent, spinules curved crescent-shaped…..*Hydroides nanhaiensis* (Fig. 3F)

48b Central tooth present..49

49a Central tooth long, verticil spine with 4–6 pairs of lateral spinules……………………………………………………………………………………………*Hydroides longispinosa* (Fig. 3G)

49b Central tooth short...50

50a Each verticil spine with 2–4 pairs of lateral spinules and 1–3 internal spinules, verticil spines fused basally, forming a wide shallow cup..51

50b Each verticil spine with 4-8 pairs of lateral spinules, verticil spines not fused basally, not forming a wide shallow cup...53

51a Incurved verticil spines with lateral spinules at basal half of each spine; with two sharp and long internal spinules, one at mid-length, one at base...........*Hydroides capensis* (Fig. 3H)

5ab Verticil spine straight or outcurved; with a row of 1–3 small internal spinules at basal half of each spine...52

52a Collar chaetae with proximal rasp...*Hydroides elegans* (Fig. 3I)

52b Collar chaetae with two knobs...*Hydroides norvegica* (Fig. 3J)

53a Spines with 4-5 pairs of lateral spinules..................................*Hydroides azorica* (Fig. 3K)

53b Spines with 6-8 pairs of lateral spinules..........................*Hydroides multispinosa* (Fig. 3L)

54a Verticil spines bulbous...55

54b Verticil spines elongated..56

55a Verticil with 3 diamond-shaped spines, becoming gradually smaller in size…………………………………………………………………………………………………*Hydroides trilobula* (Fig. 3M)

55b Verticil with 6 diamond-shaped spines, dorsal spine larger………………………………………………………………………………………………*Hydroides adamaformis* (Fig. 3N)

56a Verticil spine with pair of lateral spinules.............................*Hydroides chilensis* (Fig. 3O)

56b Verticil spine without pair of lateral spinules..57

57a Elongated slender spines pointing in one direction (dorsal inside, ventral outside), becoming gradually smaller from dorsal to ventral side……….. *Hydroides dianthus* (Fig. 3P)

57b Verticil spines all curved inwards...58

58a Verticil spine becoming smaller gradually from dorsal to ventral side, external tubercles on verticil spines present...*Hydroides nikae* (Fig. 3Q)

58b One verticil spine larger than other spines, external tubercles absent…………...........59

59a Verticil spine short, broad, slightly curved inwards................*Hydroides deleoni* (Fig. 3R)

59b Verticil spine elongated and narrower, strongly curved inwards......................................60

60a Verticil light-coloured, no black constriction………………………………………………………………………………..*Hydroides inornata* (Fig. 3S), *Hydroides operculata* (Fig. 3T)

60b Verticil black, with black opercular constriction....................... *Hydroides nigra* (Fig. 4A)

61a Dorsal verticil spine elongated...62

61b Dorsal verticil spine bulbous, swollen..76

62a Two or more curved dorsal verticil spines larger than the rest...63

62b One dorsal verticil spines larger or somehow different from the rest..............................66

63a Two larger incurved spines, smaller spines with external and/or lateral spinules, external knob absent...64

63b Two to five larger incurved spines, smaller spines with small to pronounced external knob, external and/or lateral spinules absent..65

64a Verticil spines (except dorsal) with a pair of dark lateral spinules and with an external distal spinule.....................................*Hydroides externispina* (Fig. 4B), *Hydroides ralumiana*

64b Verticil spines (except dorsal) without a pair of dark lateral spinules, but with an external spinule..*Hydroides glasbyi* (Fig. 4C)

65a 26-39 verticil spines...*Hydroides similoides* (Fig. 4D)

65b 12-22 verticil spines..*Hydroides similis* (Fig. 4E)

65c 7-10 verticil spines...*Hydroides amri* (Fig. 4F)

66a Both longer dorsal spine and rest of verticil spines bifurcated at mid-length………………...*Hydroides heterofurcata* (Fig. 4G)

66b. None of verticil spines bifurcated at mid-length...67

67a Dorsal spine narrow with pointed tip and small sub-distal internal spinule, straight or outcurved, only slightly longer than other spines; remaining verticil spines outcurved, without sub-distal spinules.. *Hydroides recta* (Fig. 4H)

67b Dorsal spine distinctly larger than other spines, raised over the rest as elongated spine ending in a hook, remaining spines the same...68

68a Dorsal spine incurved, the other verticil spines straight...69

68b Dorsal spine incurved, other verticil spines outcurved...70

68c All verticil spines incurved...74

69a Spines (except dorsal) elongated with T-shaped or bifurcate tips, internal spinule absent...*Hydroides bifurcata* (Fig. 4I)

69b Spines (except dorsal one) irregularly tetrahedral, vertex pointed upwards, internal spinule present..*Hydroides monroi* (Fig. 4J)

70a Elongated dorsal spine ending in incurved hook with two distal lateral spinules.............71

70b Elongated dorsal spine ending in incurved hook without two distal lateral spinules.......72

71a Internal spinules present..*Hydroides lirs* (Fig. 4K)

71b Internal spinules absent………………………………………...*Hydroides minax* (Fig. 4L)

72a Funnel radii with T-shaped tips....................................*Hydroides pseudexaltata* (Fig. 4M)

72b Funnel radii with pointed tips...73

73a Verticil spines without pointed basal internal spinule.............*Hydroides dipoma* (Fig. 4N)

73a Verticil spines with pointed basal internal spinule each..........*Hydroides exaltata* (Fig. 4O)

74a Spines (except dorsal one) with a pair of lateral spinules each……………………………
…………………………………………………………*Hydroides heterocera* (Fig. 4P)

74b All spines lacking lateral spinules..75

75a Verticil with 7-8 broad spines with blunt tips.........................*Hydroides inermis* (Fig. 4Q)

75b Verticil with 7-10 narrower spines with pointed tips...*Hydroides brachyacantha* (Fig. 4R)

76a Verticil consists of large bulb with 6 longitudinal groves, smaller spines absent……….
....*Hydroides steinitzi* (Fig. 4S)

76b Verticil consists of large bulbous dorsal spine and 5-14 much smaller spines…………77

77a Smaller verticil spines fused or partly fused to base of dorsal bulbous verticil spine.......78

77b Smaller verticil spines not fused to dorsal bulbous verticil spine....................................82

78a Dorsal spine convex, with five smaller spines with pointed tip, median three pressed fused to base of dorsal spine, two lateral ones positioned above median ones………………….
....*Hydroides glandifera* (Fig. 4T)

78b Dorsal spine distinctly concave or globular..79

79a Dorsal bulbous verticil spine more than five times longer than the smaller verticil spines...80

79b Dorsal bulbous verticil spine less than five times longer than the smaller verticil spines………………………………………………………………………………….81

80a Dorsal spine elongated, trilobed in cross-section, with 4–7 outwardly curved small spines with T-shaped tips pressed against base of dorsal spine.........*Hydroides trivesiculosa* (Fig. 5A)

80b Dorsal spine consisting of bulbous median part and two triangular in cross-section latero-dorsal processes. Bulb with 7-14 longitudinal groves corresponding to number of spines inserted at base...*Hydroides bulbosa* (Fig. 5B)

81a Verticil with 5-8 spines, large dorsal incurving spine with a distal knob, spines straight or directed outward with blunt tips..*Hydroides salazarvallejoi* (Fig. 5C)

81b Verticil with 9-14 spines, large dorsal spine and two smaller adjacent ones partially fused, each with a distal knob. Other spines directed outward with sharp tips……………………
....*Hydroides tenhovei* (Fig. 5D)

82a Verticil with all (5–7) bulbous spines..83

82b Verticil with conical or elongated spines with pointed or sharp tips.................................84

83a Small tubercles on outer surface on bend of verticil spines present………………………
.... *Hydroides tuberculata* (Fig. 5E)

83b Small tubercles on outer surface on bend of verticil spines absent………………………...
.... *Hydroides perezi* (Fig. 5F), *Hydroides dafnii*

84a Dorsal spine incurved, with pointed tip..85

84b Dorsal spine not incurved, with bulbous median part and two dorso-lateral processes....87

85a Dorsal verticil spine with an accessory lateral knob; other spines stubby and digitate, slightly outcurved..*Hydroides helmata* (Fig. 5G)

85b Dorsal verticil spine distinctly globular, may with external knob; other spines conical, with internal spinule..86

86a Dorsal verticil spine smooth, without external knob….*Hydroides simplidentata* (Fig. 5H)

86b Dorsal verticil spine with external knob..................................*Hydroides rostrata* (Fig. 5I)

87a Dorsal verticil spine flatten column like; other verticil spines and funnel with distinctly anchor-shaped tip...*Hydroides ancorispina* (Fig. 5J)

87b Verticil spines (except dorsal spine) and funnel with club-shaped tip.............................88

88a Dorsal spine with beak-like medial extension and bifid tip……………………………….
....*Hydroides malleolaspina* (Fig. 5K)

88b Dorsal spine without beak-like medial extension and bifid tip…………………………
.... *Hydroides albiceps* (Fig. 5L)

Taxonomic account

Family SERPULIDAE Rafinesque, 1815

Hydroides Gunnerus, 1768

Type species: *Hydroides norvegica* Gunnerus, 1768 (original binomen)

Includes *Eupomatus* Philippi, 1844, type species *E. uncinatus* Philippi, 1844 (by subsequent designation), *Eucarphus* Mörch, 1863 (as *Hydroides* (*Eucarphus*)), type species uncertain (full synonymy in WoRMS).

Remarks:

Most recently, an unusual situation has arisen concerning the correct formulation and spelling of historic species-group names in *Hydroides* Gunnerus, 1768. According to the International Code of Zoological Nomenclature (the Code, ICZN) the suffix spelling of a Latin or Latinized adjectival species-group name must agree in gender with its genus (ICZN 1999: Art.31.2). *Hydroides* was erected as feminine in 1768, followed by the matching feminine adjectival endings in most literatures for the next 200 years. The masculine ending for species of *Hydroides* was first used by Hartman for *H. pacificus* (Hartman, 1969), according to the first edition of ICZN that genus names ending in *-oides* were to be treated as masculine (ICZN, 1961: Art. 30a (ii) examples). However, both feminine and masculine endings appeared in the literature for the following 30 years until Ben-Eliahu and ten Hove (1992) correctly pointed out that the Code third edition (ICZN, 1985) had *Hydroides* as masculine. After that, serpulid taxonomists adopted the use of masculine endings, which continued until August 2015 without adoption of the new qualifying clauses in the Code fourth edition (ICZN, 1999), which reverted it back to the feminine status after almost 40 years as the opposite gender. According to the fourth edition of the Code, Article 30.1.4.4, “A compound genus-group name ending in the suffix *-ites, -oides, -ides, -odes*, or *-istes* is to be treated as masculine unless its author, when establishing the name, stated that it had another gender or treated it as such by combining it with an adjectival species-group name in another gender form.” Thus, *Hydroides* should be treated as feminine as the type species *H. norvegica* was published with a feminine ending (Gunnerus, 1768). The conflict was first reported on the website of the World Register of Marine Species (WoRMS) by Geoffrey Read in July 2015. The gender-corrected names were subsequently used in Sun et al. (2015, 2016), and then in Kupriyanova et al. (2015) for Australian species. However, an updated of *Hydroides* nomenclature is need for species described outside Australia.

1. *Hydroides adamaformis* Pillai, 2009 (original binomen) (Fig. 3N)
Diagnosis: Verticil with six thick swollen transparent spines. Dorsal verticil spine larger and elongated distally, other verticil spine similar in shape and size. All spines roughly rhomboidal, widening from base to medially towards a tiny pointed lateral knob on each side, ending in a blunt tip curved inwards. Central tooth absent. Funnel radii chitinized with T-shaped to crescent-shaped tips.
Type locality: south west corner of Lucas Island (near Dampier Archipelago) Kimberley region, Western Australia, 15°13’0.12” S, 124° 31’0.12”E. Holotype AM W.21457.
Distribution: Kimberley, Western Australia

2. *Hydroides alatalateralis* (Jones, 1962) (originally as *Eupomatus alatalateralis*) (Fig. 2E)
Diagnosis. Verticil with 13 yellowish spines (11-16), curving inwards. All verticil spines similar in shape and size, with pointed tips. Base of verticil spines flattened into wings extending to more than half of spine length. Each verticil spine with one long basal internal spinule crossing mid-point of central disk. Funnel with 26–44 radii with pointed tips, exceptionally with split tip.
Type locality: Port Royal, Jamaica. Holotype and paratypes AMNH 3617, 3618

Distribution: Jamaica, Caribbean, Colombian Pacific

3. *Hydroides albiceps* (Grube, 1870) (originally as *Serpula (Eupomatus) albiceps*) (Fig. 5L)

Diagnosis: Verticil with 6–13 spines; Dorsal verticil spine vesicular, consisting of a bulbous median part, and two latero-dorsal more or less triangular (seen from dorsal side) outpockets. Other verticil spines point outward, with rectangular to clavate tips, without any accessory spinules. Funnel with 14–31 clavate to bottle-shaped chitinized radii, base of funnel not chitinized.

Type locality: Tor, Gulf of Suez, Egypt, Red Sea. Holotype ZMB 533

Distribution: widely distributed in the Indo-West Pacific, from Zanzibar and Red Sea to South Japan and Polynesia, north Australia. A species complex.

4. *Hydroides amri* Sun, Wong, ten Hove, Hutchings, Williamson and Kupriyanova, 2015 (original binomen) (Fig. 4F)

Diagnosis: Verticil with 7–10 spines, with pointed tip, strongly curved inward, becoming smaller gradually from dorsal to ventral side; 3–5 dorsal verticil spines larger than others, smooth, covering towards central disc, other verticil spines with a sharp to round knob. Each verticil spine with one short basal internal spinule. Funnel with 27–42 sharp chitinized radii, base of funnel not chitinized.

Type locality: South Bass Point, New South Wales, Australia, 34°36’12”S, 150°53’43”E. Holotype AM W.46591

Distribution: Australia

5. *Hydroides ancorispina* Pillai, 1971 (original binomen) (Fig. 5J)

Diagnosis: Verticil with 7 spines. Dorsal spine stout, columnar, and somewhat laterally flattened, without any accessory processes. Six remaining spines with crescentic tips, gradually increase in size ventrally. Funnel with 19 radii, with transverse crescentic chitinized tips.

Type locality: Wellawate, Colombo, Sri Lanka. Holotype BMNH 1968.152

Distribution: Sri Lanka

6. *Hydroides arnoldi* Augener, 1918 (original binomen) (Fig. 1H)

Diagnosis: Verticil with 7 bottle shaped spines, with rounded tips, straight. All verticil spines similar in shape and size, each with a small rounded inner basal spinule. Central tooth absent. Funnel with 24 radii with conical tips.

Type locality: Annobón, Gulf of Guinea, West Africa. Holotype ZMH V.8724

Distribution: Annobón, Sao Tome, Congo, Zaire, Ghana, Liberia

7. *Hydroides augeneri* Zibrowius, 1973a (original binomen) (Fig. 2K)

Diagnosis: Verticil with 7–9 uniform spines. Verticil spine dark brown, elongated, slightly incurved with rounded tips. Each spine with small basal internal spinule and a large dark mid-level internal spinule pointing to central disc. Funnel with 25–43 radii with conical chitinized tips.

Type locality: Zaire, Malembe, West Africa. Holotype MRAC 34.951 and 4 paratypes MRAC 23.736, 23.764-6

Distribution: Congo, Dahomey, Ghana, Liberia, Guinea

8. *Hydroides azorica* Zibrowius, 1972a (original binomen) (Fig. 3K)

Diagnosis: Verticil with 12–21 uniform spines, almost straight or slightly curving outwards with pointed tips. Each spine with 4–10 pairs of lateral spinules and a row of 2–6 internal spinules below the most proximal lateral spinules. Central teeth dark, well developed, with pointed tip. Funnel not chitinized, with 30–40 radii with rounded tips.

Type locality: Ponta Delgada, São Miguel, Azores. Holotype MNHN, paratypes USNM

Distribution: Azores

9. *Hydroides bandaensis* Zibrowius, 1972b (original binomen) (Fig. 1K)

Diagnosis: Verticil with 7–9 uniform spines, cone-shaped, smooth, with slightly dilated or blunt tips. Spinules absent. Funnel with 16 cone-shaped radii, with dilated or blunt chitinized tips.

Type locality: Banda Sea, Indonesia. Holotype NHMW Evert. Varia 2039

Distribution: Banda Sea, Indonesia

10. *Hydroides bannerorum* Bailey-Brock, 1991 (original binomen) (Fig. 1A)

Diagnosis: Verticil with 16–19 uniform spines, light to dark brown in colour, straight, with pointed tips. Each spine with small irregular tubercles in middle sections and one small basal internal spinule. Funnel absent.

Type locality: 70 m near Sand Island and Barbers Point outfalls off Oahu, Hawaii, United States. Holotype BPBM R 2153, paratypes BMHN ZB 1990.16, ZB 1990.17, 18; BPBM R2154, USNM 136581

Distribution: Hawaii, Mariana Islands

11. *Hydroides basispinosa* Straughan, 1967a (Fig. 2I)

Diagnosis: Verticil with 7–9 similar (sometimes dorsal spine longer) elongated spines, incurved, with pointed tips. Each spine with a blunt basal internal spinule. Funnel with 36–40 radii, with pointed tips.

Type locality: Mouth of Ross River, Townsville, Queensland, Australia. Holotype AM W.4012

Distribution: Queensland, Australia

12. *Hydroides bifurcata* Pixell, 1913(originally as *Hydroides bifurcatus*) (Fig. 4I)

Diagnosis: Verticil with 7–9 spines. Dorsal spine elongated to incurved hook. Other verticil spines straight or slightly curved outwards, bifid distally ending in two sharped tips directed lateral, each with a subapical internal spinule. All verticil spines with a basal internal spinule pointed upwards. Funnel with 25 radii, with pointed tips.

Type locality: Minikoi, Maldive Islands. Holotype NHM PO01 1972.117

Distribution: Maldives

13. *Hydroides bisecta* Imajima and ten Hove, 1989 (originally as *Hydroides bisectus*) (Fig. 1N)

Diagnosis: Verticil with 7 uniform elongated spines, incurved and pointing downwards. Each spine with dark sharp tip and a distinct, subapical whip inserted to the external of the tip. Whips dark at the base and white at tips. Funnel with 27 radii ending in pointed tips.

Type locality: off Sesoko Marine Station, Okinawa, Japan. Holotype in NSMT-Pol. 256

Distribution: Okinawa, Japan

14. *Hydroides bispinosa* Bush, 1910 (original binomen) (Fig. 2T)

Diagnosis: Verticil with 6–11 (usually 7 or 8) uniform spines strongly curved inwards. Each spine with one pair of lateral spinules and one sharply pointed basal internal spinule. Funnel with 14–24 rounded radii, not chitinized.

Type locality: Bermuda. Holotype not known

Distribution: Bermuda, Gulf of Mexico, Eastern U.S.A. and Caribbean

15. *Hydroides brachyacantha* Rioja, 1941a (original binomen) (Fig. 4R)

Diagnosis: Verticil with 8 to 12 yellow to dark brown spines, curved inwards. Dorsal spine enlarged to broad hook, strongly curved, covering central disc. Other verticil spines with pointed tip and pronounced knob each. First and second pair of dorsal spines (lateral to dorsal hook) with tips and trunks wider than all other verticil spines. Funnel radii with blunt rounded tips.

Type locality: Gulf of California, Sinaloa, Mazatlán, Marina Mazatlán, Mexico, 23°16’47”N, 106°27’40”W, neotype AM W.46899

Distribution: Tropical Americas, Mediterranean

16. *Hydroides bulbosa* ten Hove, 1990 (originally as *Hydroides bulbosus*) (Fig. 5B)

Diagnosis: Verticil with large bulbous dorsal spine and 7–14 bottle shaped spines with blunt tips. Dorsal spine triangular in cross-section, consisting of bulbous median part and two latero-dorsal processes. Median bulb with 7-14 longitudinal groves, with corresponding number of verticil spines partly fused into base. Spinules absent. Funnel with 20-30 chitinized radii, with pointed tips.

Type locality: Khor Ghubb Ali, Oman. Holotype BMNH ZB 1972.42, paratypes BMNH ZB 1980.548, ZMA 3704, AM W.19862, USNM 123384

Distribution: Arabian Gulf

17. *Hydroides calopoma* Zibrowius, 1973a (original binomen) (Fig. 2P)

Diagnosis: Verticil with 8–9 spines, dorsal spine may slightly larger than others. Each spines ending in a strong incurved pointed hook with a subapical external spinule with blunt or bifurcated tip at one-fourth length of spine. All verticil spines with a second short blunt external spinule at mid length as well, and a long, erect, pointed basal internal spinule. Funnel with 29–30 radii, chitinized, long and tapering, slightly curving outwards, with a subapical denticle curving inwards.

Type locality: Isla Tortuga, Annobon, Gulf of Guinea. Holotype MRAC 34.950

Distribution: Annobón, Equatorial Guinea

18. *Hydroides capensis* Zibrowius, 1972a (original binomen) (Fig. 3H)

Diagnosis: Verticil with 8–15 uniform spines with pointed tips. Each spine with 2–4 pairs of pointed lateral spinules and a row of 1–3 internal spinules from about half of total length to base. Distal end of spines smooth, with a darker colour, and curved inwards. Funnel not chitinized, with 24–32 rounded radii.

Type locality: Lambert’s Bay, South Africa, 32°05’S, 17°56’E. Holotype and paratypes BMNH 1961.9.819-833

Distribution: West Africa: Cape Province of South Africa, Ghana, Nigeria, Senegal

19. *Hydroides chilensis* Hartmann-Schröder, 1962 (original binomen) (Fig. 3O)

Diagnosis: Verticil with 7–11 yellow spines curved inwards, with pointed tips, dorsal spine slightly longer than others. Each spine with one pair of pointed lateral spinule in middle section and a long, wart-like basal internal spinule pointed towards central disc. Funnel with 21–58 radii, with triangular tips.

Type locality: Arica, Chile. Type series ZMH V 15179, 15180 and ZMA V.Pol. 3687

Distribution: Columbia to Arica, Chile

20. *Hydroides crucigera* Mörch, 1863 (originally as *Hydroides* (*Eucarphus*) *crucigera*) (Fig. 3C)

Diagnosis: Verticil with 7–10 uniform spines, curving inwards, with pointed tips. Each spine with one pair of pointed lateral spinule in middle section and a basal internal spinule with blunt or bifurcated tip. Funnel with 22–32 radii, with pointed tips.

Type locality: Punta Arenas, Western Costa Rica. Type material unknown

Distribution: Punta Arenas Pacific, Baja California to Colombia, Hawaii

21. *Hydroides dafnii* (Amoureux, Rullier and Fishelson, 1978) (originally as *Eupomatus dafnii*)

Diagnosis: Verticil with 5 bulbous spines enlarged at the base, rounded at the border and with pointed tips. One of spines enlarged and pointing inwards. Spinules and central tooth absent. Funnel with 20 radii ending in pointed tips.

Type locality: Eilat, Israel, Red Sea. Syntypes MNHN A526 UB348

Distribution: Sea Red, Eilat Gulf

22. *Hydroides deleoni* Bastida-Zavala and ten Hove, 2003 (original binomen) (Fig. 3R)

Diagnosis: Verticil with 7–11 yellow spines, with pointed tips curving inwards. Most dorsal spine bigger than the others; other verticil spines similar in shape and size. Each spine with one short basal internal spinule. Lateral and external spinules absent. Central tooth absent. Funnel with 30–43 radii ending in pointed tips.

Type locality: Punta San Juanico, Western coast of Baja California Sur, Mexico. Holotype ZMA V Pol.3784

Distribution: Baja California Sur to Ecuador

23. *Hydroides dianthus* (Verrill, 1873) (originally as *Serpula dianthus*) (Fig. 3P)

Diagnosis: Verticil with up to 10 yellowish slender spines, with pointed tips. All verticil spines curved more or less ventrally and gradually decreasing in size ventrally, with a basal internal spinule. External and lateral spinules absent. Central spine absent. Funnel with up to 33 radii ending in pointed tips.

Type locality: Great Egg Harbor to New Haven and Cape Cod, New Jersey to Massachusetts, United States. Syntypes YPM 2698, 2699

Distribution: Along the East coast of North America down to Florida and Grand Caribbean, reportedly introduced to China, Europe, Japan, West Africa and Brazil

24. *Hydroides dipoma* (Schmarda, 1861) (originally as *Eupomatus dipoma*) (Fig. 4N)

Diagnosis: Verticil dark brown, with 9–12 slender spines with pointed tips. Dorsal spine elongated to hook, curved inwards. Other verticil spines shorter, similar in shape and size, strongly curved outwards distally. A subapical internal spinule maybe present, curved inwards, with sharp pointed tip. All verticil spines with a short triangular basal internal spinule. Funnel with 25–53 pointed radii, slightly curving outwards. Constriction between funnel and peduncle clear, brown in colour.

Type locality: Cape of Good Hope, South Africa, SE Atlantic. Types lost from NHMW

Distribution: tropical West Africa, South Africa, Suez

25. *Hydroides dirampha* Mörch, 1863 (originally as *H.* (*Eucarphus*) *dirampha*) (Fig. 1M)

Diagnosis: Verticil with 11–15 spines, straight, with T-shaped or anchor shaped tips. All verticil spines similar in shape size, with one basal internal spinule. Central tooth absent. Funnel with 20–33 pointed chitinized radii.

Type locality: St. Thomas, Lesser Antilles. Types unknown

Distribution: Mediterranean, Indo-West Pacific, tropical Pacific America (probably its native distribution), West Atlantic, East Atlantic, South Africa, New Zealand, Australia

26. *Hydroides dolabrus* Tovar-Hernández, Villalobos-Guerrero, Kupriyanova and Sun, 2015 (original binomen) (Fig. 1P)

Diagnosis: Verticil with 8–11 spines, all similar in shape and size. Verticil spine tips with medial projections, pickaxe-shaped, asymmetrical, triangular: internal ones short and pointing inwards verticil center; external projection longer than internal, pointing outwards verticil center. All verticil spines with one short basal internal spinule. External, lateral spinules and central tooth absent. Funnel with 18–31 pointed radii.

Type locality: Mazatlán, Sinaloa, Mexico, 23°16’47.42”N, 106°27’39.94”W. Holotype AM W.47012

Distribution: Southern Gulf of California

27. *Hydroides elegans* (Haswell, 1883) (Fig. 3I)

Diagnosis: Verticil with 13–21 spines, straight. All verticil spines similar in shape and size, each with pointed tip, 2–4 pairs pointed lateral spinules and 1–3 pointed internal spinules. Verticil spines widening at base and together forming a wide shallow cup. Central spine may be present. Funnel not chitinized, with 21–28 blunt radii. Collar bayonet chaetae with a well-defined serrated blade, two to four pointed, short teeth and a large proximal rasp.

Type locality: Port Jackson, New South Wales, Australia. Deposition of material is not given by Haswell (1883: 633); material of this age was not encountered by HAtH in either the AM or the BMNH

Distribution: Sub-tropical locations around the world; Mediterranean-Atlantic, Indo-West Pacific including all around Australia, tropical Pacific America, West Atlantic, East Atlantic, South Africa

28. *Hydroides elegantula* (Bush, 1910) (originally as *Eupomatus elegantulus*) (Fig. 2G)

Diagnosis: Verticil with 11 spines, curved inwards, similar in shape and size, with pointed tips. Basal part of each verticil spines into wings occupying more than half of the spine length. Each spine with a pointed basal spinule, erect at base, curved towards central disc at tips, never crossing mid-point of central disc. Central tooth absent. Funnel with 26–38 radii with pointed triangular tips.

Type locality: Bermuda. Holotype YPM IZ 001323

Distribution: Bermuda Islands

29. *Hydroides exaltata* (Marenzeller, 1885) (originally as *Eupomatus exaltatus*) (Fig. 4O)

Diagnosis: Verticil spine with a basal column and 7–9 spines, with pointed tips. Dorsal verticil spine enlarged to hook, strongly curved inward. Others spines smaller, similar in size and shape, curved outwards. All verticil spines with one erect, pointed basal internal spinule. Funnel with 20–30 chitinized radii ending in pointed tips. Constriction between funnel and peduncle elongated, chitinized.

Type locality: East Coast of Island of Enoshima, Honshu, Japan. Holotype not found

Distribution: Indo-West Pacific from Zanzibar, Red Sea, and Arabian Gulf to South Japan and Polynesia, Australia: Western Australia, Northern Territory, Queensland

30. *Hydroides externispina* Straughan, 1967b (original binomen) (Fig. 4B)

Diagnosis. Verticil with 8–10 spines, with sharply pointed tips, curved inwards. Two dorsal verticil spines elongated, covering other verticil spines. Each of other verticil spines with one sharply pointed external spinule on 1/3 of the whole length, curved outwards. All verticil spines with a pair of lateral spinules at mid-length, curved outwards, and a small blunt basal spinule. Funnel with 24–36 pointed chitinized tips.

Type locality: Heron Island, Queensland, Australia. Holotype AM W.4105

Distribution: Indo-West Pacific, Southwestern Japan, Indonesia, New Caledonia, Australia: Queensland, North Western Australia

31. *Hydroides ezoensis* Okuda, 1934 (original binomen) (Fig. 2J)

Diagnosis: Verticil with 20–30 uniform spines ending in pointed tips, straight or curved outwards at tips, fused basally for 1/3–2/3 of their length. Each verticil spine with a row of 6–8 pointed internal spinules. Central tooth of varying length present. Funnel with 23–60 radii ending with pointed chitinized tips.

Type locality: Akkeshi, Muroran and Oshoro, Hokkaido, Japan. Types not found

Distribution: Sea of Japan and north China; as introduced species in France, Southern United Kingdom and temperate Australia: New South Wales, Tasmania, Victoria

32. *Hydroides floridana* (Bush, 1910) (originally *Eupomatus floridanus*) (Fig. 2F)

Diagnosis: Verticil with 13 spines ending in sharp tips. All verticil spines incurved, similar in shape and size. Basal part of each verticil spines flattened into wings occupying less than half of the spine length. Each verticil spine with a short basal internal spinule curved towards central disc. Central tooth absent. Funnel with 26–38 radii ending in triangular tips.

Type locality: Cape Dear Rio, Florida, United States. Types not found

Distribution: Florida, Gulf of Mexico and Eastern United States

33. *Hydroides furcifera* (Grube, 1878) (originally as *Serpula furcifera*) (Fig. 1C)

Diagnosis: Verticil with 14–23 spines similar in shape and size. All verticil spines bifurcated from mid-length, ending in pointed tips. Each verticil spine with a row of up to seven internal spinules below bifurcated section. Central tooth absent. Funnel with 14–32 radii ending with slender long bifurcated tips, similar to verticil spines in shape. Constriction between funnel and peduncle clear, chitinized.

Type locality: Ubay, Pandanon, Philippines. Syntypes MNHW 383, ZMB Q 5337, MNHW MPW386

Distribution: Philippines, Palau Island, Truk, Ponape Islands, Australia: Queensland, north West Australia

34. *Hydroides fusca* Imajima, 1976a (original binomen) (Fig. 1R)

Diagnosis: Verticil with 7 glossy back slender spines, all equally in shape and size. Verticil spines recurved in clockwise direction from subapical curved part and end in sharp hook pointed to central disc. Each verticil spine with an elongated dark internal spinule reaching in verticil centre. Central tooth absent. Funnel with about 20 dark radii, outwardly curved, ending in sharp tips.

Type locality: Off Tanega-Shima, Southwestern Japan. Holotype NSMT-Pol. H 119

Distribution: South Japan, Palau Island, Red Sea, South China

35. *Hydroides fusicola* Mörch, 1863 (originally as *Hydroides* (*Eupomatus*) *fusicola*) (Fig. 1T)

Diagnosis: Verticil with 7–20 elongated slender spines with pointed tips. All verticil spines similar in shape, few spines may shorter than others, straight or slightly curved outwards. Each spine with a small conical basal internal spinule. Central tooth absent. Funnel with 34–38 radii with pointed tips.

Type locality: Japan (not further specified). Type material may have been lost

Distribution: Japan, South China

36. *Hydroides gairacensis* Augener, 1934 (originally as *Hydroides (Eupomatus) gairacensis*)

Diagnosis: Verticil with 6–7 white spines, smooth, with pointed brown tips, all equally in shape and size. Verticil spines recurved in clockwise direction from subapical curved part and end in sharp hook pointed to central disc. Spinules and central tooth absent. Funnel with 15–24 radii ending in anchor-shaped tips.

Type locality: Gairaca, Santa Marta, Caribbean Sea coast of Colombia. Holotype ZMA V.Pol. 0458

Distribution: La Guayra, Venezuela; South Florida to South Brazil, Pacific Panama

37. *Hydroides glandifera* Rioja, 1941a (originally as *Hydroides glandiferum*) (Fig. 4T)

Diagnosis: Verticil with 5 black spines. Dorsal spine bulbous, rounded and convex at the apex, with flatten pointed tip folded towards central disc. Other spines triangular, with pointed tips. Two sub dorsal spines deeply fused to dorsal spine, with tips pointed ventrally. Two ventral spines basally fused to dorsal spine. Spinules and central tooth absent. Funnel with 12–17 dark brown radii ending in pointed tips.

Type locality: Caleta (Playa Caleta), Acapulco, Mexico. Type specimens lost

Distribution: Baja California Sur to Acapulco, Guerrero

38. *Hydroides glasbyi* Sun, Wong, ten Hove, Hutchings, Williamson and Kupriyanova, 2015 (original binomen) (Fig. 4C)

Diagnosis: Verticil with 8–10 spines, ending in sharply pointed tips, curved inwards. Two dorsal verticil spines longer, covering other verticil spines. Other verticil spines similar in shape and size, with a curved subapical external spinule ending in sharp tip. Funnel with 22–26 pointed chitinized tips.

Type locality: Fort Hill Wharf, Darwin, Northern Territory, Australia, 12°28’17”S, 130°50’48”E. Holotype MAGNT W017502

Distribution: Darwin, Australia

39. *Hydroides gracilis* (Bush, 1905) (originally as *Eupomatus gracilis*) (Fig. 2I)

Diagnosis: Verticil with 9–12 yellow spines, slender, smooth, with pointed tips, curved inwards. All verticil spines similar in shape, dorsal spines maybe slightly larger in size. Spinules and central tooth absent. Funnel with 16–42 radii ending in pointed tips.

Type locality: Pacific Grove, California, United States. Two syntypes YPM 2697

Distribution: Monterey Bay, California to Bahia Tortugas, Baja California Sur

40. *Hydroides helmata* (Iroso, 1921) (originally as *Eupomatus helmatus*) (Fig. 5G)

Diagnosis: Verticil with 7 spines. Dorsal spine enlarged, helmet-like, bulbous, with incurved pointed tip and a subapical accessory lateral knob; other spines stubby and digitate, slightly curved outwards. Central tooth absent. Funnel not chitinized, with 15–20 radii ending in rounded tips.

Type locality: Gulf of Naples, Italy. Holotype not found

Distribution: Naples, Mediterranean

41. *Hydroides heterocera* (Grube, 1868) (originally as *Serpula* (*Eupomatus*) *heterocerus*) (Fig. 4P)

Diagnosis: Verticil with 10–12 elongated brown spines, curved inwards, with sharp tips. Dorsal spine larger than others, without lateral or external spinules. Other verticil spines similar in shape and size, with a pair of pointed lateral spinules curved outwards at mid-length. All verticil spines with a pointed basal internal spinule. Funnel with 19–31 chitinized radii ending in anchor-shaped tips.

Type locality: Red Sea (not further specified). Types might be in Vienna, Austria

Distribution: Red Sea, Iranian Gulf, Zanzibar, Madagascar, Sri Lanka, Lessepsian migrant to the Levant Mediterranean

42. *Hydroides heterofurcata* Pillai, 1971 (original binomen) (Fig. 4G)

Diagnosis: Verticil with 9–11 spines bifurcated at mid-length. Dorsal spine enlarged, broader than others, with two prongs curved laterally and then inwards to a pointed hook, and a straight, elongated internal spinule below the two prongs with its tip lies mid-way between the tips of the other two prongs. Other verticil spines similar in shape, gradually increased in size ventrally, V-shaped each, ending with slender tapering prongs, curved outwards. Funnel with 23–26 radii ending in conical tips.

Type locality: Talaimannar Pier, NW Sri Lanka. Holotype BMNH 1968.145, paratypes BMNH 1968.146, 147

Distribution: Sri Lanka

43. *Hydroides homoceros* Pixell, 1913 (original binomen) (Fig. 3A)

Diagnosis: Verticil with 7–10 slender spines ending in pointed tips, curved inwards, all similar in shape and size. Each spine with a pair of curved lateral spinules at mid-length and a pointed basal internal spinule. Funnel with 20–25 chitinized bottle-shaped radii with rounded tips, may with a pair of lateral spinule subapically.

Type locality: Either Maldives or off Zanzibar. Syntypes NHM 1924.6.13.147

Distribution: Arabian Sea, Arabian Gulf, Maldives, Zanzibar, Seychelles, Sudan, Lessepsian migrant to the Levant Mediterranean, Toulon, France

44. *Hydroides huanghaiensis* Sun and Yang, 2000 (original binomen)

Diagnosis: Verticil with 14 uniform slender spines, straight. All verticil spines smooth, without spinules. Central tooth hemispherical. Funnel with 40 radii with pointed tips.

Type locality: Yellow Sea, 39°00’N, 122°70’E. Holotype MBMCAS

Distribution: Yellow Sea

45. *Hydroides humilis* (Bush, 1905) (originally as *Eupomatus humilis*) (Fig. 2B)

Diagnosis: Verticil with 7–10 yellow short conical spines, with pointed tips, straight or slightly curved outwards. All verticil spines similar in shape and size, maybe sometimes with a dorsal hook, with pointed or maybe blunt tips. Spinules and central tooth absent. Funnel with 12–20 radii ending in pointed tips.

Type locality: Guaymas, Sonora, Gulf of California, Mexico. Holotype unknown, not in YPM

Distribution: Gulf of California, Baja California to Panama

46. *Hydroides inermis* Monro, 1933 (original binomen) (Fig. 4Q)

Diagnosis: Verticil with 7–8 yellow broad spines, with blunt tips, strongly curving inwards. Dorsal spine larger than the others, covered over central disc. Other verticil spines similar in shape and size, partially obscuring the central disc. Central tooth absent. Funnel with 27–35 radii with pointed tips.

Type locality: James Bay, James Island, Galapagos, Ecuador. Syntypes NHM 1933.7.10.426-430

Distribution: Galapagos, North Peru

47. *Hydroides inornata* Pillai, 1960 (original binomen) (Fig. 3S)

Diagnosis: Verticil with 7 slender elongated verticil spines with pointed tips, curved inwards, light brown in colour. Dorsal spine larger than the others, other spines similar in shape and size. Funnel with 37 pointed radii.

Type locality: north Colombo, Sri Lanka. Holotype NMNH 1959.4.14.2

Distribution: Sri Lanka, India

48. *Hydroides kimberleyensis* Pillai, 2009 (original binomen) (Fig. 1E)

Diagnosis: Verticil with 5 thick rhomboid spines, triangular in cross-section, dark brown in colour, all similar in shape and size. Each spine with a transparent blunt knob at the tip, and 4–5 minute basal inner processes. Funnel with 24 chitinized radii, with dark brown pointed tips.

Type locality: east side of Fenelon Island, Kimberley, Western Australia. Holotype AM W.21391

Distribution: Kimberley, Western Australia

49. *Hydroides lambecki* Bastida-Zavala and ten Hove, 2002 (original binomen) (Fig. 2M)

Diagnosis: Verticil with 5–7 spines, with pointed tips, curved inwards at tip, colour of the base black, gradually changing to yellowish in middle and distal parts. Some dorsal spines larger than ventral spines, exceptionally spines similar in shape and size. Each spine with groups of 2–4 small external spinules halfway, yellow; 3–5 pairs of black lateral spinules in basal-middle position. Spines with one internal spinule, directed downwards and situated in almost basal position ventrally, but displaced to about halfway in dorsal spines, where it is large and directed down or up. Central tooth absent. Funnel not chitinized, with 11–14 radii with rounded tips.

Type locality: Curacao, Netherlands Antilles. Holotype ZMA V.Pol 3411

Distribution: Curaçao, Caribbean

50. *Hydroides lirs* Kupriyanova, Sun, ten Hove, Wong and Rouse, 2015 (original binomen) (Fig. 4K)

Diagnosis: Verticil with a basal stalk and 11–12 spines with pointed tips. Dorsal verticil spine enlarged to hook, curved inward, with a pair of lateral spinules distally. Other spines similar in size and shape, strongly curved outwards. Each spine with an erect, sharp internal spinule at mid-length. Central tooth absent. Funnel with 20–30 chitinized radii ending in long pointed tips. Each radius with a small internal spinule basally. Constriction between funnel and peduncle clear and chitinized.

Type locality: Lizard Island, Queensland, Australia. Holotype AM W.43967

Distribution: Australia, Queensland

51. *Hydroides longispinosa* Imajima, 1976b (original binomen) (Fig. 3G)

Diagnosis: Verticil with 13–15 spines ending in pointed tips, curved outwards. All spines similar in shape and size. Each verticil spine with 4–6 pairs of lateral spinules directed obliquely outwards and a row of 4–6 internal spinules. Central tooth longer than verticil spines, mostly with numerous randomly placed small teeth distally, but may be smooth sometimes. Funnel not chitinized, with 15–20 blunt-tipped radii.

Type locality: Konija, Amami-Oshima, Japan. Holotype NSMT Pol. H120

Distribution: Indo-West Pacific, from Qld, Australia to South Japan and Micronesia, South China, Pohnpei Island, Tonga

52. *Hydroides longistylaris* Chen and Wu, 1980 (original binomen) (Fig. 2C)

Diagnosis: Verticil smaller than funnel, with 6–9 short, conical spines with pointed tips. All verticil spines same in shape and size. Spinules and central tooth absent. Funnel with 21–27 chitinized radii ending in pointed tips. Constriction between funnel and peduncle well chitinized, elongated to approximately three times longer than operculum, light brown in colour.

Type locality: Fujian, China. Holotype MBMCAS

Distribution: Zhangzhou, Fujian, Guangxi, South China Sea

53. *Hydroides malleolaspina* Straughan, 1967a (original binomen) (Fig. 5K)

Diagnosis: Verticil with a short basal stalk and 8–14 spines. Dorsal spine enlarged, with two lateral conical processes distally and an inner stout beak-like medial process. Other verticil spines bottle shaped, elongated with T-shaped tips and a short neck-like constriction subapical, slightly curved outward. Spinules and central tooth absent. Funnel with 20–29 chitinized radii, bottle-shaped, with T-shaped tips and short neck-like constriction. Constriction between funnel and peduncle clear, chitinized.

Type locality: Pialba, Queensland, Australia. Holotype AM W.3999

Distribution: South China Sea, Australia: Northern Territory, Queensland, West Australia

54. *Hydroides microtis* Mörch, 1863 (originally as *H.* (*Eucarphus*) *microtis*) (Fig. 1I)
Diagnosis: Verticil with 6–9 bottle-shaped spines, straight or slightly curved outwards, with globularly expanded tips. All verticil spines similar in shape and size, each with a basal internal knob. Central tooth absent. Funnel with 20–30 radii ending in pointed tips.
Type locality: North America (not further specified). Types not found
Distribution: North America, North Carolina, Gulf of Mexico, Guyana

55. *Hydroides minax* (Grube, 1878) (originally as *Serpula minax*) (Fig. 4L)
Diagnosis: Verticil with 7–11 smooth spines. Dorsal verticil spine stout, elongated, ending in sharply pointed hooks with two sharp lateral hooks distally. All three hooks strongly curved, similar in size, or with middle one slightly larger than the other two. Other verticil spines short, curved outwards, with pointed tips. Spinules and central tooth absent. Funnel with 22–26 chitinized radii ending in club-shaped or sharp tips. Constriction between funnel and peduncle elongated, chitinized.
Type locality: Philippines (not further specified). Holotype unknown
Distribution: Widely distributed in the Indo-West Pacific, Lessepsian migrant to the Levant Mediterranean.

56. *Hydroides mongeslopezi* Rioja, 1958 (original binomen) (Fig. 2N)
Diagnosis: Verticil with 8–11 elongated spines, with pointed tip, curved inwards distally. White to yellow from tip to mid-length, gradually becoming brown to the base. All verticil sines similar in shape, with dorsal spine slightly wider than ventral spines. Each verticil spine with a row of 5–13 small spinules on both external and internal side of spines from tip to mid-length, and 4–5 pairs of lateral spinules with black tips from mid-length to base. Verticil spines with one long basal internal spinule almost in a semicircle dorsally, gradually changing to nearly straight from lateral to ventral spines. Central tooth absent. Funnel not chitinized, with 17–28 radii ending in rounded tips.
Type locality: Playa Norte and Isla Santiaguillo, Vera Cruz, Gulf of Mexico. Type material lost
Distribution: Vera Cruz, Gulf of Mexico, Caribbean

57. *Hydroides monroi* Zibrowius, 1973a (original binomen) (Fig. 4J)
Diagnosis: Verticil with 7–11 yellow spines. Dorsal verticil spine slightly larger, curved inwards to a hook with pointed tip, covered over central disc, flattened laterally. Other verticil

spines similar in shape and size, straight, short and thick, conical, with blunt tips, each with a conical internal spinules at mid-length. Funnel with 31–39 radii ending in pointed tip.

Type locality: Pointe Noire, Congo (Brazzaville). Holotype MRAC 34.952 and paratypesMRAC 23.730

Distribution: Congo, Cabinda

58. *Hydroides mucronata* Rioja, 1958 (original binomen) (Fig. 3B)

Diagnosis: Verticil small, with seven straight spines, colour beige. All verticil spines similar in shape and size, with blunt tip, each with a pair of lateral spinules. Some verticil spines with one mid-internal spinules. Central tooth absent. Funnel with 14 radii with pointed tip.

Type locality: Isla de Sacrificios, Veracruz, Mexico. Types lost

Distribution: Gulf of Mexico

59. *Hydroides multispinosa* Marenzeller, 1885 (original binomen) (Fig. 3L)

Diagnosis: Verticil with 7–9 spines, straight or curved outward distally, with pointed tips. All verticil spines similar in shape and size, each with 4–5 pairs of lateral spinules and a row of 2–4 internal spinules. Verticil spines not fused basally, not forming a wide shallow cup. Central tooth short. Funnel not chitinized, with 19–21 radii ending in blunt tip.

Type locality: Eno-sima (Enoshima), Honshu, Japan. Holotype NHMW 2026

Distribution: Japan, Australia

60. *Hydroides nanhaiensis* Wu and Chen, 1981 (original binomen) (Fig. 3F)

Diagnosis: Verticil with 10 spines, with pointed tips, curved outwards. All verticil spines similar in shape and size, each with 6–8 pairs of lateral spinules and a row of 5–6 internal spinules. Verticil spines not fused basally. Central tooth absent. Funnel not chitinized, with 22 radii ending in blunt tip.

Type locality: Xi River estuary, Pearl River Delta, China. Holotype MBMCAS

Distribution: South China Sea

61. *Hydroides nigra* Zibrowius, 1971 (original binomen) (Fig. 4A)

Diagnosis: Verticil black, with 6–10 spines ending in sharply pointed tip. Doral verticil spine enlarged to hook, smoothly curved inwards. Other verticil spines similar in shape, gradually decreasing in size ventrally, strongly curved inwards from mid-length, sometimes with an external knob at mid-length. Each verticil spine with a small basal internal spinule. Funnel with 16–27 radii ending in black pointed tip. Constriction between funnel and peduncle clear, chitinized, black in colour.

Type locality: off Pointe Meloula, Tabarka, Tunisia. Holotype and paratypes MHNH

Distribution: Mediterranean

62. *Hydroides nikae* Sun, Wong, Tovar-Hernández, Williamson and Kupriyanova, 2016 (original binomen) (Fig. 3Q)

Diagnosis: Verticil with 6–9 spines, with pointed tip, strongly curved inwards, gradually becoming smaller ventrally. Each verticil spines with an external knob at mid-length and one short basal internal spinule. Central tooth absent. Funnel with 18–26 radii with sharp chitinized tip.

Type locality: Edithburgh Jetty, South Australia. Holotype SAM E3530

Distribution: Australia, South Australia

63. *Hydroides nodosa* Straughan, 1967a (original binomen) (Fig. 2R)

Diagnosis: Verticil spine with 6–10 spines, curved inwards. All verticil spines elongated conical, similar in shape and size, ending in pointed tips, with a pair of lateral spinules, one to three of the verticil spines with a second lateral spinule on one side. Each verticil spine with one sharp conical basal internal spinule, one to three verticil spines may with a small sub-basal internal spinule above the basal spinule. Central tooth short, sharp conical. Funnel not chitinized, with 9–23 radii ending in rounded tip.

Type locality: Tannum Sands, Gladstone, Queensland, Australia. Holotype AM W.4013

Distribution: Australia: Northern Territory, Queensland

64. *Hydroides norvegica* Gunnerus, 1768 (original binomen) (Fig. 3J)

Diagnosis: Verticil with 12–14 spine, straight, ending in pointed tips, fused at base forming a wide shallow cup. All verticil spines similar in shape and size, with 2–4 pairs of pointed lateral spinules and a row of 2–5 pointed internal spinules. Central tooth short, sharp conical. Funnel not chitinized, with 24–32 radii ending in rounded to blunt tips. Bayonet collar chaetae with two conical teeth on the base of smooth blade.

Type locality: Trøndelag region, Norway. Holotype not designated or lost

Distribution: North Europe

65. *Hydroides novaepommeraniae* Augener, 1925 (originally as *Hydroides* (*Eupomatus*) *novae-pommeraniae*) (Fig. 1J)

Diagnosis: Verticil with 9–10 club-shaped spines, smooth, ending in pointed to rounded tips, straight, pointed outwards. All spines similar in size. Spinules and central tooth absent. Funnel with 23–25 radii, ending in pointed to blunt tip.

Type locality: Hannan or Garua Harbour, north coast of New Britain, Papua New Guinea. Holotype ZMH V.9749

Distribution: New Britain, Palau and Chuuk Island, Philippines, South Japan

66. *Hydroides ochotereana* Rioja, 1941a (original binomen) (Fig. 3E)

Diagnosis: Verticil spine with 16–21 yellowish elongated spines with pointed tips, straight or slightly curved outwards distally. All verticil spines similar in shape and size, with 6–10 pairs of pointed lateral spinules, with or without one long basal internal spinule extended above verticil tips and straight pointed upwards. Central tooth absent. Funnel with 27–32 radii ending in chitinized sharp tips.

Type locality: La Aguada and La Quebrada beaches, Acapulco, Guerrero, Mexico. Type specimens lost

Distribution: Acapulco, Western Mexico to Pacific Colombia

67. *Hydroides operculata* (Treadwell, 1929) (originally as *Eupomatus operculata*)

Diagnosis: Verticil with 7–9 slender elongated spines ending in pointed tips, curved inwards. One or two dorsal spines larger than the others, or all verticil spine same in size. All verticil spines smooth, without any spinules. Central tooth absent. Funnel with 25–31 chitinized radii ending in pointed tips.

Type locality: Gulf of Aden, Somaliland. Holotype AMNH 1994

Distribution: Red Sea, Gulf of Aden, Arabian Gulf. Somalia, Moçambique, Lessepsian migrant to the Levant Mediterranean, Umm Rasas, Oman

68. *Hydroides panamensis* Bastida-Zavala and ten Hove, 2003 (original binomen) (Fig. 1Q)

Diagnosis: Verticil spine with 11–13 yellow spines. All verticil spines similar in shape, straight from base, ending in a sharply pointed hook strongly curved inwards. Each verticil spine with a pair of pointed lateral spinules distally at curved part, pointed laterally in dorsal spines and gradually change to pointed externally in ventral spines, as well as a pointed basal internal spinule. Dorsal spines may slightly larger than the others. Central tooth absent. Funnel with 25–29 chitinized radii ending in pointed tips.

Type locality: Paitilla Beach, Western Panama. Holotype USNM 1013581, paratypes USNM 1013582, ZMA V.Pol 5078, ECOSUR 0038

Distribution: Pacific Panama, Ecuador

69. *Hydroides parva* (Treadwell, 1902) (originally as *Eupomatus parvus*) (Fig. 3D)

Diagnosis: Verticil spine with 4–10 yellowish spines ending in pointed tips, strongly curved inwards. Dorsal verticil spines may slightly larger than the others. Each verticil spine with one conical basal internal spinule, a pair of pointed lateral spinules at mid-length, and an external knob at mid-length. Central tooth absent. Funnel with 19–26 chitinized radii ending in pointed tips.

Type locality: Boqueron Bay, Puerto Rico. Two syntypes USNM 016173

Distribution: Puerto Rico, South Florida to Brazil

70. *Hydroides perezi* Fauvel, 1918 (original binomen) (Fig. 5F)

Diagnosis: Verticil with 4–7 short bulbous spines, blunt triangular in cross section. Dorsal verticil spine slightly larger, with a pointed tip horizontally directed inwards. Central tooth absent. Funnel with 18–26 radii ending in sharp pointed tips.

Type locality: 15 miles from the coast of Oman, Persian Gulf. Syntypes MNHN 3001

Distribution: Iranian (Arabian) Gulf, Red Sea, (?)Tonga

71. *Hydroides plateni* (Kinberg, 1867) (originally as *Eupomatus plateni*) (Fig. 1B)

Diagnosis: Verticil short and small, generally not or hardly surpassing the funnel perimeter, central disc fused to funnel, with 9–11 yellow conical spines ending in pointed tips, curved inward or straight, without or with a minute basal internal spinule. Central tooth absent. Funnel not chitinized, with 29–51 radii ending in rounded to triangular tips.

Type locality: La Plata, Argentina. Type material lost

Distribution: South Brazil to Patagonia

72. *Hydroides protulicola* Benedict, 1887 (original binomen) (Fig. 2A)

Diagnosis: Verticil small, occasionally the spines hardly surpass the funnel perimeter, with 11–15 spines ending in pointed tips, straight from base to subapical, distally curved outwards, sometime twisted laterally to clockwise. All spines similar in shape and size. Each verticil spine with a basal internal spinule. Funnel with 25–37 radii ending in triangular tips.

Type locality: Cape Hatteras, North Carolina, United States. Syntypes USNM 972, ZMA V.Pol. 3211

Distribution: Cape Hatteras, North Carolina, eastern United States, northern Gulf of Mexico

73. *Hydroides pseudexaltata* Pillai, 2009 (originally as *H. pseudexaltatus*) (Fig. 4M)

Diagnosis: Verticil spine with a short basal column and 9 spines, with pointed tips. Dorsal verticil spine enlarged to hook, strongly curved inward. Others spines smaller, similar in size and shape, curved outwards. Spinules and central tooth absent. Funnel with 21 chitinized radii, ending in swollen rounded to T-shaped tips. Constriction between funnel and peduncle elongated, chitinized.

Type locality: off North East Heywood Island, Kimberley, Western Australia. Holotype AM W.202930

Distribution: Kimberley, Western Australia

74. *Hydroides pseudouncinata* Zibrowius, 1968 (original binomen) (Fig. 2H)

Diagnosis: Verticil with 8–12 long slender smooth spines, all similar in shape and size, with pointed tips, curved inwards. Spinules and central tooth absent. Funnel with 25–32 chitinized radii, ending in elongated pointed tips with an additional internal spinule each. Constriction between funnel and peduncle clear chitinized, brown in colour.

Type locality: Marseille, France. Holotype MNHN 3029

Distribution: Mediterranean, North Atlantic Ocean

75. *Hydroides qiui* Sun, Wong, ten Hove, Hutchings, Williamson and Kupriyanova, 2015 (original binomen) (Fig. 1L)

Diagnosis: Verticil with 8–9 spines, yellowish, all spines similar in shape and size, bottle shaped, with anchor-shaped tip. Central tooth absent. Funnel with 17–25 chitinized radii ending in anchor-shaped tips.

Type locality: East Arm Port, Darwin Harbour, Northern Territory, Australia, 12°29’30”S, 130°52’59”E. Holotype MAGNT W25505

Distribution: Northern Territory, Australia

76. *Hydroides ralumiana* Augener, 1927 (originally (incorrectly) as *Hydroides* (*Eupomatus*) *ralumianus*)

Diagnosis: Verticil with 9 spines with pointed tips. Two dorsal verticil spines larger, smooth, distally curved inwards to a hook. Other verticil spines similar in shape and size, with pointed tips, strongly curved inwards distally, each with a subapical external spinule curved outwards, a pair of lateral spinule slightly under the external spinule, and a pair of short basal lateral spinules. Funnel with approximately 28 radii.

Type locality: Ralum, New Britain in the Bismarck Archipelago of Papua New Guinea. Holotype not found

Distribution: New Caledonia

77. *Hydroides recta* Straughan, 1967a (original binomen) (Fig. 4H)

Diagnosis: Verticil small, with 6–10 short club-shaped spines. Dorsal verticil spine slightly larger, ending in a blunt, rectangular to dilated tip, with a small subapical internal spinule. Other verticil spines ending in pointed tips, pointed outwards or slightly curved outwards distally. Central tooth absent. Funnel with 17–22 radii ending in bluntly rounded or swollen tips, usually thinly chitinized to and including constriction.

Type locality: Pretty Beach, Cairns, Queensland, Australia. Holotype AM W.3998

Distribution: Indonesia, Australia: Northern Territory, Queensland

78. *Hydroides recurvispina* Rioja, 1941a (original binomen) (Fig. 1O)

Diagnosis: Verticil with 8–10 yellow spines, straight. All spines similar in shape and size, with pointed tips curved laterally downwards and twisted clockwise. Each verticil spine with a subapical external knob ending in rounded tip at curved part of verticil spine, and one basal internal spinule with blunt tip. Central tooth absent. Funnel with 20–29 radii with chitinized pointed tips.

Type locality: Acapulco, Guerrero, Mexico. Type specimens lost.

Distribution: Acapulco, West Mexico, Gulf of California to Panama

79. *Hydroides rhombobula* Chen and Wu, 1980 (originally as *H. rhombobulus*) (Fig. 1F)

Diagnosis: Verticil with 5–6 spines. All spines similar in shape and size, large rhomboid shaped, triangular in cross section, golden in colour, with blunt tips. Spinules and central tooth absent. Funnel with 20 radii ending in chitinized pointed tips. Bayonet collar chaetae with two large conical teeth on the base of blade.

Type locality: Dongshan, Fujian Province, China. Holotype MBMCAS

Distribution: Fujian, China

80. *Hydroides rostrata* Pillai, 1971 (original binomen) (Fig. 5I)

Diagnosis: Verticil with 7 spines. Dorsal verticil spine enlarged, distinctly globular, curved ventrally like a beak ending in pointed tip, covering to ventral side of verticil, with a blunt external accessory spinule at mid-length. Other verticil spines triangular conical, straight, ending in blunt tip, with a horizontal conical internal spinule meet at central disc. Central tooth absent. Funnel with 16–24 fleshy radii with brownish pointed tips.

Type locality: Colombo, Sri Lanka. Holotype BMNH 1968.148, paratypes 1968.149–151

Distribution: Sri Lanka

81. *Hydroides salazarvallejoi* Bastida-Zavala and ten Hove, 2002 (original binomen) (Fig. 5C)

Diagnosis: Verticil with 5–8 spines, yellow to dark brown in colour. Dorsal verticil spine bulbous, with the tip curved strongly inward, almost reaching the base, like a strongly bent pick-axe; with an external knob at sharply curved part. Other verticil spines club-shaped, with swollen rounded to blunt tips ventrally, directed ventrally, with one basal internal spinule each. Two sub-dorsal verticil spines partly fused to dorsal spine at base. Central tooth absent. Funnel with 14–24 chitinized radii with pointed tips.

Type locality: Cabo de la Aguja, Santa Marta, Colombia (Atlantic side). Holotype and paratype ZMA V.Pol. 3776

Distribution: Colombia, Caribbean and Pacific Costa Rica to Ecuador

82. *Hydroides sanctaecrucis* Krøyer in Mörch, 1863 (originally *Hydroides* (*Eucarphus*) *sanctae crucis*) (Fig. 2O)

Diagnosis: Verticil with 10–14 elongated slender spines, all curved ventrally, with pointed tip. Dorsal spines slightly larger than ventral ones. Each verticil spine with narrow wings extending to around half of spine length, and one external spinule at mid-length, larger in ventral spines than in dorsal ones. All verticil spines may with one basal internal spinule. Central tooth absent. Funnel with 17–34 radii with pointed tips.

Type locality: Saint Croix, Lesser Antilles. Five syntypes probably in ZMC, labelled: “Kr. St. Croix, legit Oerstedt” (pers. comm. Mary Petersen to HAtH, 12 Febr 2002)

Distribution: St. Croix, Caribbean, both sides of Mexico and Panama, ship-transported to Singapore and tropical Australia

83. *Hydroides similis* (Treadwell, 1929) (originally as *Eupomatus similis*) (Fig. 4E)

Diagnosis: Verticil with 12–16 yellow to light brown spines strongly curved inwards, with pointed tips. Three to four dorsal verticil spines bigger than the others. Other verticil spines similar in shape, with small to pronounced knob. All verticil spines with one basal internal spinule. Funnel with 25–39 radii with pointed tips.

Type locality: Baja California, Western Mexico. Holotype AMNH 1993

Distribution: Baja California to Panama

84. *Hydroides similoides* Bastida-Zavala and ten Hove, 2002 (original binomen) (Fig. 4D)

Diagnosis: Verticil with 8–13 spines, yellow to dark brown in colour. Two or three dorsal verticil spines larger than the others, curved inwards smoothly, with pointed tips. Other verticil spines curved abruptly, geniculate, ending in pointed tip, decreased in size ventrally, with one prominent conical external knob at geniculate bend part increasing in size ventrally.

All verticil spines with one pointed basal internal spinule. Central tooth absent. Funnel with 12–22 radii with blunt tips.

Type locality: La Parguera, Isla Magueyes, Puerto Rico. Holotype and 7 paratypes ZMA V.Pol. 5038

Distribution: Puerto Rico, Caribbean

85. *Hydroides simplidentata* Pillai, 2009 (originally as *Hydroides simplidentatus*) (Fig. 5H)

Diagnosis: Verticil with 7 spines. Dorsal verticil spine vesicular, curved ventrally like a swollen beak ending in pointed tip, covering to ventral side of verticil. Other verticil spines conical, straight, ending in pointed tip, with a horizontal conical internal spinule meet at central disc. Central tooth absent. Funnel with 16 radii with brownish pointed tips.

Type locality: reef north-west of Buffon Island, Kimberley, Western Australia. Holotype AM W.21415

Distribution: Kimberley, Western Australia

86. *Hydroides sinensis* Zibrowius, 1972b (original binomen) (Fig. 2L)

Diagnosis: Verticil with 8–10 dark brown spines, all similar in shape and size, thick, bottle shaped, with bulbous T-shaped tips, straight pointed outwards. Each verticil spine with one large subapical internal spinule with bulbous base and pointed tip directed to centre of verticil, as well as a row of 4–6 smaller pointed internal spinule blew the large one extended to base. Funnel with 40–50 radii with blunt or rounded tips.

Type locality: Qingdao, China. Holotype ZINRAS AN 1/10 1930

Distribution: Yellow Sea

87. *Hydroides spongicola* Benedict, 1887 (original binomen) (Fig. 2D)

Diagnosis: Verticil 14–16 slender spines, smoothly curved inwards, with pointed tips. Dorsal spines slightly larger. Basal part of each verticil spines flattened into narrow wings. Spinules and central tooth absent. Funnel with 45–61 sharply elongated radii with pointed tips.

Type locality: off Venice, Florida, United States, 27°04’N, 83°21’15”W. Syntypes USNM 975, ZMA V.Pol. 3209

Distribution: Gulf of Mexico, Caribbean, Bahamas

88. *Hydroides steinitzi* Ben-Eliahu, 1972 (original binomen) (Fig. 4S)

Diagnosis: Verticil only with one large bulb with 6 longitudinal groves, any other verticil spines absent. Funnel with 12 pointed to blunt radii.

Type locality: Little Bitter Lake of Suez Canal, Egypt, Red Sea. Holotype and 2 paratypes HUJ SLC 85/P116

Distribution: Suez Canal, Philippines, found once on ship's hull in Toulon, Mediterranean

89. *Hydroides stoichadon* Zibrowius, 1971 (original binomen) (Fig. 2S)

Diagnosis: Verticil with 10 thick spines ending in pointed tips, curved inwards distally. All verticil spines similar in size and shape, each with a pair of pointed lateral spinules at mid-length. Funnel with 15–20 radii with rounded tips.

Type locality: Cap du Merlan, Parc Nacional de Port Cros, (at Port-Cros Island), France. Holotype and 2 paratypes MNHN

Distribution: Provence, Mediterranean

90. *Hydroides tambalagamensis* Pillai, 1961 (original binomen) (Fig. 2Q)

Diagnosis: Verticil with 7–8 spines all similar in shape and size, white to black in colour. Each verticil spine curved outwards and ending in pointed tip, with a pair of outwardly curved lateral spinules at mid-length, a pointed internal spinule curved towards central disc at the same level or slightly above, and a small basal internal spinule. Funnel with 24–39 sharply pointed dark-brown chitinized radii.

Type locality: Tambalagam Lake, Sri Lanka. Holotype BMNH 1960.3.13.30

Distribution: Indo-West Pacific

91. *Hydroides tenhovei* Bastida-Zavala and Leon-Gonzalez, 2002 (original binomen) (Fig. 5D)

Diagnosis: Verticil with 9–14 spines, yellow in colour. Three dorsal verticil spines enlarged, bulbous, partly fused at base, each with globular expanded tip. Internal part of each of the three dorsal verticil spines swollen extended from subapical, forming a plane extended over central disc at base. Most dorsal verticil spines largest, two sub-dorsal ones smaller in size. Other verticil spines decreased in size ventrally, similar in shape, thick bottle shaped ending in pointed tip, with one basal internal spinule each. Central tooth absent. Funnel with 20–42 radii with pointed tips.

Type locality: Cabo San Lazaro, Western coast of Baja California Sur, Mexico, 24°50'N, 112°15'W. Holotype and 4 paratypes ZMA V.Pol. 4994

Distribution: Western coast of Baja California Sur, Mexico

92. *Hydroides trilobula* Chen and Wu, 1978 (originally *H. trilobulus*) (Fig. 3M)

Diagnosis: Verticil with three thick, roughly rhomboidal spines, widen from base to medially towards a tiny pointed lateral knob on each side, then narrowed from medially to distally, ending in blunt tip curved inwards. Verticil spines decreased in size ventrally. Funnel with 26 sharp radii.

Type locality: Xisha Island, China. Holotype MBMCAS

Distribution: South China Sea

93. *Hydroides trivesiculosa* Straughan, 1967b (originally *H. trivesiculosus*) (Fig. 5A)

Diagnosis: Verticil with 5–8 spines. Dorsal verticil spine bulbous, enlarged to more than five times to other verticil spines, with a bulbous median part and two latero-dorsal extensions. Other verticil spines small, positioned at base of dorsal verticil spine and partly fused to dorsal verticil spine at the base, bottle shaped, with T-shaped tips, straight, directed outwardly. Funnel with 18–25 chitinized bottle shaped radii with rounded or T-shaped tips.

Type locality: Heron Island, Queensland, Australia. Holotype AM W.4111

Distribution: Queensland, tropical Australia, Tanzania, Red Sea, Seychelles, Indonesia

94. *Hydroides trompi* Bastida-Zavala and ten Hove, 2003 (original binomen) (Fig. 1S)

Diagnosis: Verticil with 5–10 yellow spines, curved inwards with tips twisted clockwise. All verticil spines similar in shape and size, with black sharply pointed tips. Each spine with one basal internal spinule with bifid tip. Central tooth absent. Funnel with 16–22 club-shaped radii with blunt, sometimes bifid tips.

Type locality: Miraflores Locks, Western Panama. Holotype USNM 58605

Distribution: Baja California to Panama

95. *Hydroides tuberculata* Imajima, 1976a (original binomen) (Fig. 5E)

Diagnosis: Verticil with 5–6 triangular spines with blunt tips, curved inwards distally. Dorsal verticil spine larger than others, with elongated sharp tip. All spines with a small subapical external knob. Funnel with 18–25 radii with pointed tips.

Type locality: Urata, Tanega-shima, Japan. Holotype NSMT-Pol. H120, 13 paratypes NSMT-Pol. P121

Distribution: Indo-West Pacific: Micronesia, Melanesia, Southern Japan, North Australia

96. *Hydroides uniformis* Imajima and ten Hove, 1986 (original binomen) (Fig. 1D)

Diagnosis: Verticil with 7–8 spines, straight. All verticil spines thick, conical, blunt triangular in cross section, with rounded to blunt tips, equally in size or with dorsal verticil spine slightly larger. Each verticil spine with a rounded basal internal spinule. Funnel with 19–29 club-shaped radii with blunt to pointed tips.

Type locality: Keaso, Solomon Islands. Holotype NSMT-Pol. H195, 2 paratypes NSMT-Pol, P196

Distribution: Solomon Islands

97. *Hydroides vizagensis* Lakshmana Rao, 1969 (original binomen)

Diagnosis: Verticil with 9–10 elongated slender spines all curved ventrally, with pointed tip. Each spine with a broad proximal part and a narrow distal part, with a small outwardly directed blunt external spinule at the junction of these two parts. Central tooth absent. Funnel with 25 radii with pointed tips.

Type locality: Naval Base, Visakhapatnam Harbour. Types lost

Distribution: East Coast of India

98. *Hydroides xishaensis* Chen and Wu, 1978 (original binomen) (Fig. 1G)

Diagnosis: Verticil with 5 bulbous triangular spines, straight, with blunt tip. All verticil spines equal in size and shape, without spinules. Funnel with 26–30 club-shaped radii with blunt radii. Collar chaetae with two large conical teeth and 2 to 3 small teeth.

Type locality: Xisha Island, China. Holotype MBMCAS

Distribution: Guandong, China

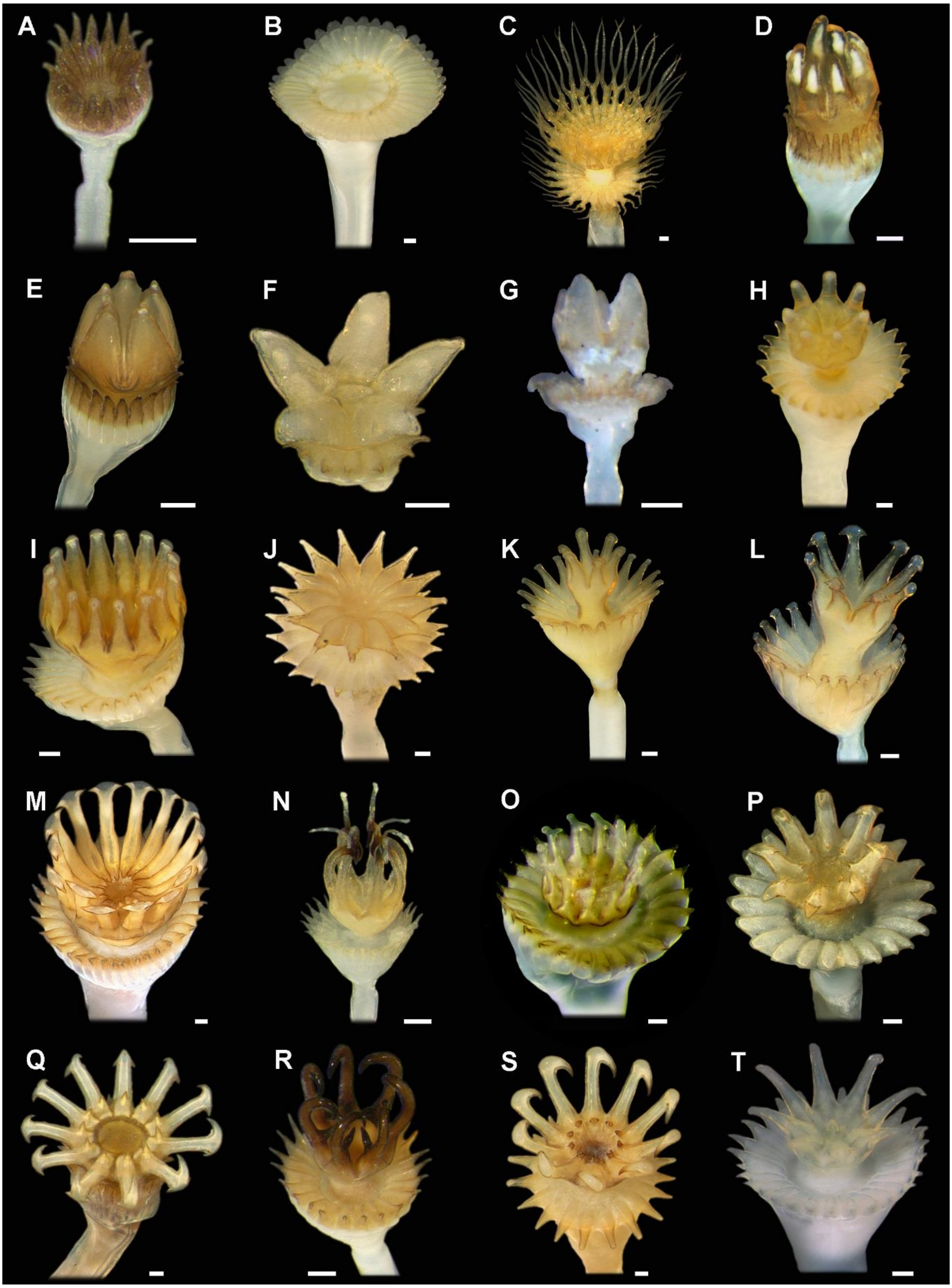


Fig. 1. Operculum A. *Hydroides bannerorum*, B. *H. plateni*, C. *H. furcifera*, D. *H. uniformis*, E. *H. kimberleynsis*, F. *H. rhombobula*, G. *H. xishaensis*, H. *H. arnoldi*, I. *H. microtis*, J. *H. novaepommeraniae*, K. *H. bandaensis*, L. *H. qiui*, M. *H. dirampha*, N. *H. bisecta*, O. *H. recurvispina*, P. *H. dolabrus*, Q. *H. panamensis*, R. *H. fusca*, S. *H. trompi*, T. *H. fusicola*. Scale bars = 0.1 mm.

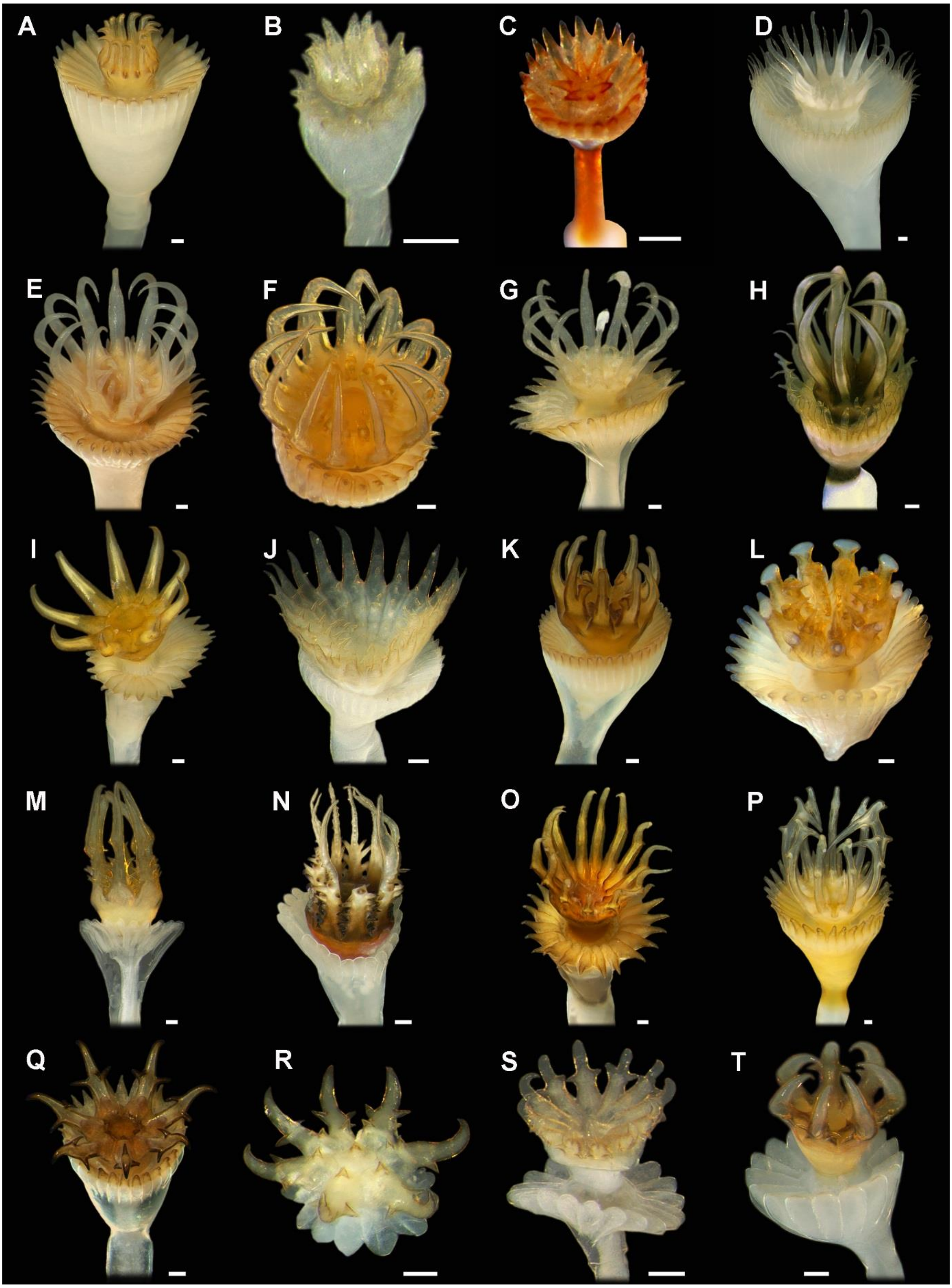


Fig. 2. Operculum A. *Hydroides protulicola*, B. *H. humilis*, C. *H. longistylaris*, D. *H. spongicola*, E. *H. alatalateralis*, F. *H. floridana*, G. *H. elegantula*, H. *H. pseudouncinata*, I. *H. gracilis*, J. *H. ezoensis*, K. *H. augeneri*, L. *H. sinensis*, M. *H. lambecki*, N. *H. mongeslopezi*, O. *H. sanctaecrucis*, P. *H. calopoma*, Q. *H. tambalagamensis*, R. *H. nodosa*, S. *H. stoichadon*, T. *H. bispinosa*. Scale bars = 0.1 mm.

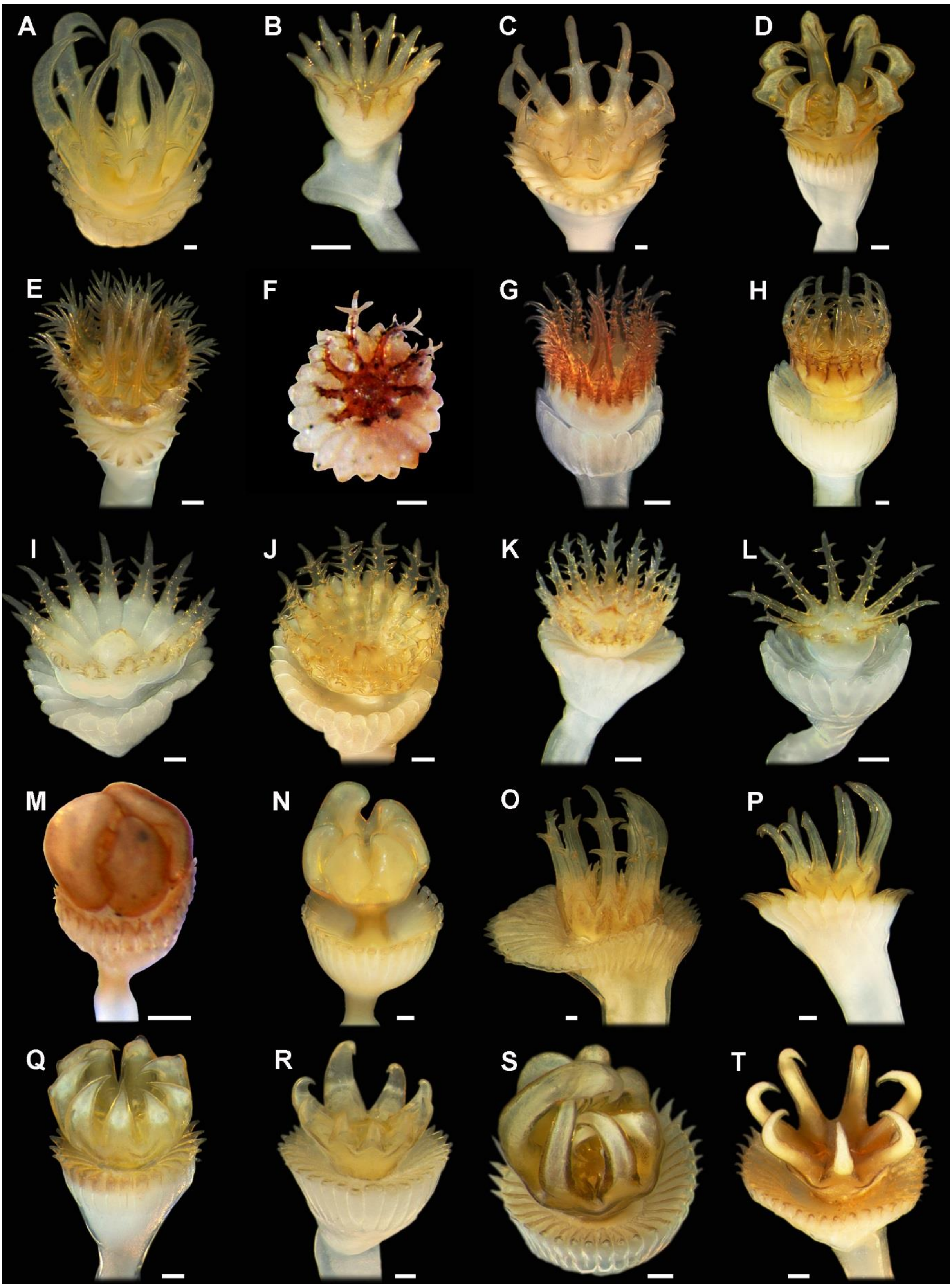


Fig. 3. Operculum A. *Hydroides homoceros*, B. *H. mucronata*, C. *H. crucigera*, D. *H. parva*, E. *H. ochotereana*, F. *H. nanhaiensis*, G. *H. longispinosa*, H. *H. capensis*, I. *H. elegans*, J. *H. norvegica*, K. *H. azorica*, L. *H. multispinosa*, M. *H. trilobula*, N. *H. adamaformis*, O. *H. chilensis*, P. *H. dianthus*, Q. *H. nikae*, R. *H. deleoni*, S. *H. inornata*, T. *H. operculata*. Scale bars = 0.1 mm.

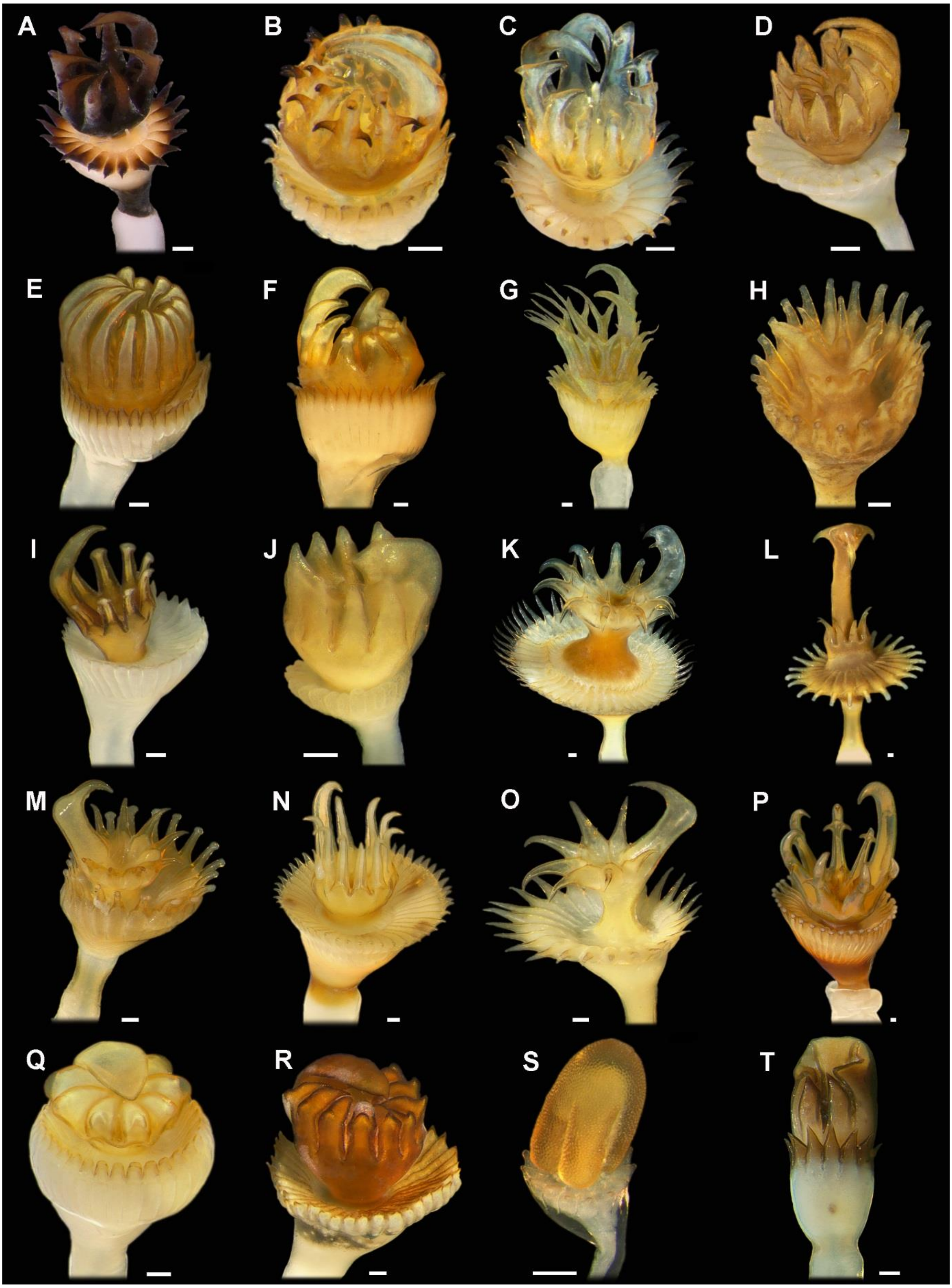


Fig. 4. Operculum A. *Hydroides nigra*, B. *H. externispina*, C. *H. glasbyi*, D. *H. similoides*, E. *H. similis*, F. *H. amri*, G. *H. heterofurcata*, H. *H. recta*, I. *H. bifurcata*, J. *H. monroi*, K. *H. lirs*, L. *H. minax*, M. *H. pseudexaltata*, N. *H. dipoma*, O. *H. exaltata*, P. *H. heterocera*, Q. *H. inermis*, R. *H. brachyacantha*, S. *H. steinitzi*, T. *H. glandifera*. Scale bars = 0.1 mm.

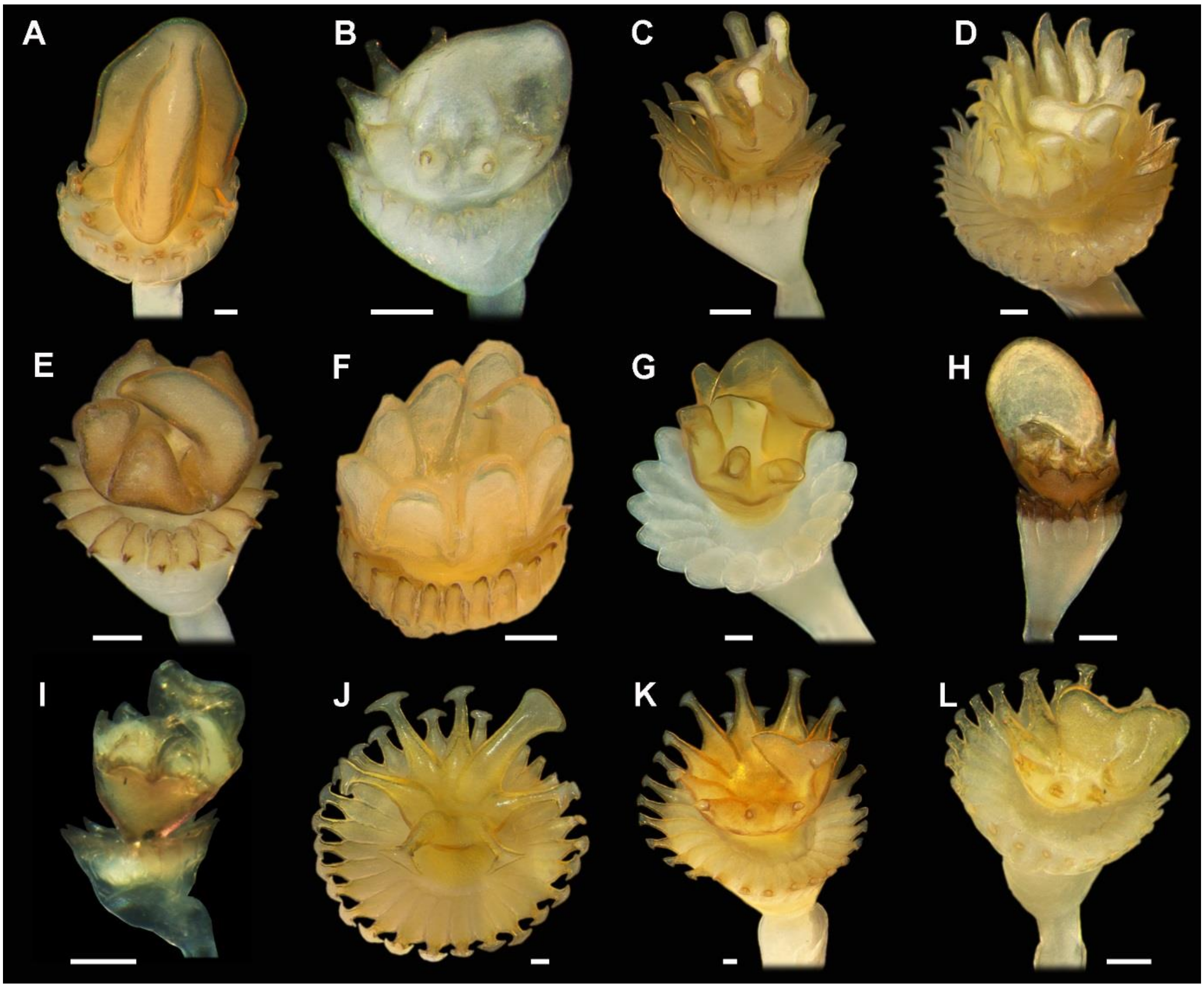


Fig. 5. Operculum A. *Hydroides trivesiculosa*, B. *H. bulbosa*, C. *H. salazarvallejoi*, D. *H. tenhovei*, E. *H. tuberculata*, F. *H. perezi*, G. *H. helmata*, H. *H. simplidentata*, I. *H. rostrata*, J. *H. ancorispina*, K. *H. malleolaspina*, L. *H. albiceps*. Scale bars = 0.1 mm.

References

Amoureux L, Rullier F, Fishelson, L (1978) Systématique et Écologie d'Annélides Polychètes de la presqu'îl du Sinai. Israel Journal of Zoology 27(2–3): 57–163.

Augener H (1918) Polychaeta. Beiträge zur Kenntnis der Meeresfauna Westafrikas 2(2): 67–625.

Augener H (1925) Die Polychaeten der Südsee-Expedition der Hamburgischen wissenschaftliche Stiftung 1908–1909. Mitteilungen der Zoologisches Staatsinstitut und zoologisches Museum, Hamburg 41: 53–70.

Augener H (1927) Polychaeten von Neu-Pommern. Sitzungsberichte der Gesellschaft der naturforschende Freunde zu Berlin 1926: 119–152.

Augener H (1934) Polychaeten aus den Zoologischen Museen von Leiden und Amsterdam. IV (Schluss). Zoölogische Mededeelingen Rijks Museum van Natuurlijke Historie Leiden 17: 67–160.

Bailey-Brock JH (1991) Tubeworms (Serpulidae, Polychaeta) collected from sewage outfalls, coral reefs and deep waters off the Hawaiian Islands, including a new *Hydroides* species. Bulletin of Marine Science 48(2): 198–207.

Bastida-Zavala JR (2008) Serpulids (Annelida: Polychaeta) from the Eastern Pacific, including a short mention of Hawaiian serpulids. Zootaxa 1722: 1–61.

Bastida-Zavala JR and ten Hove HA (2002) Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the Western Atlantic Region. Beaufortia 52(9): 103–178.

Bastida-Zavala JR and ten Hove HA (2003) Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the Eastern Pacific Region and Hawaii. Beaufortia 53(4): 67–110.

Bastida-Zavala JR and de Leon Gonzalez JA (2002) A new species of *Hydroides* (Polychaeta: Serpulidae) from western Mexico. Journal of the Marine Biological Association of the United Kingdom 82(3): 389–393.

Benedict JE (1887) Descriptions of ten species and one new genus of annelids from the dredgings of the U.S. Fish Commission Steamer Albatross. Proceedings of the United States National Museum 9: 547–553.

Ben-Eliahu MN (1972) A description of *Hydroides steinitzi* n. sp. (Polychaeta: Serpulidae) from the Suez Canal with remarks on the serpulid fauna of the Canal. Israel Journal of Zoology 21(2): 77–81.

Ben-Eliahu MN, ten Hove HA (1992) Serpulids, Annelida: Polychaeta, along the Mediterranean coast of Israel—New population build-ups of Lessepsian migrants. Israel Journal of Zoology 38(1): 35–53.

Bush KJ (1905) Tubicolous annelids of the tribes Sabellides and Serpulides from the Pacific Ocean. Harriman Alaska Expedition 12: 169–346.

Bush KJ (1910) Description of new serpulids from Bermuda with notes on known forms from adjacent regions. Proceedings of the Academy of Natural Sciences, Philadelphia 62: 490–501.

Chen M and Wu BL (1978) Two new species of the genus *Hydroides* (Polychaeta, Serpulidae) from the Xisha Islands, Guandong Province, China. Studia Marina Sinica 12: 141–145.

Chen M and Wu BL (1980) Two new species of the genus *Hydroides* (Polychaeta, Serpulidae). Oceanologia et Limnologia Sinica 11(3): 247–250.

Dew B (1959) Serpulidae (Polychaeta) from Australia. Records of the Australian Museum 25: 19–56.

Fauvel P (1918) Annélides polychètes des côtes d'Arabie récoltées par M. Ch. Pérez. Bulletin du Muséum d'Histoire Naturelle, Paris 24(5): 329–344.

Fiege D and Sun R (1999) Polychaeta from Hainan Island, South China Sea Part I: Serpulidae. Seckenbergiana Biologia 79: 109–141.

Grube AE (1868) Beschreibungen einiger von Georg Ritter von Frauenfeld gesammelter Anneliden und Gephyreen des rothen Meeres. Verhandlungen der kaiserlich–königlichen zoologisch–botanischen Gesellschaft in Wien 18: 629–650.

Grube AE (1870) Beschreibungen neuer oder weniger bekannter von Hrn. Ehrenberg gesammelter Anneliden des rothen Meeres. Monatsbericht der Koniglich Preussischer Akademie der Wissenschaften zu Berlin 1869: 484–521.

Grube AE (1878) Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen. Memoires de L'Académie Impériale.des Sciences de St.-Pétersbourg VII Série 25(8): 1–300.

Gunnerus JE (1768) Om nogle Norske Coraller. Det Kongelige Norske Videnskabernes Selskabs Skrifter 4: 38–73.

Hartman O (1959) Catalogue of the polychaetous annelids of the world. Part II. Occasional Paper, Allan Hancock Foundation Publications 23 (2): 354–628.

Hartman O (1969) Atlas of the sedentariate polychaetous annelids from California. Allan Hancock Foundation, University of Southern California, 812 pp.

Hartmann-Schröder G (1962) Die Polychaeten des Eulitorals. In: Hartmann-Schröder, G. and Gerd Hartmann. Zur Kenntnis des Eulitorals der chilenischen Pazifikküste und der argentinischen Küste Südpatagoniens unter besonderer Berücksichtigung der Polychaeten und Ostracoden. Mitteilungen aus dem Hamburgischen zoologischen Museum und Institut 60: 57–270.

Haswell WA (1883) On some new Australian tubicolous annelids. Proceedings of the Linnean Society of New South Wales 7: 633–638.

Hove HA ten (1970) Serpulinae (Polychaeta) from the Caribbean: I - the genus *Spirobranchus*. Studies on the Fauna of Curaçao and other Caribbean Islands 32: 1–57.

Hove HA ten (1990) Description of *Hydroides bulbosus* sp. nov. (Polychaeta, Serpulidae), from the Iranian Gulf, with a terminology for opercula of *Hydroides*. Beaufortia 41(16): 115–120.

Hove HA ten and Kupriyanova EK (2009) Taxonomy of. Serpulidae (Annelida, Polychaeta): the state of affairs. Zootaxa 2036: 1–126.

Imajima M (1976a) Serpulid polychaetes from Tanega-shima, southwest Japan. Memoirs of the National Science Museum 9: 123–143.

Imajima M (1976b) Serpulinae (Annelida, Polychaeta) from Japan I. the genus *Hydroides*. Bulletin of the National Science Museum, Tokyo, Series A (Zoology) 2(4): 229–248.

Imajima M (1978) Serpulidae (Annelida, Polychaeta) collected around Nii-jima and O-shima, Izu Island. Memoirs of the National Science Museum (Tokyo) 11: 49–72.

Imajima M (1982) Serpulinae (Polychaetous Annelids) from the Palau and Yap Islands, Micronesia. Proceedings of the Japanese Society of Systematic Zoology 23: 37–55.

Imajima M and ten Hove HA (1984) Serpulinae (Annelida, Polychaeta) from the Truk Island, Ponape and Majuro Atoll, with some other new Indo-Pacific records. Proceedings of the Japanese Society of Systematic Zoology 27: 35–66.

Imajima M and ten Hove HA (1986) Serpulinae (Annelida, Polychaeta) from Nauru, the Gilbert Islands (Kiribati) and the Solomon Islands. Proceedings of the Japanese Society of Systematic Zoology 32: 1–16.

Imajima M and ten Hove HA (1989) Two new species of serpulids (Annelida, Polychaeta) from Sesoko Island, Okinawa. Bulletin of the National Science Museum, Tokyo, Series A (Zoology) 15(1): 11–17.

International Commission on Zoological Nomenclature [ICZN] (1961) International Code of Zoological Nomenclature, adopted by the XV International congress of Zoology. The International Trust for Zoological Nomenclature, London. 176 pp.

ICZN (1985) International Code of Zoological Nomenclature Third Edition adopted by the XX General Assembly of the International Union of Biological Sciences. The International Trust for Zoological Nomenclature, London. 338 pp.

ICZN (1999) International Code of Zoological Nomenclature Fourth edition adopted by the International Union of Biological Sciences. The International Trust for Zoological Nomenclature, London. 306 pp.

Iroso I (1921) Revisione dei Serpulidi e Sabellidi del Golfo di Napoli. Pubblicazioni della Stazione Zoologica di Napoli 3: 47–91.

Jones ML (1962) On some polychaetous annelids from Jamaica, the West Indies. Bulletin of the American Museum of Natural History 124(5): 169–212.

Kinberg JGH (1867) Annulata nova. [Continuatio.]. Öfversigt af Kongl. [sic] Vetenskapsakademiens förhandlingar, Stockholm 23(9): 337–357.

Kupriyanova EK, Sun Y, Hove, HA ten, Wong E, Rouse GW (2015) Serpulidae (Annelida) of Lizard Island, Australia. Zootaxa 4019(1): 275–353.

Lakshmana Rao MV (1969). Fouling serpulids from some Indian harbours. Journal of the Timber Development Association of India 15(2): 1–20.

Marenzeller E von (1885) Südjapanische Anneliden. II. Ampharetea, Terebellacea, Sabellacea, Serpulacea. Denkschriften der Akademie der Wissenschaften Mathematisch-Naturwissenschaftliche Classe, Wien 49(2): 197–224.

Monro CCA (1933) The Polychaeta Sedentaria collected by Dr. C. Crossland at Colón, in the Panama Region, and the Galapagos Islands during the Expedition of the S.Y. 'St. George'. Proceedings of the Zoological Society of London 103(4): 1039–1092.

Mörch OAL (1863) Revisio critica Serpulidarum. Et Bidrag til Rørormenes Naturhistorie. Naturhistorisk Tidsskrift, København 3(1): 347–470.

Okuda S (1934) Some tubicolous annelids from Hokkaido. Journal of the Faculty of Science, Hokkaido University, Series 6, Zoology 3(4): 233–246.

Philippi A (1844) Einige Bemerkungen über die Gattung *Serpula*, nebst Aufzählung der von mir im Mittelmeer mit dem beobachteten Arten. Archiv für Naturgeschichte, Berlin 10: 186–198. (translated in: Annals and Magazine of Natural History London (1) 14: 153–162.

Pillai TG (1960) Some marine and brackish water serpulid Polychaeta from Ceylon, including new genera and species. Ceylon Journal of Science (Biological Sciences) 3(1): 1–40.

Pillai TG (1961) Annelida Polychaeta of Tambalagam Lake, Ceylon. Ceylon Journal of Science (Biological Sciences) 4(1): 1–40.

Pillai TG (1971) Studies on a collection of marine and brackish-water polychaete annelids of the family Serpulidae from Ceylon. Ceylon Journal of Science (Biological Sciences) 9(2): 88–130.

Pillai TG (1972) A review and revision of the systematics of the genera *Hydroides* and *Eupomatus* together with an account of their phylogeny and zoogeography. Ceylon Journal of Science (Biological Sciences) 10(1): 7–31.

Pillai TG (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. Records of the Australian Museum 61(2): 93–199.

Pixell HLM (1913) Polychaeta of the Indian Ocean, together with some species from the Cape Verde Islands. The Serpulidae, with a classification of the genera *Hydroides* and *Eupomatus*. Transactions of the Linnean Society of London 2(16): 69–92.

Rafinesque CS (1815) Analyse de la Nature ou Tableau de l'univers et des corps organisés. Palerme 1–224.

Rioja E (1941a) Estudios Anelidologicos. II. Observaciones acerca de varias especies del genero *Hydroides* Gunnerus (sensu Fauvel) de las costas Mexicanas del Pacifico. Anales del Instituto de Biologia, Mexico 12(1): 161–175.

Rioja E (1941b) Estudios Anelidologicos. III. Datos para el conocimiento de la fauna de poliquetos de las costas del pacifico de Mexico. Anales del Instituto de Biologia, Mexico 12(2): 669–746.

Rioja E (1958) Estudios Anelidologicos. XXI. Observaciones acerca de algunas especies de serpulidos de los generos *Hydroides* y *Eupomatus* de las costas Mexicanas del Golfo de Mexico. Anales del Instituto de Biologia, Mexico 28(1/2): 247–266 [1957 issue].

Schmarda LK (1861) Neue wirbellose Thiere beobachtet und gesammelt auf einer Reise um die Erdr [sic] 1853 bis 1857 von Ludwig K. Schmarda. Erster Band. Turbellarien, Rotatorien und Anneliden. Zweite Hälfte. Wilhelm Engelmann, Leipzig. 164 pp.

Straughan D (1967a) Marine Serpulidae (Annelida: Polychaeta) of eastern Queensland and New South Wales. Australian Journal of Zoology 15(1): 201–261.

Straughan D (1967b) Some Serpulidae (Annelida: Polychaeta) from Heron Island, Queensland. University of Queensland Papers [Great Barrier Reef Committee, Heron Island Research Station] 1(2): 27–45.

Sun R, Yang D (2000) Study on *Hydroides* (Polychaeta: Serpulidae) from waters off China. I. Studia Marina Sinica 42: 116–135. [Chinese with English summary]

Sun R, Yang D (2014) Annelida Polychaeta III Sabellida. Fauna Sinica Invertebrata 54: 1–493.

Sun Y, ten Hove HA and Qiu JW (2012) Serpulid polychaetes from Hong Kong. Zootaxa 3424: 1–42.

Sun Y, Wong E, ten Hove HA, Hutchings PA, Williamson JE, Kupriyanova EK (2015) Revision of the genus *Hydroides* (Annelida: Serpulidae) from Australia. Zootaxa 4009(1): 1–99.

Sun Y, Wong E, Tovar-Hernández MA, Williamson JE, Kupriyanova EK (2016). Is *Hydroides brachyacantha* (Serpulidae: Annelida) a widespread species? Invertebrate Systematics 30(1): 41–59.

Tovar-Hernández MA, Villalobos-Guerrero TF, Kupriyanova EK, Sun Y (2016 [online 2015]). A new fouling *Hydroides* (Annelida, Sabellida, Serpulidae) from southern Gulf of California. Journal of the Marine Biological Association of the United Kingdom 96(3): 693–705.

Treadwell AL (1902) The polychaetous annelids of Porto Rico. Bulletin of the United States Fish Commission 20 (2): 181–210.

Treadwell AL (1929) New species of polychaetous annelids in the collections of the American Museum of Natural History from Porto Rico, Florida, Lower California, and British Somaliland. American Museum Novitates 392: 1–13.

Uchida H (1978) Serpulid tube worms (Polychaeta, Sedentaria) from Japan with the systematic review of the group. Bulletin of the Marine Park Research Stations 2: 1–98.

Verrill AE (1873) VIII. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. Report of the United States Commission of Fish and Fisheries. 757 pp.

WoRMS (2016) *Hydroides* Gunnerus, 1768. In: Read G; Fauchald K (Ed) (2016) World Polychaeta database. Accessed through: Read G; Fauchald K (Ed) (2016) World Polychaeta database at http://www.marinespecies.org/polychaeta/aphia.php?p=taxdetailsandid=129566 on 2016-10-23.

Wu BL and Chen M (1981) Two new species of *Hydroides* (Polychaeta: Serpulidae) from South China Sea. Oceanologia et Limnologia Sinica 12(4): 354–357.

Zibrowius H (1968) Étude morphologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille. Recueil des Travaux de la Station Marine d'Endoume 43(59): 81–252.

Zibrowius H (1971) Les espèces Méditerranéennes du genre *Hydroides* (Polychaeta Serpulidae). Remarques sur le pretendu polymorphisme de *Hydroides uncinata*. Tethys 2: 691–746.

Zibrowius H (1972a) *Hydroides norvegica* Gunnerus, *Hydroides azorica* n.sp. et *Hydroides capensis* n. sp. (Polychaeta Serpulidae) espèces vicariantes dans l'Atlantique. Bulletin du Muséum d'Histoire Naturelle, Paris, Series 3 33(39): 433–446.

Zibrowius H (1972b) Deux espèces nouvelles du genre *Hydroides* (Polychaeta, Serpulidae) de la Mer Jaune et des Iles Banda. Bulletin de la Société Zoologique de France 97(1): 89–93.

Zibrowius H (1973a) Serpulidae (Annelida Polychaeta) des côtes ouest de l'Afrique et des archipels voisins. Annales Musee Royal de l'Afrique Centrale, Ser. 8, Sciences Zoologique 207: 1–93.

Zibrowius H (1973b) Remarques sur trois espèces de Serpulidae acclimatées en Méditerranée: *Hydroides dianthus* (Verrill, 1873), *Hydroides dirampha* Mörch, 1863, et *Hydroides elegans* (Haswell, 1883). Rapport Commission international Mer Méditerranée 21(9): 683–686.

Chapter 4

Is *Hydroides brachyacantha* (Serpulidae : Annelida) a widespread species?

Authors:
Yanan Sun [A,B,D], Eunice Wong[A], María Ana Tovar-Hernández[C], Jane E. Williamson[B] and Elena K. Kupriyanova[A]

[A]Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia.
[B]Department of Biological Sciences, Macquarie University, NSW 2109, Australia.
[C]El Colegio de Sinaloa, Gral. Antonio Rosales 435 Poniente, C. P. 80000, Culiacán, Sinaloa, México.
[D]Corresponding author. Email: yanan.sun@austmus.gov.au

Published Journal Article
Journal: *Invertebrate Systematics*
Published online: 16 March 2016
Printed in Volume 30 (2016), 41–59
http://dx.doi.org/10.1071/IS15015

Abstract

Hydroides brachyacantha Rioja, 1941, an important fouling serpulid species originally described from Mazatlán (Southern Gulf of California, Mexico) and Acapulco (southern Mexican Pacific), has been reported from the Mexican Pacific and numerous tropical and subtropical localities. However, a recent description of *H. amri* Sun, Wong, ten Hove, Hutchings, Williamson & Kupriyanova, 2015 from Australia, which was historically misidentified as *H. brachyacantha*, suggested that the widespread '*H. brachyacantha*' is indeed a species complex. To test the status of *H. amri*, we conducted phylogenetic analyses based on a combined dataset of 18S rRNA, internal transcribed spacer-2, and cytochrome *b* sequences of *H. brachyacantha* from the type locality in Mexico with those of *H. amri* from Australia. Our molecular data supported the morphology-based hypothesis of *H. amri* and *H. brachyacantha sensu stricto* as two distinct species. Furthermore, *H. amri* comprises two non-sister well-supported clades. *Hydroides amri* thus comprises what we consider two cryptic species with long-term isolation. Here we describe the genetic lineage in South Australia as *Hydroides nikae*, sp. nov. Given the absence of a holotype of *H. brachyacantha*, we designate a neotype collected from the type locality (Mazatlán, Mexico). This study calls for a worldwide revision of the *H. brachyacantha*-complex.

Keywords: fouling, cryptic species, 18S, ITS2, cytochrome *b*, species-complex.

Introduction

Polychaetes, with over 10 000 described species (Rouse and Pleijel 2001), were historically considered as a group consisting of numerous species characterised by having wide or even cosmopolitan distributions (Fauvel 1953; Day 1967; Briggs 1974). However, species with perceived extensive distributions are often simply a reflection of poor taxonomic understanding of the taxa, a situation coined 'the cosmopolitan syndrome' (Fauchald 1984; Hutchings and Glasby 1991). Not surprisingly, careful morphological revisions of such taxa routinely reveal several species with geographically restricted distributions under one 'cosmopolitan' name (e.g. *Terebellides stroemii* Sars, 1835: Williams 1984; *Microphthalmus listensis* Westheide, 1967: Westheide and Rieger 1987; *Serpula vermicularis* Linnaeus, 1767: Kupriyanova 1999; *Owenia fusiformis* Delle Chiaje, 1844: Koh and Bhaud 2001). Recent applications of molecular techniques have revealed further hidden diversity. Numerous supposedly widespread polychaete species have been shown to consist of multiple morphologically indistinguishable cryptic species (e.g. Grassle and Grassle 1976; Soosten *et al*. 1998; Scaps *et al*. 2000; Bleidorn *et al*. 2006; Jolly *et al*. 2006; Blake *et al*. 2009; Halt *et al*. 2009; Barroso *et al*. 2010; Borda *et al*. 2013; Nygren 2014; Kawauchi and Giribet 2014).

Although natural cosmopolitan distributions of polychaetes now seem unlikely, genetic studies show that distribution ranges can be significantly enhanced as a result of human introductions, thus appearing widespread (Westheide *et al*. 2003; Meyer *et al*. 2008). For instance, biofouling has been a major mode of dispersal for *Hydroides elegans* (Haswell, 1883), one of the most important invasive tubeworms globally (Pettengill *et al*. 2007). Given that incomplete morphological taxonomic studies, the presence of cryptic species and anthropogenic translocations can co-occur (Zhan *et al*. 2010; Bock *et al*. 2012; Capa *et al*. 2013), combined morphological and genetic analysis appears the most powerful approach to reveal the true distribution of species, particularly if they appear to have extensive distribution ranges.

The serpulid tubeworm *Hydroides brachyacantha* Rioja, 1941 is an example of a questionable widely distributed species. Originally described from Mazatlán, Mexico, this species is commonly found intertidally on rocks and molluscs (Rioja 1941). Its operculum is characterised by a verticil with several incurved spines, one of which is enlarged into a dorsal hook (Fig. 5). Since its original description, *H. brachyacantha* has been reported from tropical and subtropical localities around the world, such as Hawaii (Straughan 1969; Coles *et al*. 1999; Coles *et al*. 2002, 2004; Carlton and Eldredge 2009), Brazil (Zibrowius 1970; Rullier and Amoureux 1979; Knight-Jones and Knight-Jones 1991), Yap and Chuuk islands in Micronesia (Imajima 1982; Imajima and ten Hove 1984; Bailey-Brock and Hartman 1987), Japan (Imajima 1987, 1996), the Mediterranean coast of Israel and Turkey (Ben-Eliahu 1991; Ben-Eliahu and ten Hove 1992; Çinar 2006; Galil 2008; Katsanevakis *et al*. 2012), Venezuela (Díaz Díaz and Liñero Arana 2001), southern California, the western coast of Baja California Sur, Gulf of California to Ecuador (Bastida-Zavala and ten Hove 2003), and India (Pati *et al*. 2015). Also recognised was *H.* cf *brachyacantha* from the Caribbean Sea and Brazil (Bastida-Zavala and ten Hove 2002). It has been reported from both natural habitats and artificial substrates and is considered an important introduced species in the Mediterranean (Ben-Eliahu and ten Hove 1992; Çinar 2006). Several studies have, however, questioned the wide distribution of *H. brachyacantha*. Imajima and ten Hove (1984) and then Bastida-Zavala and ten Hove (2003) identified *H. brachyacantha* as either being a taxon with a long record of dispersal through shipping (see also Lewis *et al*. 2006 and Streftaris and Zenetos 2006), or belonging to a complex of morphologically similar species. Moreover, it has been proposed that the records from Hawaii, the tropical Indo-Pacific and western Pacific are likely to be one or more separate species.

In Australia, *H. brachyacantha* was first recorded (as *H. brachyacanthus*) from Botany Bay, New South Wales, by Dew (1959). Straughan (1967) followed Dew's identification, reporting the species in Moreton Bay, Queensland. Subsequently, *H. brachyacantha* was consistently reported from Australia (Queensland and Western Australia: Imajima and ten Hove

1984; Edithburgh, South Australia: Kupriyanova *et al*. 2006, 2008; Sydney Harbour: Lewis *et al*. 2006; Hutchings *et al*. 2013). However, there is no indication that Dew or Straughan or any subsequent authors studied material from Australia and compared it with the original records from Mexico. Some of the specimens recorded as *H. brachyacantha* by Dew (1959) and Straughan (1967) have subsequently been confirmed to be *Hydroides tuberculata* Imajima, 1976 (Imajima and ten Hove 1984). The only likely record of *H. brachyacantha sensu stricto* in Australia is that of Lewis *et al*. (2006), which referred to specimens scraped from ship hulls in Sydney after the Australian Bicentennial Tall Ships race in 1988. These specimens were assumed to settle on the ship during a three-week stay in Acapulco, Mexico. Most recently, as a result of morphological comparative studies of specimens collected from Australia and Mexico, Sun *et al*. (2015) re-described '*H. brachyacantha*' from Australia (southern Queensland to South Australia) as a new species, *H. amri*, thus supporting the hypothesis that *H. brachyacantha* is a species complex.

Given the broad distribution of the *H. brachyacantha*-complex and uncertainty as to the number of species within the species complex, the aim of this study was to provide molecular support for the morphology-based separation of *H. amri* from *H. brachyacantha sensu stricto* and to investigate the presence of potential cryptic species. As Rioja (1941) did not leave any collections and thus the type material of *H. brachyacantha* is not available, here we designate a neotype in accordance with the International Code of Zoological Nomenclature (ICZN 1999), Article 75.3.

Materials and methods

Sample collection

Specimens were collected from seven locations in New South Wales, Victoria, South Australia and Mazatlán, Mexico (see Fig. 1 and Table 1 for details). From each location, tubes with worms were carefully scraped off rocks and docks. Specimens used for morphological examination were fixed in 4% formalin and then preserved in 80% ethanol. Specimens used for molecular work were fixed and preserved in 96% ethanol and stored at –20°C. Type specimens and specimens used for molecular work were deposited in the Australian Museum (AM) collection (Table 1) and the rest of the specimens were deposited in the Colección de Referencia de Invertebrados, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (EMU).

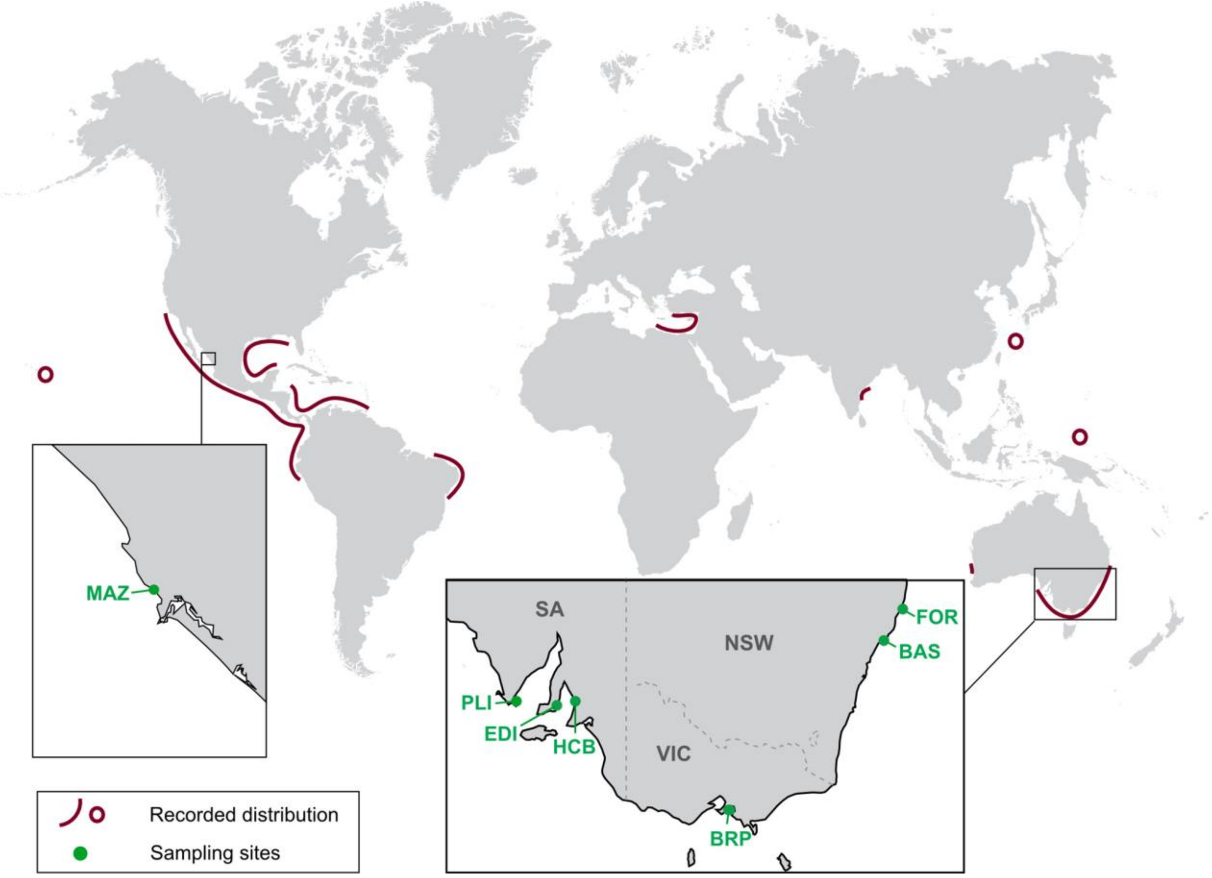


Fig. 1 World map indicating the known distribution of the Hydroides brachyacantha-*complex. The magnified maps show the sample sites in south-eastern Australia and Mexico with the sample code of each site. BAS, Bass Point; BRP, Black Rock Pier; EDI, Edithburgh Jetty; FOR, Forster; HCB, Hallett Cove Beach; MAZ, Mazatlán Port; PLI, Port Lincoln.*

DNA extraction, amplification and sequencing

A total of 78 individuals from the *H. brachyacantha*-complex were used for molecular work. Sequences from *H. tuberculata*, a species morphologically similar to *H. brachyacantha*, as well as those of *Galeolaria caespitosa* Lamarck, 1818 and *Spirobranchus cariniferus* (Gray, 1843), were used as outgroups in the phylogenetic analysis (Table 1).

Details of taxon names, collection localities, voucher numbers, and GenBank accession numbers are given in Table 1. For each individual, the posterior end was cut for DNA extraction. The tissue was washed with 0.5 mL TE buffer three times to remove any ethanol in the tissue. A DNeasy Blood and Tissue Kit (Qiagen, Duesseldorf, Germany) was used to extract genomic DNA according to the manufacturer's protocol. Fragments of two nuclear genes, 18S rRNA (18S) and the internal transcribed spacer-2 (ITS2) region, and one mitochondrial gene, cytochrome *b* (cyt *b*), were used as molecular markers in this study. Gene regions were amplified by polymerase chain reaction (PCR) using the primers listed in Table 2. Polymerase chain reactions were performed in a total volume of 15 µL with 10× PCR buffer (1.5 µL), 25 mM $MgCl_2$ (1.8 µL), 10 mM of each primer (0.25 µL), 2.5 mM dNTPs (1 µL), Milli-Q H_2O

(9.12 µL), Qiagen Taq DNA Polymerase (0.08 µL) and template (1 µL). Polymerase chain reactions were carried out in an Eppendorf Mastercycler Pro using the following PCR protocol: initialisation at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 51°C to 58°C for 30 s, with different annealing temperatures according to primer combinations, extension at 72°C for 20s to 70s, according to the length of each fragment (see Table 2), repeated for 40 cycles, and a final extension at 72°C for 5 min. Polymerase chain reaction products were separated by electrophoresis using a 1.0% agarose gel. Successfully amplified products were purified with ExoSAP-IT (Affymetrix, California, United States), and an Applied Biosystems 3730 xl DNA Sequencer was used for bidirectional sequencing by Macrogen Inc., South Korea.

Table 2. Primers used for PCR and their sources

Primer	Sequence 5' to 3'	T_a (°C)	References
18S1			
TimA	AMCTGGTTGATCCTGCCAG	58	Nóren and Jondelius, 1999
1100R2	CGGTATCTGATCGTCTTCGA	58	Nóren and Jondelius, 1999
Cyt-b			
424-f	GGWTAYGTWYTWCCWTGRGGWCARAT	47	Boore and Brown (2000)
COBr825	AARTAYCAYTCIGGYTTRATRTG	47	Burnette *et al.* (2005)
ITS2			
ITS3	GCATCGATGAAGAACGCAGC	54	White *et al.* (1990)
ITS4	TCCTCCGCTTATTGATATGC	54	White *et al.* (1990)

Sequence alignment and phylogenetic analyses

All sequences generated were checked for contaminations with BLAST searches (Altschul *et al*. 1990). Sequences from both directions of target fragments were assembled using CodonCode Aligner v. 1.2.4 (CodonCode Corporation, Dedham, MA). The details of these taxa, including taxon name, collection localities, voucher numbers and GenBank accession numbers are given in Table 1. Alignment was performed with ClustalX (Larkin *et al*. 2007) using default settings (15 gap opening penalty and 6.66 gap extension penalty), and subsequently edited by eye using BioEdit (Hall 1999). Approximately 980 bp of 18S, 300 bp of cyt *b*, and 700 bp of ITS2 were used for phylogenetic and genetic structure analyses.

Bayesian phylogenetic analyses were performed with MrBayes ver. 3.1 (Ronquist and Huelsenbeck 2003). Models for the sequence data partitions were chosen using MrModelTest ver. 2.3 (Nylander 2004) according to hierarchical likelihood ratio tests (Frati *et al*. 1997; Huelsenbeck and Crandall 1997). The model chosen was TrN + G for 18S, HKY + G for the 1st and 2nd codon positions of cyt *b*, TVM + G for the 3rd codon position of cyt *b*, HKY + G for ITS2. MrBayes ver 3.1 was used to construct the tree under the selected models. Parameters were unlinked in different genes so the genes could be assigned to different evolutionary models. Two simultaneous runs with four Markov chains each ran for 4 000 000 generations, sampling every 1000 trees. Parameter stationarity was confirmed using Tracer (Rambaut and

Drummond 2007) by examining the values for all parameters (including tree –lnL) converged across the two analyses. Based on this, the first 1 000 000 generations (1000 trees) were discarded as burn-in. The majority rule consensus tree of the remaining 3000 trees for each analysis gave the posterior probabilities for each clade.

Genetic diversity and population structure

Uncorrected pairwise distance and corrected pairwise distances using the TrN model among cyt *b* and ITS2 sequences of each population were calculated in MEGA 6 (Tamura *et al*. 2013). Haplotype networks were generated for both cyt *b* and ITS2 in TCS ver 1.21 (Clement *et al*. 2000) following the statistical parsimony criterion (Templeton *et al*. 1992) with 95% probability (4-step connection limit) and fixed step connection limits ranging from 5 to 50.

Morphological examination

Specimens used in the morphological studies were observed under a dissecting microscope, focusing on the operculum, the most important feature used for species-level identification for this group of organisms. Selected specimens were photographed with a Spot Flex CCD 15.2 camera fitted on a Leica MZ16 dissection microscope. Helicon Focus 5.3 Pro software was used to create photomontage using the layers of partially focused images captured. One specimen of each species was dehydrated in ethanol, critical-point dried, coated with 20 µm of gold and examined under a Zeiss EVO LS15 scanning electron microscope at AM. The terminology used in the species descriptions follows that in ten Hove and Kupriyanova (2009).

Results

Phylogenetic analyses

The phylogenetic relationships of *H. brachyacantha*-complex specimens were inferred from concatenated fragments of 18S (66 sequences, 980 bp), cyt *b* (45 sequences, ~309 bp) and ITS2 (67 sequences, ~686 bp) (Table 1). The majority ruled consensus tree supported four major clades in the *H. brachyacantha*-complex, clades I–IV, with high posterior probability (pp) support (Fig. 2). Clades I (pp = 0.99) and II (pp = 0.99) included Mexican *H. brachyacantha* specimens from Marina Mazatlán and were recovered as well-supported sister clades (pp = 0.99). Clade III (pp = 1.0) included *H. amri* specimens from Forster and Bass Point (New South Wales), Black Rock Pier, Melbourne (Victoria), Hallett Cove Beach, Adelaide (South Australia) and Port Lincoln (South Australia). Clade IV (pp = 1.0) included specimens from Hallett Cove Beach and Edithburgh Jetty (South Australia) and was a sister group of *H. tuberculata* (pp = 0.89). Monophyly of the two Australian clades (clades III and IV) was not supported.

Genetic diversity and population structure

Comparison of uncorrected and corrected pairwise distances of both cyt *b* and ITS2 sequences indicated high divergence among all four clades, which was greater than intra-clade distances (Table 3). The mean cyt *b* interclade-corrected genetic distance was 23.9% and ranged from 18.3% to 29.1%. The intraclade-corrected genetic distance was 0% in clades I, II and IV, and was 10.3% in clade III. The mean ITS2 interclade-corrected genetic distance was 20.1%, with a range of 10.9% to 28.8%, and the intraclade-corrected genetic distance was 0% in clades I, II and III, and 0.3% in clade IV.

Haplotype network analysis of cyt *b* further reflected the higher genetic variation in clade III, in contrast to the low genetic variation in clades I, II and IV. Eleven haplotypes of cyt *b* were detected in clade III in a single network (*n* = 31, fixed 15-step limit connection; Fig. 3A), covering all specimens from NSW to SA, while only one haplotype was detected in each of the other three clades under the same steps of limit connection setting. However, the two cyt *b* haplotypes of clades I and II were merged into one network with a fixed 26-step connection limit. Haplotype network analysis of ITS2 detected low genetic variations in each of the four clades (Fig. 3B). Only one ITS2 haplotype was observed for each of clade I (*n* = 8), clade II (*n* = 3) and clade III (*n* = 52), while three different haplotypes were observed for clade IV (*n* = 4).

Table 3. *Hydroides brachyacantha* complex pairwise distances. Mean Tamura Nei (TrN; below diagonal) and uncorrected (above diagonal) interclade and intraclade (TrN; italics along diagonal) pairwise distances for cyt *b* and ITS2 (bold)

	***H. brachyacantha* (Clade I)**	***H.* cf. *brachyacantha* (Clade II)**	***H. amri* (Clade III)**	***H. nikae* (Clade IV)**
***H. brachyacantha* (Clade I)**	*0.000* ***0.000***	0.194 **0.126**	0.176 **0.101**	0.194 **0.227**
***H.* cf. *brachyacantha* (Clade II)**	0.238 **0.140**	*0.000* ***0.000***	0.158 **0.140**	0.224 **0.237**
***H. amri* (Clade III)**	0.212 **0.109**	0.183 **0.156**	*0.103\0.084* ***0.000***	0.231 **0.204**
***H. nikae* (Clade IV)**	0.231 **0.273**	0.280 **0.288**	0.291 **0.240**	*0.000* ***0.003\0.003***

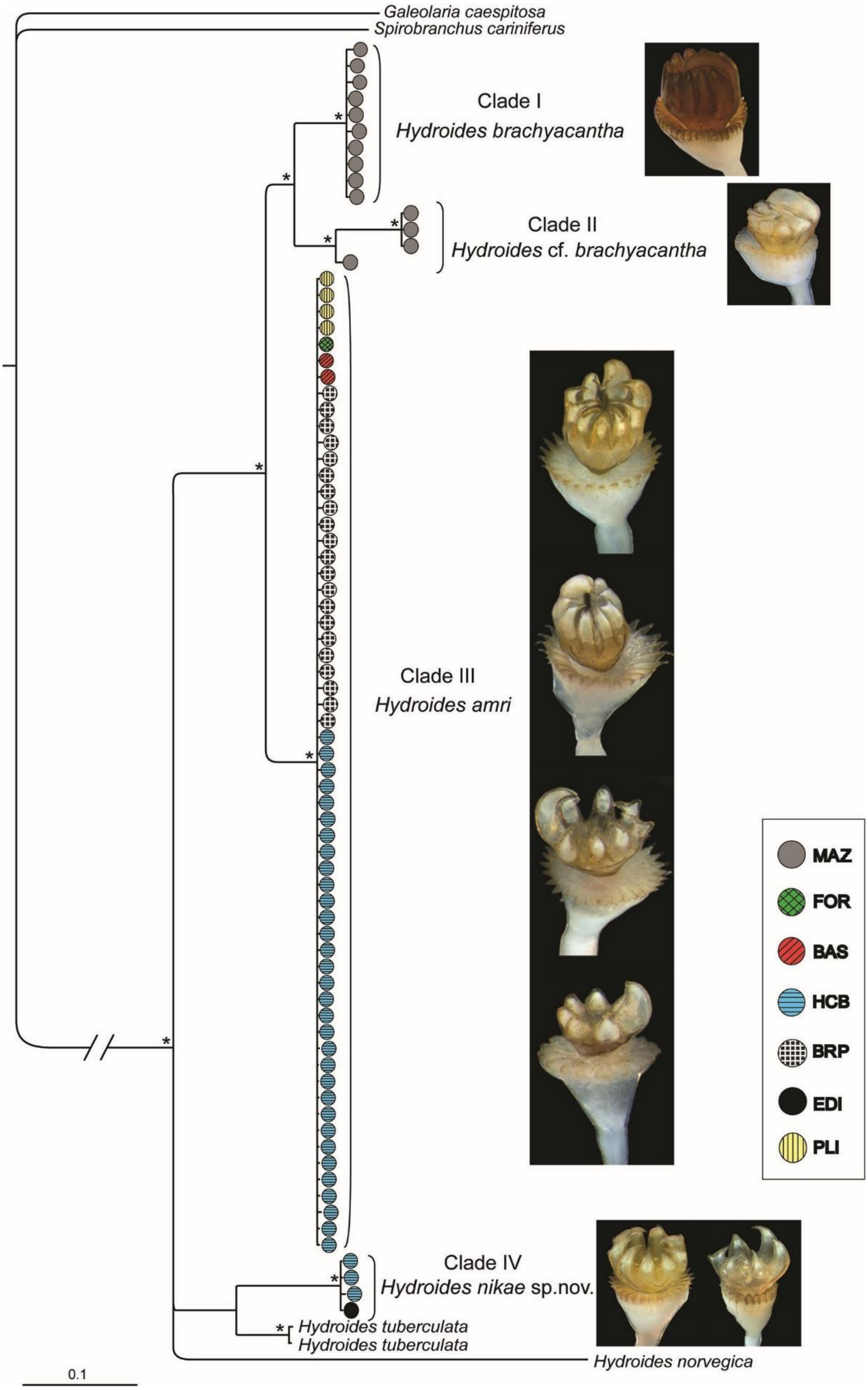


Fig. 2. MrBayes tree of Hydroides brachyacantha*-complex constructed from combined dataset of 18S, cyt b and ITS2. Bayesian inference posterior probabilities (pp) values >0.95 indicated above nodes as asterisk. Opercular types of each species shown after species name. Sample codes are the same as those explained in Table 1. BAS, Bass Point; BRP, Black Rock Pier; EDI, Edithburgh Jetty; FOR, Forster; HCB, Hallett Cove Beach; MAZ, Mazatlán Port; PLI, Port Lincoln.*

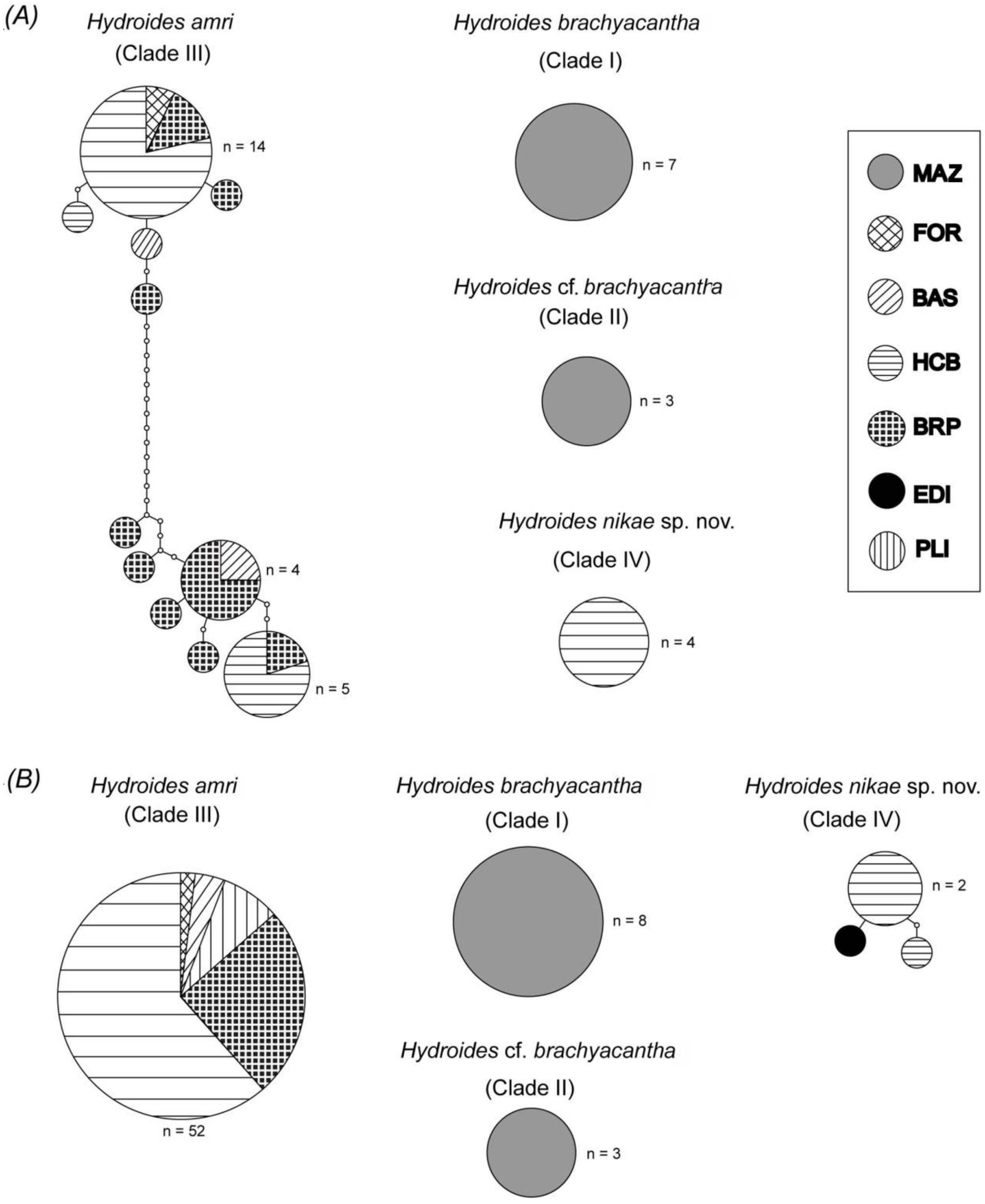


Fig. 3 Haplotype network for Hydroides brachyacantha-*complex based on cyt b and ITS2 sequence data. Areas of the circles are proportional to the total number of individuals of each clade used in network analyses, haplotype frequencies (n) >1 are indicated next to each circle. Partitions in the circles represent the proportion of each population in each haplotype. Lines between circles represent one mutational step. (A) Network for cyt b haplotypes; (B) network for ITS2 haplotypes.BAS, Bass Point; BRP, Black Rock Pier; EDI, Edithburgh Jetty; FOR, Forster; HCB, Hallett Cove Beach; MAZ, Mazatlán Port; PLI, Port Lincoln.*

Discussion

Our molecular data clearly support the hypothesis previously suggested by Bastida-Zavala and ten Hove (2003) that *H. brachyacantha* represents a species complex. Moreover, it provides molecular support for the establishment of *H. amri* based on traditional morphological taxonomic characteristics (Sun *et al*. 2015).

Based on morphological taxonomy, *H. amri* has recently been described as a species distributed from southern Queensland to South Australia (Sun *et al*. 2015). However, our phylogenetic analyses on the combined dataset of three genes provide strong evidence for the existence of two groups within organisms with a *H. amri* morphotype (clades III and IV; Fig. 2). Clade III comprises specimens from NSW, VIC and SA, including the specimen of *H. amri sensu stricto* collected from the type locality (Base Port, NSW). Clade IV comprises specimens collected from South Australia (Adelaide and Edithburgh) only. The sister group relationship between clade IV and *H. tuberculata* suggests a long-term isolation of clades III and IV. Furthermore, these two clades are not connected with the current threshold in the haplotype networks (Fig. 3). They show approximate 30% cyt *b* divergence and 24% ITS2 divergence (Table 3), both of which are higher than those found in serpulids (e.g. Halt *et al*. 2009) and other polychaetes (e.g. Bleidorn *et al*. 2006; Drake *et al*. 2007). Although no clear morphological distinctions were observed among the Australian specimens during morphological examination, based on the phylogenetic species concept *sensu* Mishler and Theriot (2000), we accept specimens in clade III, which include sequence data from the holotype, as *H. amri*, and name specimens in clade IV as a distinct species – *Hydroides nikae*, sp. nov. This species is described in the 'Taxonomy' section of this paper.

Phylogenetic analyses also support the monophyly of *H. brachyacantha* from the type locality, and it consists of two clades (clades I and II; Fig. 2). These two clades show nearly 24% cyt *b* divergence and 14% ITS2 divergence, which are lower than those between clades III and IV, but comparable with that displayed for other serpulids (e.g. Halt *et al*. 2009). However, the cyt *b* haplotypes of clades I and II can be merged into one network under a high number of connection limit steps. Given that the specimens of clade II were collected from the same locality as *H. brachyacantha*, the high divergence in cyt *b* and ITS2 may reflect a high intraspecific genetic diversity. In light of these results, we suggest that these two clades belong to the same species (*H. brachyacantha*).

Oceanographic processes such as historical geological events and currents are routinely used to explain the distribution patterns of marine invertebrates (e.g. Teske *et al*. 2011; Waters *et al*. 2014). The south-eastern Australian biogeographic boundary, in the vicinity of Bass Strait, is one of the major biogeographic barriers of the continent. The boundary was highly influenced by a historical land bridge from Tasmania to mainland Australia during times of lower sea levels in the early- to mid-Pleistocene, which resulted in physical isolation for many subpopulations of invertebrates during that time (Hill *et al*. 1993; O'Hara and Poore 2000). This biogeographic boundary has resulted in a well-documented distribution limit in many intertidal species that are most affected by sea level fluctuations (Hidas *et al*. 2007; York *et al*. 2008; Halt *et al*. 2009). Subtidal species, however, do not consistently show such separation and are commonly distributed across the Bass Strait boundary (e.g. O'Loughlin *et al*. 2003; Waters *et al*. 2004; Waters 2008). Oceanic currents such as the East Australian Current and Leeuwin Current systems are thought to drive the distribution of subtidal species in south-eastern Australia (Waters *et al*. 2004; Dawson 2005; Coleman *et al*. 2011). A subtidal pattern of distribution was observed for *H. amri*, which extends across Bass Strait and along the entire south-eastern Australian coast from NSW to at least Port Lincoln, SA. The highest number of haplotypes was observed at Bass Strait (Fig. 3), where we sampled nine of 11 observed haplotypes. Such population connectivity is most likely facilitated by a combination of currents such as the strong summer flow of the surface East Australian Current towards Bass Strait (Colgan and da Costa 2013), complex eddies in Bass Strait, and seasonal inshore currents off Victoria and South Australia (Middleton and Bye 2007) (Fig. 4).

Unlike *H. amri*, *H. nikae*, sp. nov. occurs only in Gulf St Vincent (Edithburgh and Adelaide) and is in sympatry with *H. amri* at Hallett Cove Beach in Adelaide. Given its low density (three specimens out of 35 collected specimens at Hallett Cove Beach) and morphological similarity with *H. amri*, this species could easily be overlooked. Alternatively, this demography could be indicative of a recent colonisation of *H. nikae*, sp. nov. to the Gulf St Vincent. Given that *H. nikae*, sp. nov. did not occur east to Gulf St Vincent, this species might be a south-western species penetrating to Gulf St Vincent via the eastward flowing Leeuwin Current from Western Australia, together with deep upwelling off the coast of Kangaroo Island (Middleton and Bye 2007; Condie *et al*. 2011) (Fig. 4). *Hydroides nikae*, sp. nov. may also be a member of the *H. brachyacantha*-complex, which was introduced from a distant geographic region by human-driven transport, as already shown for some members of the *H. brachyacantha*-complex (Çinar 2006; Lewis *et al*. 2006). Further sampling west of Port Lincoln, South Australia and throughout Western Australia, also with a large scale revision of the *H. brachyacantha*-complex are needed to test these concepts and to determine whether *H. nikae*, sp. nov. is native or an introduced species.

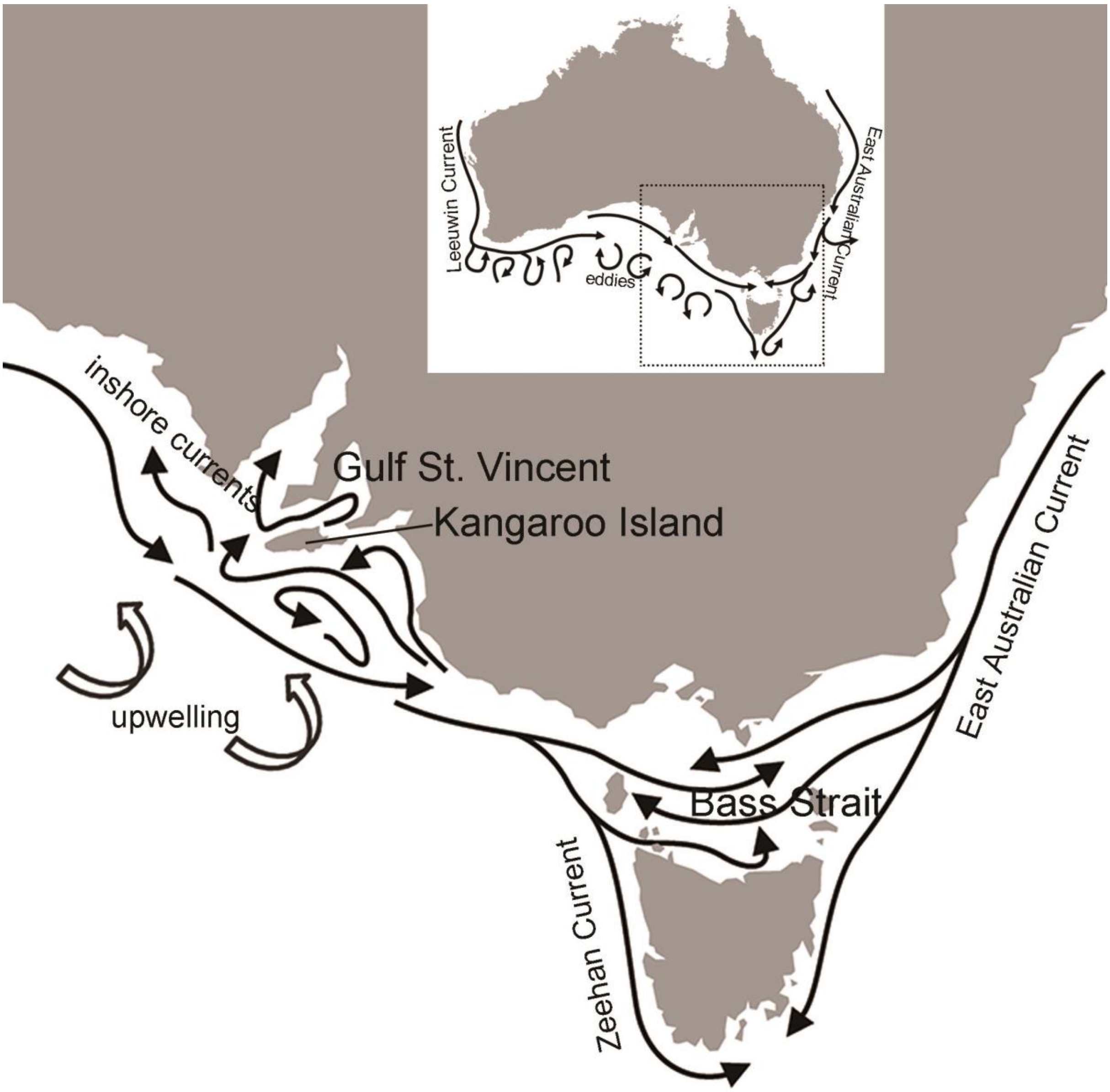


Fig. 4 Dominant currents for southern Australia, particularly for south-east Australia.

In conclusion, the natural distribution of *H. brachyacantha sensu stricto* is not as widespread as previously thought. This study supports the hypothesis of Bastida-Zavala and ten Hove (2003) that several species have been classified under the name '*H. brachyacantha*'. The previously reported wide distribution of *H. brachyacantha* is the result of insufficient taxonomic studies of a species complex. Any future reports of '*H. brachyacantha*' from outside Mexico should be treated with caution as a critical re-examination of historical specimens will most likely reveal additional morphospecies or misidentifications in the species complex (e.g. Sun *et al*. 2015). As our study also shows, however, the *H. brachyacantha*-complex does include true cryptic species that are indistinguishable without the application of molecular methods. Further molecular studies should include '*H. brachyacantha*' reported from Brazil, Hawaii, India, Japan, the Mediterranean and Micronesia. Genetic data coupled with morphological descriptions can reveal potential cryptic species and assist in the understanding of their geographic distribution patterns and native ranges.

Taxonomy

Both mitochondrial and nuclear sequence data allowed the recognition of a new taxon among the Australian specimens. Therefore, despite the apparent lack of morphological distinction between the two clades (clades III and IV), we provide the full morphological description of the new species *H. nikae*, sp. nov. according to the International Code of Zoological Nomenclature (ICZN 1999), and give molecular characters for differential diagnoses of both *H. amri* and *H. nikae*, sp. nov. Due to the high variation of cyt *b* within *H. amri*, we did not find a consistently conserved section as an identical character. A 26-bp subsection of ITS2 (positions 293–318 from the 5′ end of the reference alignment matrix), which was consistently conserved among each of the three species, was used as the diagnostic character here.

Hydroides brachyacantha Rioja, 1941

(Figs 5, 6A–F)

Hydroides brachyacantha Rioja, 1941: 169–172, pl. 3, fig. 2, pl. 4, figs 1–9.

Hydroides brachyacanthus – Bastida-Zavala and ten Hove, 2003: 73–76, figs 3a–m, 7a–f; Bastida-Zavala, 2008: 22–23, fig. 6c; Tovar-Hernández *et al*. 2009: 328–330, figs 3j, 8d–f.

Material examined

Neotype. Mexico: Gulf of California, Sinaloa, Mazatlán, Marina Mazatlán, 23°16′47″N, 106°27′40″W, dock fouling, 0.3–0.5 m, legit M. Tovar, 17.iv.2014 (AM W46899).

Additional material examined. Mexico: Gulf of California, Sinaloa, Mazatlán port, 23°10′58.4″N, 106°25′28.3″W, hull fouling, 1 m, fixed in 10% formalin, 12.i.2010, legit M. Tovar (1, EMU–10819A). Mexico: Gulf of California, Sinaloa, Mazatlán, Facimar, Pinitos beach, on rocks, fixed in 96% ethanol, legit R. Ramírez 14.vii.2010 (5, EMU–10820A). Mexico: Gulf of California, Sinaloa, Mazatlán, Mazatlán Marina, 23°16′47.42″N, 106°27′39.94″W, dock fouling, 0.5 m, fixed in 96% ethanol, legit M. Tovar, 3.xi.2012 (38, EMU–10820B); same, fixed in 96% ethanol, legit M. Tovar 22.iv.2013 (5, EMU–10820C); same, fixed in 96% ethanol, legit M. Tovar 17.iv.2014 (1, AM W46900; 1, AM W46901; 1, AM W46902; 1, AM W46913; 1, AM W46914; 1, AM W46915; 1, AM W46916; 1, AM W46917; 1, AM W46918; 1, AM W46919; 1, AM W46920; 1, AM W46921; 1, AM W46922; 1, AM W46923; 1, AM W46924; 1, AM W46925; 16, EMU–10820D); same, fixed in 10% formalin, 17.iv.2014 (10, EMU–10818); same, fixed in 96% ethanol, legit M. Tovar 15.viii.2015 (1, EMU–10820E).

Diagnosis

Verticil with 8 to 12 yellow to dark brown spines unequal in size. Dorsal hook broad, curved, bigger than all other spines, covering central disc. Other spines with pointed tip and pronounced knob each. First and second pair of dorsal spines (lateral to dorsal hook) with tips and trunks wider than all other spines. Collar chaetae bayonet with two blunt teeth; distal blade smooth.

The molecular diagnostic characters of ITS2: GTCTCTCGGA----TGG--GCCTGTC. Position 293, G (guanine); 296–298, GCT (guanine-cytosine-thymine); 300, G; 303–306, delete; 314–316, CTG (cytosine-thymine-guanine); 318, C (cytosine). Accession numbers of reference sequences used for alignment are shown in Table 1.

Description

Measurements based on neotype and 10 additional specimens, but other features based on the examination of 50 additional specimens from the type locality.

Tube: white, sinuous or coiled clockwise. Usually occurring in patches with several dozen conspecifics. All tubes lack peristomes and longitudinal ridges; transversal, shallow ridges are present at the anterior part of tubes.

Branchiae: radioles arranged in semicircles, with 11 radioles on left lobe and 11 on right lobe (12 ± 3, n = 10, 7–16 radioles on left lobe; 12 ± 2, n = 10, 7–16 radioles on right lobe). Radioles 2.58 mm long (1.93 ± 0.64 mm, n = 9, 0.8–3 mm). Filamentous tips up to 0.21 mm long (0.18 ± 0.04 mm, n = 10, 0.1–0.2 mm). Radiolar eyes absent.

Peduncle: cylindrical, smooth, with a shallow constriction. Peduncle plus operculum length 2.35 mm (2.6 ± 0.75 mm, n = 9, 1.3–3.9 mm). Operculum placed on the right side in neotype.

Operculum: with distal verticil inserted on short stalk into proximal oblique radial symmetrical funnel. Verticil with 11 amber or brown spines (10 ± 1 spine, n = 10, 8–12 spines). Dorsal hook present, broad, curved, covering the central disc. Other spines with a knob and pointed tips; first and second pair of dorsal spines (lateral pairs to dorsal hook) wider than lateral and ventral spines (Fig. 5). A basal internal spinule present in each spine, triangular. Funnel with 42 radii with pointed tips (32 ± 5 radii, n = 10, 26–42 radii). Length 1.22 mm (1.25 ± 0.54 mm, n = 10, 1.0–2.5 mm), opercular diameter 0.65 mm (0.61 ± 0.13 mm, n = 10, 0.5–0.9 mm). Pseudoperculum present, in left side in neotype. Pseudoperculum very short in most specimens examined, as long as collar segment or slightly longer, digitiform, without verticil and spines; pseudoperculum well developed only in EMU–10820E, with verticil and spines and as long as 3/4 of the peduncle plus operculum length.

Collar and thoracic membranes: collar segment long (1 mm), as long as the length of four thoracic segments, trilobed, sub-divided into one long medio-ventral and two latero-dorsal lobes, the latter shorter than medio-ventral lobe. Medio-ventral lobe usually folded at middle.

Thoracic membranes well developed forming a ventral apron, extending towards second abdominal chaetiger.

Thorax: collar chaetae of two types: bayonet with two blunt-elongate teeth and smooth distal blade, and limbate chaetae (Fig. 6A). Six thoracic chaetigers with narrowly limbate chaetae of two sizes (Fig. 6B); saw-shaped uncini with 6–7 teeth, all equal in size (Fig. 6C).

Abdomen: with 92 chaetigers (83 ± 15, *n* = 3, 68–98 chaetigers). Anterior and mid-abdominal chaetigers with flat, trumpet-shaped chaetae (Fig. 6D); asymmetrical with denticulate edge; uncini saw-shaped with five teeth, equal size (Fig. 6E). Posterior chaetigers with capillary chaetae and uncini rasp-shaped with 7–8 rows of teeth (Fig. 6F), equal in size.

Size: total body length 10.2 mm (13.6 ± 2.08 mm, *n* = 3, 12–16 mm). Width of thorax 1.6 mm (1.6 ± 0.08 mm, *n* = 3, 1.57–1.67 mm).

Colour: base of the branchial crown purple. Brown or purple bands (occupying the space of three pinnules) alternating with thin white bands (one pinnule) along radioles, except for the middle region where a wide pink band (five pinnules) is present. Colour of all bands extends towards pinnules. Peduncle with a purple base, then brown or purple bands alternating with white bands until the base of verticil. Thorax mostly red, orange in a few specimens. Abdomen yellow to cream towards posterior end. Each thoracic uncinal torus with an anterior, transverse dark brown band. Brownish pigmentation located transversely across ventral side of abdominal segments and fainter bands dorsally in posterior part of abdomen. Posterior abdominal segments with dark spots below chaetigers.

After fixation in formalin or ethanol only transverse brown bands anterior to each thoracic uncinal torus are still present. Verticil spines amber or brown. Some funnels with scattered white spots.

Ecology

The species occurs among littoral rocks (Rioja 1941) and is also a part of fouling communities on buoys, piers, docks and ship hulls (Tovar-Hernández *et al*. 2009). At the type locality it is associated with algae, chaetopterid and sabellariid tubes, *Bisoarca*, *Spondylus* and *Crassostrea* molluscs (Rioja 1941). At other localities from the Mexican Pacific it has been reported in coralline algal rubble fields, on dead coral *Pocillopora* spp., on shells of molluscs *Muricanthus* and *Spondylus* spp. (Bastida-Zavala and ten Hove 2003; Bastida-Zavala 2008). At the Mazatlán Port the mean annual density of *H. brachyacantha* during 2009 was 80 individualsm^{-2} (fouling assemblages in metallic buoys), with a minimum of 4 ind m^{-2} in November and a maximum of 304 ind m^{-2} in March.

Fig. 5 Operculum of the neotype of Hydroides brachyacantha *AM W46899, various views of the same operculum. Scale bar = 0.1 mm.*

Remarks

Examination of specimens collected from the type locality of *H. brachyacantha* showed that the first and second pairs of dorsal verticil spines (lateral to dorsal hook) are wider than lateral and ventral spines (contrary to descriptions by Bastida-Zavala and ten Hove 2003, and Tovar-Hernández *et al*. 2009, but the same as Rioja's original description and figures by Bastida-Zavala and ten Hove 2003). The presence of a short pseudoperculum is the most common pattern in *H. brachyacantha*. Among ~60 specimens reviewed here, only one had a long pseudoperculum extending 3/4 of the branchial crown length. In the remaining specimens, pseudoperculum occupied ~1/8 of the branchial crown length (as well as reported by Rioja, although he reported one specimen with pseudoperculum extending 1/2 of the branchial crown length).

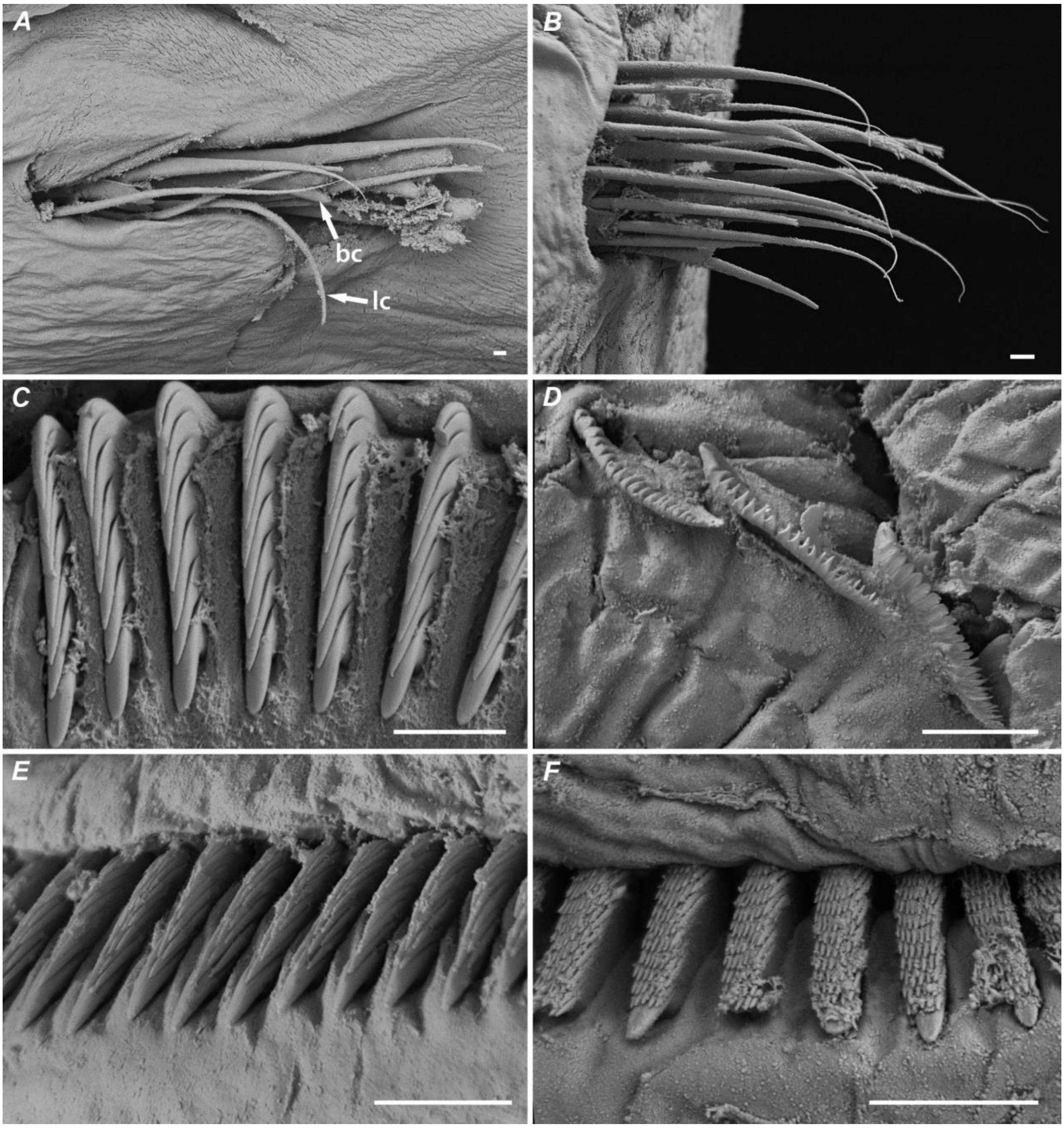


Fig. 6 Scanning electron microscopy images of Hydroides brachyacantha*, paratype, AM W46940. (A) Close-up view of bayonet collar chaetae; (B) chaetae of first thoracic chaetiger; (C) uncini of thoracic chaetiger; (D) flat trumpet-shaped chaetae of abdominal chaetiger; (E) mid-abdominal uncini; (F) posterior abdominal uncini. Scale bars = 10 mm.*

Hydroides amri Sun, Wong, ten Hove, Hutchings, Williamson & Kupriyanova, 2015

Material used for molecular diagnosis

Paratypes. Australia: New South Wales, Port Kembla, north-east end of Martin Island, 34°29′30.3″S 150°55′45.5″E, from scallop shell, 6 m, legit E. Wong 3.v.2014 (1, AM W46426); Australia, New South Wales, Bass Point, 34°35′42.2″S 150°53′56.8″E, on rocks, legit A. Hegedus and P. Berents 25.v.2014 (1, AM W46591).

Additional material examined. Australia: Victoria, Black Rock Pier, 37°58′9.48″S, 145°0′34.92″E, legit E. Kupriyanova 18.vi.2014 (total 13: AM W46683, AM W46684, AM W46687, AM W46688, AM W46690, AM W46692, AM W46693, AM W46695, AM W46696, AM W46697, AM W46698, AM W46699, AM W46702). South Australia, Hallett Cove Beach, 35°13′17.7″S 138°27′35.4″E, on rocks, legit E. Kupriyanova 14.ix.2014 (total 32: AM W46911, AM W46912, AM W46913, AM W46915, AM W46917, AM W46918, AM W46919, AM W46920, AM W46921, AM W46922, AM W46924, AM W46925, AM W46926, AM W46929, AM W46930, AM W46931, AM W46932, AM W46933, AM W46934, AM W46936, AM W46937, AM W46938, AM W46927, AM W47076, AM W47077, AM W47078, AM W47079, AM W47080, AM W47081, AM W47082, AM W47083, AM W47084).

Description

See Sun *et al*. (2015).

Molecular diagnosis

The molecular diagnostic characters of ITS2: GTCACTCTGATAGATGG--GCCTGTC. Position 293, G; 296–298, ACT (adenine-cytosine-thymine); 300, T (thymine); 303–306, TAGA (thymine-adenine-guanine-adenine); 314–316, CTG; 318, C. Accession numbers of reference sequences used for alignment are shown in Table 1.

Hydroides nikae, sp. nov.

(Figs 7, 8A–F)

http://zoobank.org/lsid/urn:lsid:zoobank.org:act:F64DAA11-FFCD-447D-A859-A04E712E6BE6

Material examined

Holotype. Australia: South Australia, under Edithburgh Jetty, 35°5′S, 137°44′E, legit G. Rouse and E. Kupriyanova 13.ii.2005 (SAM E3530).

Paratypes. Australia: South Australia, Hallett Cove Beach, 35°13′17.7″S, 138°27′35.4″E, on rocks during low tide, legit E. Kupriyanova 14.ix.2014 (1, AM W46916; 1, AM W46923; 1, AM W46935).

Diagnosis

Verticil with six to nine yellow or dark brown spines strongly curved inwards, becoming smaller gradually from dorsal to ventral side. One to four dorsal hooks broader than other verticil spines. Dorsal hooks with or without external knob, other verticil spines with external knob. Special collar chaetae bayonet with two short cylindrical round-tip teeth.

The molecular diagnostic characters of ITS2: ATCATGCCG-TAGTTGG--GCACTTT. Position 293, A (adenine); 296–298, ATG (adenine-thymine-guanine); 300, T; 302, delete; 303–306, TAGA; 314–316, ACT; 318, T. Accession numbers of reference sequences used for alignment are shown in Table 1.

Description

Tube: white, 0.8 (up to 0.8) mm wide with lumen of 0.5 (up to 0.5) mm, sub-trapezoidal in cross section, with two longitudinal ridges.

Branchiae: each lobe with seven radioles on each side (7.5 ± 0.84, *n* = 3, 7–9 radioles on each side), arranged in semicircles in short spiral pectinately, not connected by branchiae. Pinnules increasing in length distally, terminal filament long. Branchial eyes absent.

Peduncle: smooth, circular in cross section, inserted just below first and second normal radiole; constriction between peduncle and funnel present.

Operculum: with distal verticil inserted on short stalk into proximal oblique radial symmetrical funnel. Verticil with nine spines (7.5 ± 1.3, *n* = 4, 6–9 spines), with pointed tip, strongly curved inwards, becoming smaller gradually from dorsal to ventral side; in holotype, four dorsal verticil spines larger than others, with small external knob, covering towards central disc, other verticil spines smaller, with round knob (Fig. 7A), in paratypes, AM W46916 (Fig. 7B) and AM W46935 (Fig. 7D) with two smooth dorsal hooks, and four to five smaller vertical spines with external knob, AM W46923 with one smooth dorsal hook, and five smaller vertical spines with external knob (Fig. 7C). Each verticil spine with one short basal internal spinule. Central tooth absent. Funnel with 26 (23 ± 3.5, *n* = 4, 18–26 radii) sharp chitinised radii; base of funnel not chitinised. Grooves separating radii extending 1/3 to 1/2 of funnel length. Length of operculum 1 mm (0.73 ± 0.18 mm, *n* = 4, 0.6–1 mm), width 0.6 (0.34 ± 0.18 mm, *n* = 4, 0.17–1 mm). Pseudoperculum present, in left side in holotype (right insertion *n* = 2, left side *n* = 2).

Collar and thoracic membranes: collar low, continuous with thoracic membranes, forming apron across anterior abdominal chaetigers.

Thorax: with collar chaetiger and six uncinigerous chaetigers. Collar chaetae of two types: bayonet with two short cylindrical round-tip teeth (Fig. 8A) and limbate. Subsequent chaetae limbate, of two sizes (Fig. 8B). Uncini along the entire thorax saw-shaped with six to seven teeth (Fig. 8C). Prostomial eyes absent.

Abdomen: with appreciately 60 chaetigers (55 ± 8.9, *n* = 3). Chaetae flat, trumpet-shaped (Fig. 8D). Uncini saw-shaped anteriorly, with pointed fang and 4–5 teeth (Fig. 8E); rasp-shaped with 2–5 rows of teeth and fang, and up to 5–6 teeth in profile view posteriorly (Fig. 8F). Simple capillaries present posteriorly.

Size: length 8.6 mm (6.9 ± 1.5 mm, *n* = 3, 5.7–8.6 mm). Width of thorax 0.57 mm (0.62 ± 0.08 mm, *n* = 3, 0.57–0.71 mm). Branchiae and operculum accounting for 1/5 of entire length.

Colour: verticil spines and tips of funnel radii yellow to dark brown.

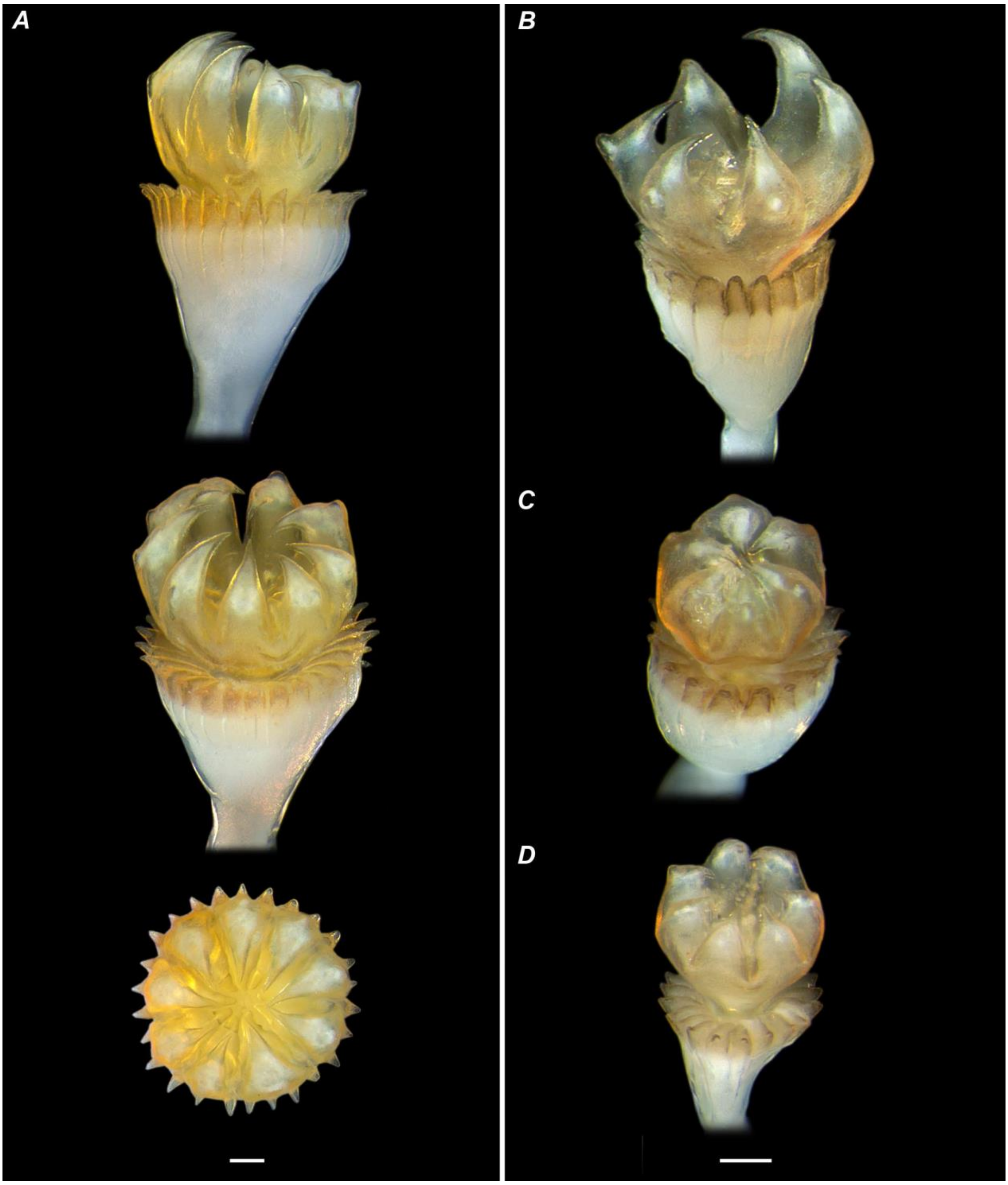


Fig. 7 Operculum of Hydroides nikae*, sp. nov. (A) HolotypeSAME3830, various views of the same operculum; paratypes (B) AM W46916; (C) AM W46923; (D) AM W46935. Scale bars = 0.1 mm.*

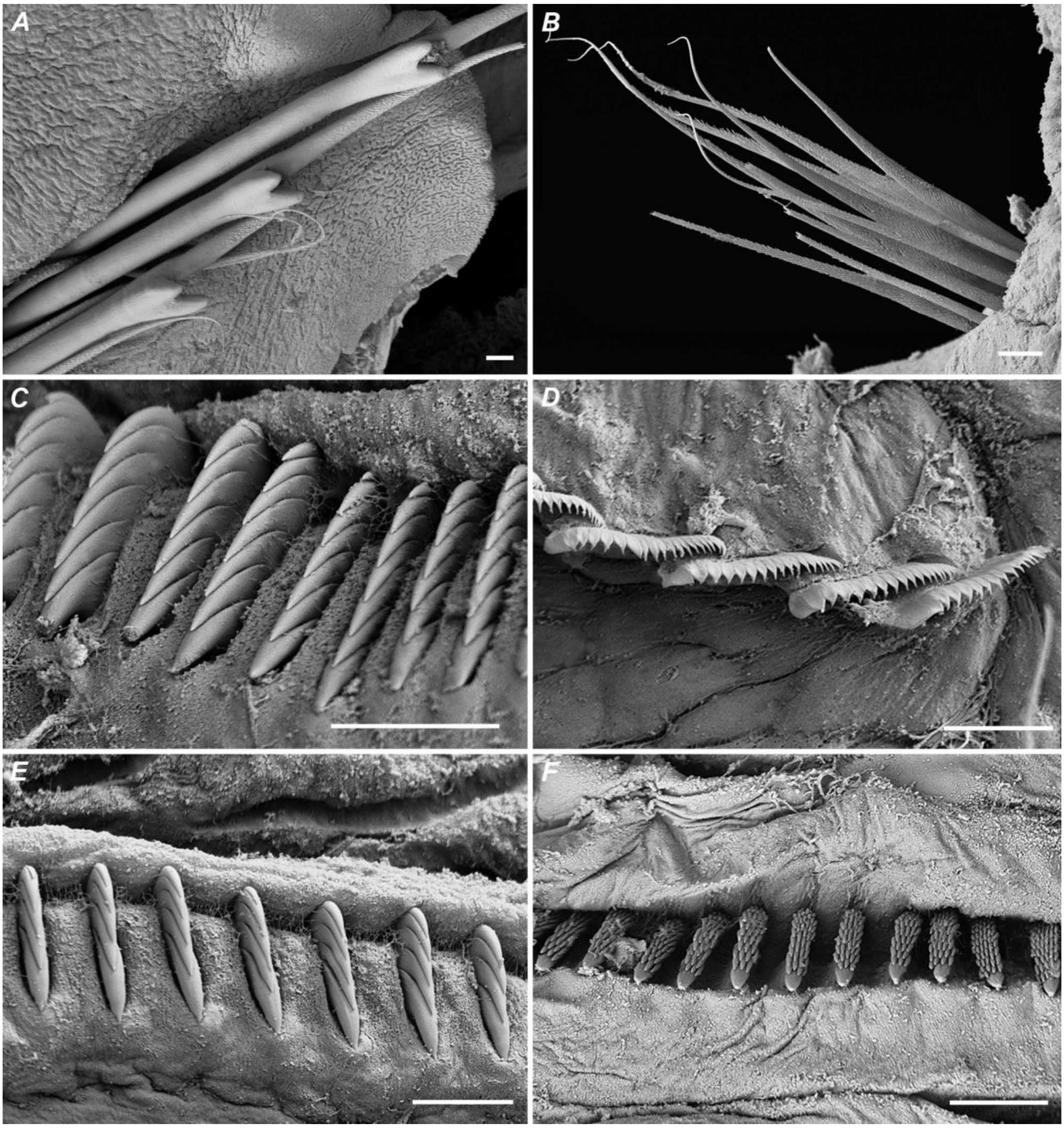


Fig. 8 Scanning electron microscopy images of Hydroides nikae*, sp. nov., paratype, AMW46916. (A) Close-up view of collar chaetae; bc, bayonet chaeta; lc, limbate chaeta; (B) chaetae of first thoracic chaetiger; (C) uncini of thoracic chaetiger; (D) flat trumpet-shaped chaetae of abdominal chaetiger; (E) mid-abdominal uncini; (F) posterior abdominal uncini. Scale bars = 10 mm.*

Ecology

Attached to subtidal rocks, usually to the bottom surface of the rocks.

Etymology

The species is named in honour of Nika Mikhin, the daughter of Elena Kupriyanova, for her help in specimen collecting in Adelaide.

Distribution

Australia: Gulf St Vincent, SA.

Remarks

The four specimens show high variation in the morphology of the operculum (Fig. 7). The holotype (SAM E3530) has an external knob on the dorsal verticil hook that is absent in the paratypes. The presence and absence of an external knob on the dorsal verticil hook was also observed in the *H. amri* population from Victoria. The number of dorsal verticil hooks varies from one to four in *H. nikae*, in contrast to one to five in *H. amri*, making them difficult to assess morphologically. Thus, molecular characters are a more effective method to diagnose these two species.

Table 1. *Hydroides brachyacantha* group sampling locations, dates of collection and total sample sizes used for haplotype, molecular and morphological analyses.

Species	**Geographical location**	**Sample code**	**Latitude, Longitude**	**Sampling date**	**Registration number**	**GenBank accession number**		
						18S1	**cyt*b***	**ITS2**
Hydroides amri	Forster, NSW, Australia	FOR	32°10'50"S, 152°30'42"E	3/05/2014	AM W46426	KR095107	KR095054	KR094992
	Bass Point, NSW, Australia	BAS	34°35'50"S, 150°54'00"E	23/05/2014	AM W46591	KR095108	KR095055	KR094993
					AM W46592	KR095109	KR095056	KR094994
	Black Rock Pier, Port Phillip, VIC, Australia	BRP	37°58'09"S, 145°00'35"E	18/06/2014	AM W46682	KR095110		
					AM W46683	KR095111		KR094995
					AM W46684	KR095112	KR095057	KR094996
					AM W46685	KR095113	KR095058	
					AM W46686	KR095114		
					AM W46687	KR095115	KR095059	KR094997
					AM W46688	KR095116	KR095060	KR094998
					AM W46689	KR095117		
					AM W46690	KR095118		KR094999
					AM W46691	KR095119		
					AM W46692	KR095120	KR095061	KR095000
					AM W46693	KR095121	KR095062	KR095001
					AM W46694	KR095122	KR095063	
					AM W46695	KR095123	KR095064	KR095002
					AM W46696	KR095124		KR095003
					AM W46697	KR095125	KR095065	KR095004
					AM W46698	KR095126	KR095066	KR095005
					AM W46699	KR095127		KR095006
					AM W46700	KR095128		
					AM W46701	KR095129		
					AM W46702	KR095130	KR095067	KR095007
	Hallett Cove Beach, SA, Australia	HCB	35°04'37"S, 138°30'07"E	14/09/2014	AM W46911	KR095133	KR095071	KR095011
					AM W46912	KR095134	KR095072	KR095012
					AM W46913	KR095135		KR095013
					AM W46915	KR095136		KR095014
					AM W46917	KR095138	KR095074	KR095016
					AM W46918	KR095139	KR095075	KR095017
					AM W46919	KR095140		KR095018
					AM W46920	KR095141	KR095076	KR095019
					AM W46921	KR095142	KR095077	KR095020
					AM W46922	KR095143		KR095021
					AM W46924	KR095145		KR095023
					AM W46925	KR095146		KR095024
					AM W46926	KR095147	KR095079	KR095025
					AM W46929	KR095148	KR095080	KR095027

Table 1 (continued)

Species	Geographical location	Sample code	Latitude, Longitude	Sampling date	Registration number	GenBank accession number		
						18S1	cyt*b*	ITS2
					AM W46930	KR095149		KR095028
					AM W46931	KR095150	KR095081	KR095029
					AM W46932	KR095151	KR095082	KR095030
					AM W46933	KR095152		KR095031
					AM W46934	KR095153	KR095083	KR095032
					AM W46936	KR095155	KR095085	KR095034
					AM W46937	KR095156	KR095086	KR095035
					AM W46938	KR095157		KR095036
					AM W46927			KR095026
					AM W47076		KR095093	KR095043
					AM W47077		KR095094	KR095044
					AM W47078		KR095095	KR095045
					AM W47079			KR095046
					AM W47080			KR095047
					AM W47081			KR095048
					AM W47082		KR095096	KR095049
					AM W47083			KR095050
					AM W47084			KR095052
	Port Lincoln, SA, Australia	PLI	34°43'34"S, 135°53'10"E	6/3/2015	AM W47474	KR095100		KR094986
					AM W47475	KR095101		KR094987
					AM W47476	KR095102		KR094988
					AM W47477	KR095103		KR094990
Hydroides brachyacantha	Marina Mazatlán, Mazatlán, Sinaloa, Mexico	MAZ	23°16'47"N, 106°27'40"W	3/11/2011	AM W45577	KR095104	KR095053	KR094990
					AM W45582	KR095105		KR094991
					AM W45584	KR095106		
				18/08/2014	AM W46899	KR095131	KR095068	KR095008
					AM W46900		KR095069	KR095009
					AM W46903	KR095132	KR095070	KR095010
					AM W47013	KR095158		
					AM W47014	KR095159	KR095087	KR095037
					AM W47015	KR095160	KR095088	KR095038
					AM W47016	KR095161	KR095089	KR095039
					AM W47017	KR095162		
					AM W47020	KR095163	KR095090	KR095040
					AM W47021	KR095164	KR095091	KR095041
					AM W47022	KR095165	KR095092	KR095042
***Hydroides nikae* sp. nov.**	Edithburgh Jetty, SA, Australia	EDI	35°05'26"S, 137°44'51"E		SAM E3530	**DQ317117***	**EU190466**	KR095051
	Hallett Cove Beach, SA, Australia	HCB	35°04'37"S, 138°30'07"E	14/09/2014	AM W46916	KR095137	KR095073	KR095015
					AM W46923	KR095144	KR095078	KR095022
					AM W46935	KR095154	KR095084	KR095033
Outgroups								
Hydroides norvegica	Mølnbukt, Agdenes, Norway		63°37'21.3"N 9°38'06.5"E	2/09/2014	AM W46897	**AY611452**	KR095097	
Hydroides tuberculata	Turtle Beach, Lizard Island, Queensland, Australia		14°39'07"S, 145°27'02"E	24/08/2013	AM W45414	KR095166	KR095098	
Hydroides tuberculata	Turtle Beach, Lizard Island, Queensland, Australia		14°39'07"S, 145°27'02"E	25/08/2013	AM W45419	KR095167	KR095099	
Galeolaria caespitosa						**AB106257**	**FJ646546**	**FJ646558**
Spirobranchus cariniferus						**JX144817**	**JX144878**	

*** : GenBank accession numbers in Bold indicate sequences downloaded from Genbank.**

Acknowledgements

The study was funded by Australian Biological Resource Survey (ABRS) grant RF213–19 to EKK. We are grateful to Tulio F. Villalobos-Guerrero (ECOSUR, Mexico) who generously dedicated his time to collect and send samples, Robin Wilson and Julian Finn who hosted EKK's visit to Melbourne Museum, Dmitry Mikhin who helped with collecting in Adelaide and Port Lincoln, and Jan Rykr who helped with the map. We thank two anonymous reviewers who provided constructive criticism for the manuscript. We also thank the Department of Biological Sciences at Macquarie University for an International Macquarie University Research Excellence Scholarship (iMQRES) awarded to YS.

References

Altschul, S., Gish, W., Miller, W., Myers, E., and Lipman, D. (1990). Basic local alignment search tool. *Journal of Molecular Biology* **215**(3), 403–410. doi:10.1016/S0022-2836(05)80360-2

Bailey-Brock, J. H., and Hartman, O. (1987). Class Polychaeta. In 'Reef and Shore Fauna of Hawaii'. (Eds D. M. Devaney and L. G. Eldredge.) pp. 216–220. (Bishop Museum: Honolulu, HI.)

Banks, S. C., Piggott, M. P., Williamson, J. E., Bove, U., Holbrook, N. J., and Beheregaray, L. B. (2006). Oceanic variability and coastal topography shape local genetic structure in a long-dispersing marine invertebrate. *Ecology* **88**, 3055–3064.

Barroso, R., Klautau, M., Solé-Cava, A. M., and Paiva, P. C. (2010). *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. *Marine Biology* **157**, 69–80. doi:10.1007/s00227-009-1296-9

Bastida-Zavala, J. R. (2008). Serpulids (Annelida: Polychaeta) from the eastern Pacific, including a short mention of Hawaiian serpulids. *Zootaxa* **1722**, 1–61.

Bastida-Zavala, J. R., and ten Hove, H. A. (2002). Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the western Atlantic region. *Beaufortia* **53**, 103–173.

Bastida-Zavala, J. R., and ten Hove, H. A. (2003). Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the eastern Pacific region and Hawaii. *Beaufortia* **53**, 67–110.

Ben-Eliahu, M. N. (1991). Red Sea serpulids (Polychaeta) in the eastern Mediterranean. In 'Ophelia Supplement Volume 5, Systematic Biology and Morphology of World Polychaeta: Proceedings of the 2nd International Polychaete Conference, Copenhagen 1986'. (Eds M. E. Petersen and J. B. Kirkegaard.) pp. 515–528. (Ophelia Publications: Helsingør, Denmark)

Ben-Eliahu, M. N., and ten Hove, H. A. (1992). Serpulids (Annelida: Polychaeta) along the Mediterranean coast of Israel – new population build-ups of Lessepsian migrants. *Israel Journal of Zoology* **38**, 35–53.

Blake, J.A.,Grassle, J. P., and Eckelbarger,K. J. (2009). *Capitella teleta*, a new species designation for the opportunistic and experimental *Capitella* sp. I, with a reviewof the literature for confirmed records. *Zoosymposia* **2**, 25–53.

Bleidorn, C., Kruse, I., Albrecht, S., and Bartolomaeus, T. (2006). Mitochondrial sequence data expose the putative cosmopolitan polychaete *Scoloplos armiger* (Annelida, Orbiniidae) as a species complex.*BMCEvolutionary Biology* **6**, 47. doi:10.1186/1471-2148-6-47

Bock, D. G., MacIsaac, H. J., and Cristescu, M. E. (2012). Multilocus genetic analyses differentiate between widespread and spatially restricted cryptic species in a model ascidian. *Proceedings of the Royal Society of London. Series A* **279**, 2377–2385. doi:10.1098/rspb.2011.2610

Boore, J. L., and Brown, W. M. (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. *Molecular Biology and Evolution* **17**(1), 87–106. doi:10.1093/oxfordjournals.molbev.a026241

Borda, E., Kudenov, J. D., Chevaldonné, P., Blake, J. A., Desbruyères, D., Fabri, M., Hourdez, S., Pleijel, F., Shank, T. M., Wilson, N. G., Schulze, A., and Rouse, G. W. (2013). Cryptic species of *Archinome* (Annelida: Amphinomida) from vents and seeps. *Proceedings. Biological Sciences* **280**, 20131876. doi:10.1098/rspb.2013.1876

Briggs, J. C. (1974). ‘Marine Zoogeography.’ (MacGraw-Hill: New York, NY.)

Burnette, A. B., Struck, T. H., and Halanych, K. M. (2005). Holopelagic *Poeobius meseres* (‘Poeobiidae’, Annelida) is derived from benthic flabelligerid worms. *The Biological Bulletin* **208**, 213–220. doi:10.2307/3593153

Capa, M., Pons, J., and Hutchings, P. (2013). Cryptic diversity, intraspecific phenetic plasticity and recent geographical translocations in *Branchiomma* (Sabellidae, Annelida). *Zoologica Scripta* **42**, 637–655.

Carlton, J. T., and Eldredge, L. G. (2009). ‘The Introduced and Cryptogenic Marine and Estuarine Animals and Plants of the Hawaiian Archipelago. Marine Bioinvasions of Hawai‘i. Bishop MuseumBulletin in Cultural and Environmental Studies 4.’ (Bishop Museum Press: Honolulu, HI.)

Cinar, M. E. (2006). Serpulid species (Polychaeta: Sabellidae) from the Levantine coast of Turkey (eastern Mediterranean), with special emphasis on alien species. *Aquatic Invasions* **1**, 223–240. doi:10.3391/ai.2006.1.4.6

Clement, M., Posada, D., and Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **4**, 331–346.

Coleman, M. A., Roughan, M., Macdonald, H., Connell, S. D., Gillanders, B., Kelaher, B. P., and Steinberg, P. D. (2011). Variation in the strength of continental boundary currents determines continent-wide connectivity in kelp. *Journal of Ecology* **99**, 1026–1032. doi:10.1111/j.1365-2745.2011. 01822.x

Coles, S. L., DeFelice, R. C., and Eldredge, L. G. (1999). Nonindigenous marine species introductions in the harbors of the south and west shores of Oʻahu, Hawaiʻi. Final report prepared for the David and Lucile Packard Foundation. *Bishop Museum Technical Report* **15**.

Coles, S. L., DeFelice, R. C., and Eldredge, L. G. (2002). Nonindigenous marine species in Kaneʻohe Bay, Oʻahu, Hawaiʻi. *Bishop Museum Technical Report* **24**.

Coles, S. L., Reath, P. A., Longenecker, K., Bolick, H., and Eldredge,L. G. (2004). Assessment of nonindigenous marine species in harbors and on nearby coral reefs on Kauaʻi, Molokaʻi, Maui, and Hawaiʻi. *Bishop Museum Technical Report* **29**.

Colgan, D. J., and da Costa, P. (2013). Possible drivers of biodiversity generation in the *Siphonaria* of southeastern Australia. *Marine Biodiversity* **43**, 73–85. doi:10.1007/s12526-012-0127-2

Condie, S. A., Mansbridge, J. V., and Cahill, M. L. (2011). Contrasting local retention and cross-shore transports of the East Australian Current and the Leeuwin Current and their relative influences on the life histories of small pelagic fishes. *Deep-sea Research. Part II, Topical Studies in Oceanography* **58**, 606–615. doi:10.1016/j.dsr2.2010.06.003

Dawson, M. N. (2005). Incipient speciation of *Catostylus mosaicus* (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. *Journal of Biogeography* **32**, 515–533. doi:10.1111/j.1365-2699.2004.01193.x

Day, J. H. (1967). ‘A Monograph on the Polychaeta of Southern Africa. Volume British Museum (Natural History) Publication 656.’ (British Museum (Natural History): London, UK.)

Delle Chiaje, S. (1844). ‘Animali Senza Vertebre del Regno di Napoli. Descrizione e Notomia Degli Animali Invertebrati Della Sicilia Citeriore Osservati Vivi Negli Anni 1822–1830. 8 Tomes.’ (C. Batelli: Naples, Italy.)

Dew, B. (1959). Serpulidae (Polychaeta) from Australia. *Records of the Australian Museum* **25**, 19–56. doi:10.3853/j.0067-1975.25.1959.654

Díaz Díaz, O., and Liñero-Arana, L. (2001). Poliquetos asociados a substratos artificiales sumergidos en la costa nororiental de Venezuela. II: Serpulidae y Spirorbidae. *Boletín del Instituto Oceanográfico de Venezuela, Universidad de Oriente* **40**(1, 2), 9–20.

Drake, C. A., McCarthy, D. A., and von Dohlen, C. D. (2007). Molecular relationships and species divergence among *Phragmatopoma* spp. (Polychaeta: Sabellaridae) in the Americas. *Marine Biology* **150**, 345–358. doi:10.1007/s00227-006-0373-6

Fauchald, K. (1984). Polychaete distribution patterns, or: can animals with Palaeozoic cousins show large-scale geographical patterns? In 'Proceedings of the First International Polychaete Conference, Sydney, Australia, July 1983'. (Ed. P. Hutchings.) pp. 1–6. (The Linnean Society of New South Wales: Sydney, Australia.)

Fauvel, P. (1953). 'Annelida Polychaeta. The Fauna of India, Including Pakistan, Ceylon, Burma and Malaya.' (The Indian Press: Allahabad, India.)

Frati, F., Simon, C., Sullivan, J., and Swofford, D. L. (1997). Gene evolution and phylogeny of the mitochondrial cytochrome oxidase gene in Collembola. *Journal of Molecular Evolution* **44**, 145–158. doi:10.1007/PL00006131

Galil, B. S. (2008). Alien species in the Mediterranean Sea – which, when, where, why? *Developments in Hydrobiology* **606**, 105–116. doi:10.1007/s10750-008-9342-z

Grassle, J. F., and Grassle, J. P. (1976). Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science* **192**, 567–569. doi:10.1126/science.1257794

Gray, J. E. (1843). Fauna of New Zealand. In 'Travels in New Zealand: With Contributions to the Geography, Geology, Botany, and Natural History of That Country, Vol 2'. (Ed. E. Dieffenbach.) pp. 177–296. (John Murray: London, UK.)

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Halt, M. N., Kupriyanova, E. K., Cooper, S. B., and 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. *Invertebrate Systematics* **23**, 205–222. doi:10.1071/IS09003

Haswell, W. A. (1883). On some new Australian tubicolous annelids. *Proceedings of the Linnean Society of New South Wales* **7**, 633–638.

Hidas, E. Z., Costa, T. L., Ayre, D. J., and Minchinton, T. E. (2007). Is the species composition of rocky intertidal invertebrates across a biogeographical barrier in south-eastern Australia related to their potential for dispersal? *Marine and Freshwater Research* **58**, 835–842. doi:10.1071/MF06235

Hill, R. S., Carpenter, R. J., Jordan, G. J., and Smith, S. J. (1993). Past environments and the Gondwana connection. In 'Tasmanian Wilderness –World Heritage Values'. (Eds S. J. Smith and M. R. Banks.) pp. 38–48. (Royal Society of Tasmania: Hobart, Australia.)

Huelsenbeck, J. P., and Crandall, K. A. (1997). Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* **28**, 437–466. doi:10.1146/annurev.ecolsys.28.1.437

Hutchings, P. A., and Glasby, C. J. (1991). Phylogenetic implications of the biogeography of Australian Terebellidae (Polychaeta). *Ophelia* **5**, 565–572.

Hutchings, P. A., Ahyong, S. T., Ashcroft, M. B., McGrouther, M. A., and Reid, A. L. (2013). Sydney Harbour: its diverse biodiversity. *Australian Zoologist* **36**, 255–320. doi:10.7882/AZ.2012.031

ICZN (1999). 'International Code of Zoological Nomenclature, 4th edition.' (The International Trust for Zoological Nomenclature: London, UK.) Available at http://www.nhm.ac.uk/hosted-sites/iczn/code/. Accessed 12/11/2015.

Imajima, M. (1976). Serpulinae (Annelida, Polychaeta) from Japan. I. The genus *Hydroides. Bulletin of the National Science Museum, Tokyo, Ser. Zoology (Jena, Germany)* **2**(4), 229–248.

Imajima, M. (1982). Serpulinae (Polychaetous annelids) from the Palau and Yap islands, Micronesia. *Proceedings of the Japanese Society of Systematic Zoology* **23**, 37–55.

Imajima, M. (1987). Serpulidae (Annelida, Polychaeta) collected around Sesoko Island and Bise, Okinawa. *Galaxea* **6**, 75–82.

Imajima, M. (1996). 'Polychaeta.' (Seibutsu Kenkyusha Col.: Tokyo, Japan.) [In Japanese.]

Imajima, M., and ten Hove, H. A. (1984). Serpulinae (Annelida, Polychaeta)from the Truk Islands, Ponape and Majuro Atoll, with some other new Indo-Pacific records. *Proceedings of the Japanese Society of Systematic Zoology* **27**, 35–66.

Jolly, M. T., Viard, F., Gentil, F., Thiébaut, E., and Jollivet, D. (2006). Comparative phylogeography of two coastal polychaete tubeworms in the northeast Atlantic supports shared history and vicariant events. *Molecular Ecology* **15**, 1841–1855. doi:10.1111/j.1365-294X.2006.02910.x

Katsanevakis, S., Bogucarskis, K., Gatto, F., Vandekerkhove, J., Deriu, I., and Cardoso, A. C. (2012). Building the European alien species information network (EASIN): a novel approach for the exploration of distributed alien species data. *BioInvasions Records* **1**, 235–245. doi:10.3391/bir.2012.1.4.01

Kawauchi, G. Y., and Giribet, G. (2014). *Sipunculus nudus* Linnaeus, 1766 (Sipuncula): cosmopolitan or a group of pseudo-cryptic species? An integrated molecular and morphological approach. *Marine Ecology (Berlin)* **35**, 478–491. doi:10.1111/maec.12104

Knight-Jones, P., and Knight-Jones, E.W.(1991). Ecology and distribution of Serpuloidea Polychaeta round South America. *Ophelia. Supplementum* **5**, 579–586.

Koh, B. S., and Bhaud, M. (2001). Description of *Owenia gomsoni* n. sp. (Oweniidae, Annelida, Polychaeta) from the Yellow Sea and evidence that *Owenia fusiformis* is not a cosmopolitan species. *Vie et Milieu* **51**, 77–86.

Kupriyanova, E. K. (1999). The taxonomic status of *Serpula* cf. *columbiana* Johnson, 1901 from the American and Asian coasts of the North Pacific Ocean. *Ophelia* **50**, 21–34. doi:10.1080/00785326.1999.10409386

Kupriyanova,E. K., McDonald, T. A., and Rouse, G.W.(2006). Phylogenetic relationships within Serpulidae (Annelida: Polychaeta) inferred frommolecular and morphological data. *Zoologica Scripta* **35**, 421–439. doi:10.1111/j.1463-6409.2006.00244.x

Kupriyanova, E. K., Bastida-Zavala, R., Halt, M. N., Lee, M., and Rouse, G. W. (2008). Phylogeny of the *Serpula-Crucigera-Hydroides* clade (Serpulidae; Annelida) using molecular and morphological data: implications for operculum evolution. *Invertebrate Systematics* **22**, 425–437. doi:10.1071/IS08011

Lamarck, J. B. (1818). 'Histoire Naturelle des Animaux Sans Vertèbres, Présentant les Caractères Généraux et Particuliers de ces Animaux, Leur Distribution, Leur Classe, Leur Familles; Leurs Genres, et la Citation des Principales Espèces qui s'yRapportent.' (Déterville: Paris, France.)

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**(21), 2947–2948. doi:10.1093/bioinformatics/btm404

Lewis, J. A., Watson, C., and ten Hove, H. A. (2006). Establishment of the Caribbean serpulid tubeworm *Hydroides sanctaecrucis* Krøyer [in] Mörch, 1863, in northern Australia. *Biological Invasions* **8**, 665–671. doi:10.1007/s10530-005-2062-7

Linnaeus, C. (1767). 'Systema naturae sive regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.' pp 533–1327. (Laurentii Salvii: Stockholm, Sweden)

Meyer, A., Bleidorn, C., Rouse, G. W., and Hausen, H. (2008). Morphological and molecular data suggest a cosmopolitan distribution of the polychaete *Proscoloplos cygnochaetus* Day, 1954 (Annelida, Orbiniidae). *Marine Biology* **153**, 879–889. doi:10.1007/s00227-007-0860-4

Middleton, J. F., and Bye, J. A. T. (2007). A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. *Progress in Oceanography* **75**, 1–41. doi:10.1016/j.pocean.2007.07.001

Mishler, B. D., and Theriot, E. C. (2000). The phylogenetic species concept (*sensu* Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. In 'Species

Concepts and Phylogenetic Theory. A Debate'. (Eds Q. D. Wheeler and and R. Meier.) pp. 44–54. (Columbia University Press: New York, NY.)

Nóren, M., and Jordelius, U. (1999). Phylogeny of Prolecithophora (Platyhelminthes) inferred from 18S rDNA sequences. *Cladistics* **15**, 103–112. doi:10.1111/j.1096-0031.1999.tb00252.x

Nygren, A. (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta* **43**, 172–183. doi:10.1111/zsc.12044

Nylander, J. A. A. (2004). 'MrModeltest v2.2. Program Distributed by the Author. (Uppsala University, Evolutionary Biology Centre: Uppsala, Sweden.)

O'Hara, T. D., and Poore, G. C. B. (2000). Patterns of distribution for southern Australian marine echinoderms and decapods. *Journal of Biogeography* **27**, 1321–1335. doi:10.1046/j.1365-2699.2000.00499.x

O'Loughlin, P. M., Waters, J. M., and Roy, M. S. (2003). A molecular and morphological review of the asterinid, *Patiriella gunnii* (Gray) (Echinodermata: Asteroidea). *Memoirs of the Museum of Victoria* **60** (2), 181–195.

Pati, S. K., Rao, M. V., and Balaji, M. (2015). Spatial and temporal changes in biofouling community structure at Visakhapatnam harbour, east coast of India. *Tropical Ecology* **56**(2), 139–154.

Pettengill, J. B., Wendt, D. E., Schug, M. D., and Hadfield, M. G. (2007). Biofouling likely serves as a major mode of dispersal for the polychaete tubeworm *Hydroides elegans* as inferred from microsatellite loci. *Biofouling* **23**, 161–169. doi:10.1080/08927010701218952

Rambaut, A. J., and Drummond, A. (2007). Tracer v1.4: MCMC trace analyses tool. Available at http://beast.bio.ed.ac.uk/Tracer. Accessed 12/11/2015.

Rioja, E. (1941). Estudios anelidológicos, 2. Observaciones de varias especiesdel género *Hydroides* Gunnerus (sensu Fauvel) de las costas mexicanas del Pacífico. *Anales del Instituto de Biología. Universidad Nacional Autónoma de México* **12**, 161–175.

Ronquist, F., and Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi:10.1093/bioinformatics/btg180

Rouse, G. W., and Pleijel, F. (2001). 'Polychaetes.' (Oxford University Press: Oxford, UK.)

Rullier, F., and Amoureux, L. (1979). Campagne de la Calypso au large des côtes Atlantiques de l'Amérique du Sud (1961–1962). I. 33. Annélides Polychètes. *Annales de l'Institut Océanographique* **55**, 145–206.

Sars, M. (1835). 'Beskrivelser og Iagttagelser Over Nogle Maerkelige Eller Nye i Havet Ved den Bergenske Kyst Levende Dyr af Polypernes, Acalephernes, Radiaternes, Annelidernes og Molluskernes Classer, Med en Kort Oversigt Over de Hidtil af Forfatteren Sammesteds Fundne Arter og Deres Forekommen.’ (Thorstein Hallegers Forlag: Bergen, Norway.)

Scaps, P., Rouabah, A., and Lepretre, A. (2000). Morphological and biochemical evidence that *Perinereis cultrifera* (Polychaeta: Nereididae) is a complex of species. *Journal of the Marine Biological Association of the United Kingdom* **80**, 735–736. doi:10.1017/S0025315400002587

Straughan, D. (1967). Marine Serpulidae (Annelida: Polychaeta) of eastern Queensland and New South Wales. *Australian Journal of Zoology* **15**, 201–261. doi:10.1071/ZO9670201

Straughan, D. (1969). Serpulidae (Annelida, Polychaeta) from Oahu, Hawaii. *Bulletin of the Southern California Academy of Sciences* **68**, 229–240.

Streftaris, A., and Zenetos, N. (2006). Alien marine species in the Mediterranean – the 100 ‘worst invasives’ and their impacts. *Mediterranean Marine Science* **7**, 87–118. doi:10.12681/mms.180

Sun, Y., Wong, E., ten Hove, H. A., Hutchings, P. A., Williamson, J., and Kupriyanova, E.K. (2015). Revision of the genus *Hydroides* (Serpulidae, Annelida) from Australia. *Zootaxa* **4009**, 1–99. doi:10.11646/zootaxa. 4009.1.1

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6:molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729. doi:10.1093/molbev/mst197

Templeton, A. R., Crandall, K. A., and Sing, C. F. (1992). Acladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. Cladogram estimation. *Genetics* **132**, 619–633.

ten Hove, H. A., and Kupriyanova, E. K. (2009). Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs. *Zootaxa* **2036**, 1–126.

Teske, P. R., Rius, M., McQuaid, C. D., Styan, C. A., Piggott, M. P., Benhissoune, S., Fuentes-Grünewald, C., Walls, K., Page, M., Attard, C. R. M., Cooke, G. M., McClusky, C. F., Banks, S. C., Barker, N. P., and Beheregaray, L. B. (2011). ‘Nested’ cryptic diversity in a widespread marine ecosystem engineer: a challenge for detecting biological invasions. *BMC Evolutionary Biology* **11**, 176. doi:10.1186/1471-2148-11-176

Tovar-Hernández, M. A., Méndez, N., and Villalobos-Guerrero, T. F. (2009). Fouling polychaete worms from the Southern Gulf of California: Sabellidae and Serpulidae. *Systematics and Biodiversity* **7**, 319–336. doi:10.1017/S1477200009990041

von Soosten, C., Schmidt, H., and Westheide, W. (1998). Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma*

(Polychaeta: Syllidae). Results from RAPD-PCR investigations. *Marine Biology* **131**, 659–669. doi:10.1007/s002270050358

Waters, J. M. (2008). Marine biogeographical disjunction in temperate Australia: historical landbridge, contemporary currents, or both? *Diversity and Distributions* **14**, 692–700. doi:10.1111/j.1472-4642.2008.00481.x

Waters, J. M., O'Loughlin, P. M., and Roy, M. S. (2004). Cladogenesis in a starfish species complex from southern Australia: evidence for vicariant speciation? *Molecular Phylogenetics and Evolution* **32**, 236–245. doi:10.1016/j.ympev.2003.11.014

Waters, J. M., Condie, S. A., and Beheregaray, L. B. (2014). Does coastal topography constrain marine biogeography at an oceanographic interface? *Marine and Freshwater Research* **65**(11), 969–977. doi:10.1071/MF13307

Westheide,W. (1967). Monographie der gattungen *Hesionides* Friedrich und *Microphthalmus* Mecznikow (Polychaeta, Hesionidae). Ein beitrag zur organisation und biologie psammobionter polychaeten. *Zeitschrift fur Morphologie und Oekologie der Tiere* **61**(1), 1–159. doi:10.1007/BF00298775

Westheide, W., and Rieger, R. M. (1987). Systematics of the amphiatlantic *Microphthalmus-listensis*-species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. *Journal of Zoological Systematics and Evolutionary Research* **25**(1), 12–39. doi:10.1111/j.1439-0469.1987.tb00911.x

Westheide, W., Haß-Cordes, E., Krabusch, M., and Müller, M. (2003). *Ctenodrilus serratus* (Polychaeta: Ctenodrilidae) is a truly amphi-Atlantic meiofauna species – evidence from molecular data. *Marine Biology* **142**, 637–642.

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR Protocols: a Guide to Methods and Applications'. (Eds M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White.) pp. 315–322. (Academic Press: New York, NY.)

Williams, S. J. (1984). The status of *Terebellides stroemi* (Polychaeta, Trichobranchidae) as a cosmopolitan species on a worldwide morphological survey, including description of a new species. In 'Proceedings of 1st International Polychaete Conference'. (Ed. P. A. Hutchings.) pp. 118–142. (Linnean Society of New South Wales: Sydney, Australia.)

York, K. L., Blacket, M. J., and Appleton, B. R. (2008). The Bassian Isthmus and the major ocean currents of southeast Australia influence the phylogeography and population structure of a southern Australian intertidal barnacle *Catomerus polymerus* (Darwin). *Molecular Ecology* **17**, 1948–1961. doi:10.1111/j.1365-294X.2008.03735.x

Zhan, A., Macisaac, H. J., and Cristescu,M.E. (2010). Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology* **19**, 4678–4694. doi:10.1111/j.1365-294X.2010.04837.x

Zibrowius, H. (1970). Contribution à l’etude des Serpulidae (Polychaeta Sedentaria) du Brésil. *Boletim do Instituto Oceanográfico de São Paulo* **19**, 1–32. doi:10.1590/S0373-55241970000100001

Chapter 5

A global invader or a complex of regionally distributed species? Clarifying the status of an invasive calcareous tubeworm *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) using DNA barcoding

Authors:
Yanan Sun[1, 2, *], Eunice Wong[1], Erica Keppel[3, 4], Jane E. Williamson[2], Elena Kupriyanova[1, 2]

[1]*Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia.*
[2]*Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.*
[3]*Marine Invasion Research Laboratory, Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, Maryland 21037, USA.*
[4]*Italian National Research Council (CNR), ISMAR Institute of Marine Sciences, Arsenale di Venezia, Tesa 104, Venice I–30122, Italy.*
**Corresponding author E-mail: yanan.sun@austmus.gov.au*

Accepted by *Marine Biology*

Abstract

Clarifying taxonomic status is essential to understanding invasion source and the spread of invasive species. Here we used barcoding gene cytochrome *c* oxidase subunit I (COI) to explore the issue in a common fouling invasive species *Hydroides dianthus*. The species was originally described from off New England USA, reported along the East coast of North America down to Florida and the Caribbean region, introduced to China, Europe, Japan, and West Africa via anthropogenic transport and is now collected in Brazil for the first time. Unlike most congeners, *Hydroides dianthus* has tolerance for a wide temperature range, being distributed from temperate to subtropical waters. Our results based on 112 specimens collected from 17 localities worldwide confirmed that *H. dianthus sensu stricto* is indeed a global invader. Observed higher haplotypes diversity in the Mediterranean seems to contradict the currently accepted native range of *H. dianthus sensu stricto* in the United States. The study also revealed the existence of a potential cryptic species *H.* cf. *dianthus* with a genetic distance of 5.6%. The cryptic lineage found in Texas was evidently introduced to the Black Sea only recently. Given that both lineages within *H. dianthus* are invasive, a greater emphasis on adequate monitoring and management of the routes responsible for introductions of this species is needed.

Keywords: COI, cryptic species, invasive dynamic, non-indigenous species, biogeography

Introduction

Among the family Serpulidae, known as calcareous tube worms, the largest genus *Hydroides*, with almost 1/5 total species in Serpulidae, includes a number of notorious biofoulers and invaders (e.g., *Hydroides dianthus* (Verrill, 1873), *H. diramphus* Mörch, 1863, *H. elegans* (Haswell, 1883), *H. ezoensis* Okuda, 1934, *H. sanctaecrucis* Krøyer in Mörch, 1863 (Lewis et al. 2006; Link et al. 2009; Breton 2014; Fofonoff et al. 2016; Kupriyanova et al. 2016). With free-swimming planktotrophic larval and sessile adult stages, invasive *Hydroides* species can be easily transported in ballast water, on vessel hulls and via fouling on aquaculture infrastructure, and thus be introduced to new localities throughout their life history where they quickly attain large population sizes in favourable environmental settings (Lewis et al. 2006; Pettengill et al. 2007; Link et al. 2009). Dense aggregates formed by *Hydroides* spp. on underwater structures such as aquaculture nets, seawater intake pipes (Qiu and Qian 1997) and ship hulls and buoys (Wang and Huang 1993) constitute a substantial financial burden to marine aquaculture, navigation, shipping industries and power plants. An introduction of non-native populations of *Hydroides* can also cost millions of dollars to aquaculture industries as well as to local authorities investing in eradication and monitoring (Hirata and Akashige 2004; Dürr and Watson 2009; Katsanevakis et al. 2014).

One of the most documented invasive foulers in the eastern Atlantic is *Hydroides dianthus*, characterised by an operculum with spines that are gradually reduced in size and curved in one direction. The species was originally described from the coast of Massachusetts, USA (Verrill 1873). Its distribution spans along the east coast of North America down to Florida and Caribbean Sea, and it occurs in a variety of habitats including natural open coasts, lagoons and ports (Hedgpeth 1950; ten Hove and Wolf 1984; Bastida-Zavala and Salazar-Vallejo 2000; Bastida-Zavala and ten Hove 2002; Trott 2004; Link et al. 2009). With a long recorded history of introductions, *H. dianthus* was reported from the Mediterranean in the nineteenth century (Zibrowius 1973; Zibrowius and Thorp 1989; Zenetos et al. 2010), and subsequently recorded on the Atlantic coasts of Europe (Streftaris et al. 2005), English Channel (Zibrowius 1978; Eno et al. 1997; Dauvin et al. 2003; Minchin et al. 2013), West Africa (Zibrowius 1971), northern China (Sun and Yang 2000), Japan (Link et al. 2009) and Black Sea (Boltachova et al. 2011).

However, for many marine invasions, the wide distribution also reflects an incomplete understanding of the invaders' biodiversity and geography at the species level (Geller et al. 2010). Seemingly 'cosmopolitan' invasive species, even ones that are considered to be well-known taxonomically, often dissolve into species complexes or cryptic clades when assessed via molecular techniques (e.g., spionid *Marenzelleria viridis* (Verrill, 1873): Bastrop et al. 1998; amphinomid *Eurythoe complanata* (Pallas, 1766): Barroso et al. 2000; mussel *Mytilus edulis* Linnaeus, 1758: Geller et al. 2010; solitary ascidian *Ciona intestinalis* (Linnaeus, 1767): Zhan et al. 2012). Because wide distributions of some *Hydroides* species are often considered a result of anthropogenic transport, slight morphological differences are often attributed to intraspecific variability (Bastida-Zavala and ten Hove 2003; Nishi and Tanaka 2008). Recently, however, highly divergent genetic lineages have been detected in supposedly widespread *H. brachyacantha* Rioja, 1941 (Sun et al. 2015, 2016), which casts doubt on the status of other widespread species of *Hydroides*, such as *H. dianthus*. Moreover, unlike most *Hydroides* that are restricted in their distribution by temperature (e.g., *H. elegans* occurs in tropical and subtropical waters (Qiu and Qian 1997); *H. ezoensis* occurs in temperate waters (Zibrowius and Thorp 1989)), *H. dianthus* is reported from localities across a broad temperature range that extends from temperate to tropical waters (Zibrowius and Thorp 1989). Since temperature is an important factor limiting the distribution ranges of marine organisms, an unusual wide temperature tolerance within a species might indicate the presence of cryptic diversity (Miller et al. 2009; Dennis and Hellberg 2010).

In the present paper, we use barcoding gene cytochrome *c* oxidase I (COI) sequences to analyse the genetic divergence among *H. dianthus* populations world-wide and to assess whether these populations comprise a single species or a species complex. We reflect on what is known of its invasion history to provide insight into the invasive routes and patterns for these populations.

Material and methods

Samples collection

Specimens of *H. dianthus* were collected in 2012–2016 from 17 localities representative of the currently known species distribution range, and also from Cabo Frio, Brazil which is a new sighting (Fig. 1). Specimens were removed from their tubes under a dissecting microscope and preserved in 95% ethanol. For each specimen, the posterior end was cut for DNA extraction. A total of 112 individuals of *H. dianthus* were used for molecular analyses. Details of specimens, including collection localities, voucher numbers and GenBank accession numbers are given in Table 1.

DNA extraction and sequence alignment

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's protocol. An approximate 500 bp fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) was sequenced with primers Hydro-COIF/Hydro-COIR (Sun et al. 2012). Polymerase chain reactions (PCR) were performed in a total volume of 20 µl with Invitrogen 10x PCR buffer (2.0 µl), 50 mM $MgCl_2$ (1.5 µl), 10 nM each primer (0.4 µl), 2.5 µM dNTPs (1.5 µl), Milli-Q H_2O (13.1 µl), Invitrogen Taq DNA Polymerase (0.1 µl) and genomic DNA template (1 µl) in an Eppendorf Mastercycler® pro using the thermal cycle profiles described in Sun et al. (2012). PCR products were separated by electrophoresis using 1.0% agarose gel. Successfully amplified products were purified with the USB ExoSAP-IT PCR clean-up kit, and bidirectionally sequenced at Macrogen Inc. (South Korea) using an Applied Biosystems 3730 xl DNA Sequencer.

Nucleotide sequences were assembled using CodonCode Aligner v. 1.2.4 (CodonCode Corporation Dedham, MA, USA) and checked for potential contaminations with BLAST searches (Altschul et al. 1990). Alignments were performed with BioEdit v. 7.0.5.3 (Hall 1999) using default settings (15 gap opening penalty and 6.66 gap extension penalty) and subsequently edited manually. A dataset of 380 bp COI sequences was used for phylogenetic and genetic structure analyses.

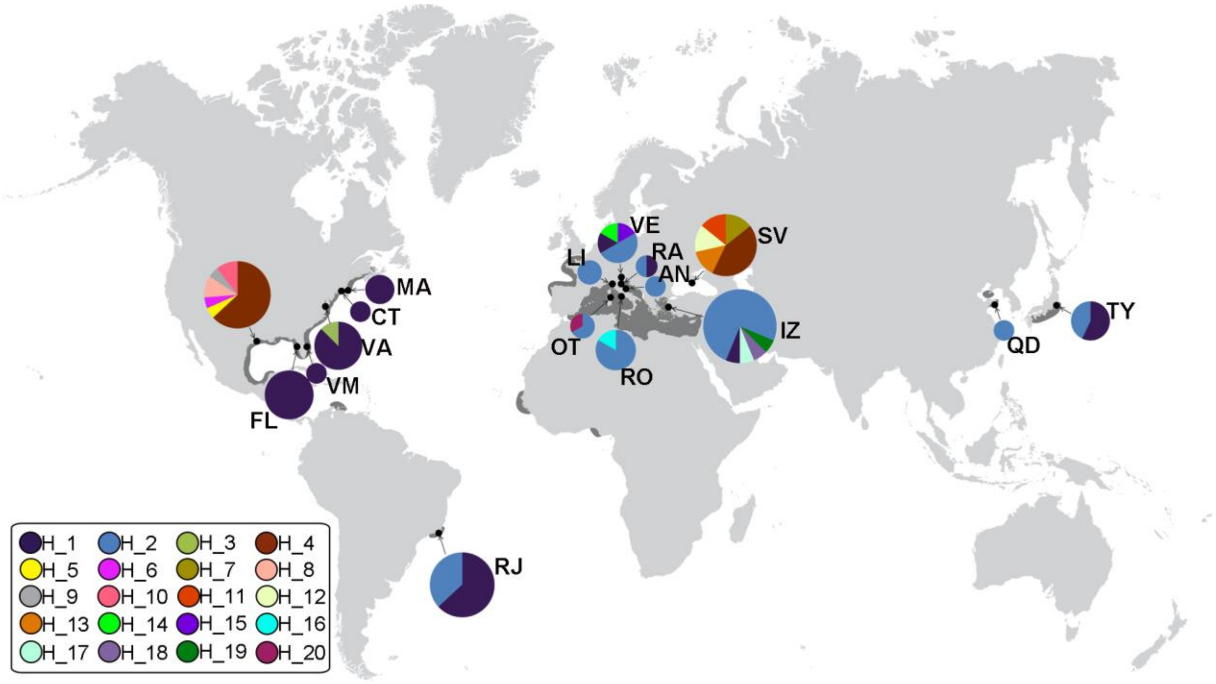


Fig. 1 Hydroides dianthus *distribution. Black spots indicate sampling locations. Dark grey shadow indicates known distribution of the species. Pie charts on the map represent haplotype frequencies for each locality; pie size is proportional to sample size. AN: Ancona, Italy; CT: Old Saybrook, Connecticut, USA; FL: Tampa, Florida, USA; IZ: Izmir, Turkey; LI: Livorno, Italy; MA: Woods Hole, Massachusetts, USA; OT: Olbia-Tempio, Italy; QD: Qingdao, Shandong, China; RA: Ravenna, Italy; RJ: Cabo Frio, Rio de Janeiro, Brazil; RO: Rome, Italy; SV: Sevastopol, Crimea, Ukraine; TX: Galveston, Texas, USA; TY: Tokyo, Japan; VA: Hampton, Virginia, USA; VE: Venice, Italy; VM: Village Marina, Florida, USA. H: Haplotype.*

Phylogenetic analyses and genetic diversity

Bayesian analyses were performed with MrBayes ver. 3.1 (Ronquist and Huelsenbeck 2003). Sequences from *Hydroides sanctaecrucis* (a species morphologically similar to *H. dianthus*), *H. elegans* and *H. ezoensis*, were used as outgroups. Models for the sequence data partitions for Bayesian analyses were TrNef for the 1st codon, TIM for the 2nd codon, and GTR for the 3rd codon according to MrModelTest ver. 2.3 (Nylander 2004). The parameters were unlinked in different partitions so each partition could have different models. Two simultaneous runs with four Markov chains each ran for 4000000 generations, sampling every 1000 trees. Based on this, the first 1000000 generations (1000 trees) were discarded as burn-in. The majority rule consensus tree of the remaining 3000 trees for each analysis gave the posterior probabilities for each clade.

Genetic divergences among phylogenetic clades obtained from the reconstructed tree and specimens of *H. dianthus* from different localities were calculated in MEGA 6 (Tamura et al. 2013) with uncorrected pairwise distance.

A dataset of 380 bp COI sequences from 112 specimens of *H. dianthus* was used to construct an unrooted network in TCS ver 1.21 (Clement et al. 2000) following the statistical parsimony criterion (Templeton et al. 1992) with 95% probability (4-step connection limit) and fixed step connection limits ranging from 5 to 20.

Results

A total length of approximately 506 bp fragment of the mitochondrial gene COI was sequenced from 112 individuals of *Hydroides dianthus* collected from 17 localities (Table 1). Twenty haplotypes were found from the sequences analysed (Table 1). Haplotype composition in each locality is shown in Fig. 1.

Based on a dataset of 380 bp COI sequence fragments, the Bayesian Inference (BI) tree grouped *H. dianthus* into two major clades (posterior probabilities pp = 1 and 0.82 respectively) henceforth named as Clade A and B (Fig. 2). Interclade genetic distance between clade A and B was 5.6%, and intraclade variabilities were comparatively lower (0.2% and 0.4% in each clade, Table 2).

The unrooted haplotype network also supported the existence of two divergent groups within *H. dianthus* (Fig. 3). Each haplotype group consisted of the same individuals forming Clades A and B, respectively. All haplotypes in each group were closely related. The nearest haplotypes between the two clades were separated by 18 mutation steps. Clade A included ten haplotypes covering 15 localities across the east coast of the United States (CT, FL, MA, VA, VM), and in Italy (AN, LI, OT, RA, RO, VE), Turkey (IZ), Japan (TY), China (QD) and Brazil (RJ). Two distant haplotypes (H_1 and H_2) were the most frequent. H_1 and H_2 appeared in similar frequency in Tokyo Bay and Rio de Janeiro, but beyond this, H_1 appeared mainly in the United States, except for Galveston (TX), while H_2 was most common in the Mediterranean. Clade A grouped individuals from four different marine basins (North America, South America, Mediterranean, Asia), but nine of the ten Clade A haplotypes appeared in the Mediterranean basin only, while only two haplotypes were found in each of the other three basins. Clade B also included ten haplotypes occurring only in Galveston (TX) and Sevastopol (SV), while one haplotype (H_4) was shared by 15 of 26 specimens from these localities.

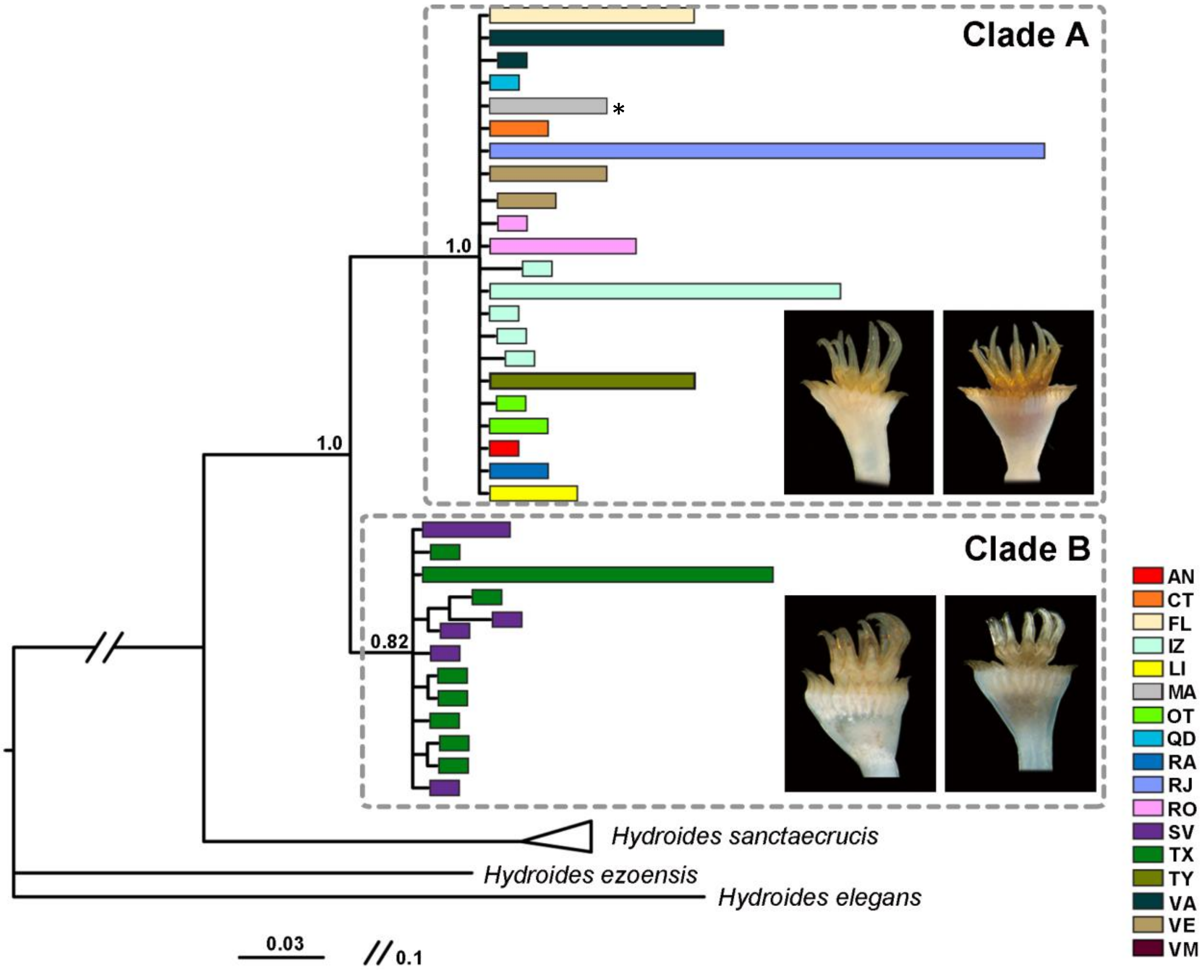


Fig. 2 *Bayesian majority ruled consensus tree of* Hydroides dianthus *based on COI sequences. Two main clades are highlighted. Values represent posterior probabilities (pp) > 0.7. Length of bar at each branch indicates number of individuals in that branch. Asterisk indicates branch formed by specimens from type locality.*

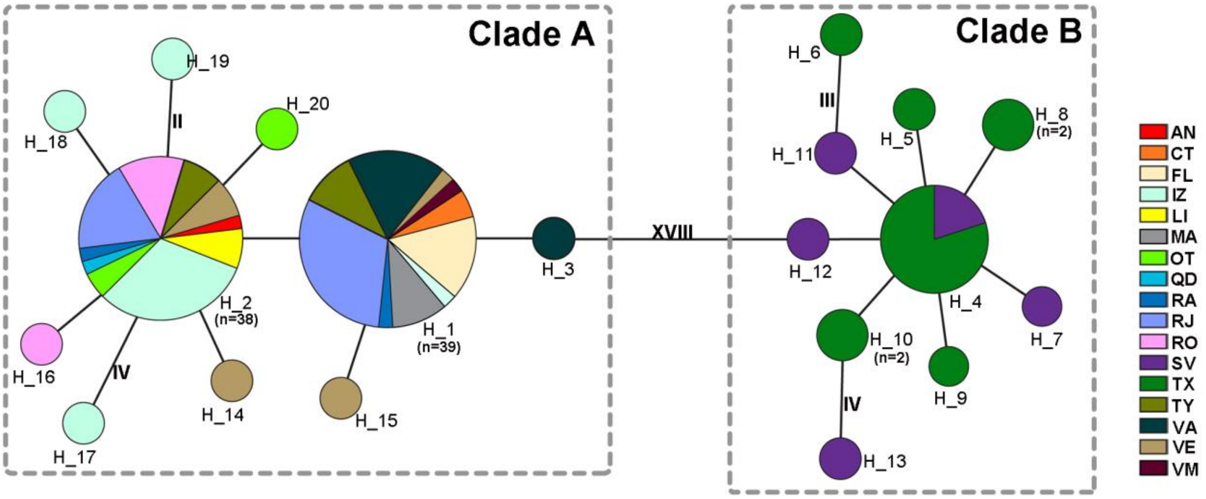


Fig. 3 *Haplotype network for* Hydroides dianthus *from COI data. Haplotypes are marked as H_1 to H_20. Numbers of specimens are given for haplotypes with more than one specimen. Lines between circles represent one mutational step unless marked with Roman numerals.*

Discussion

The high genetic homogeneity of the barcoding region of COI gene at a global scale supports our previous understanding of *Hydroides dianthus* as a global invader that has been greatly impacted by human-mediated transport in its dispersal and range extension. Contrary to our expectations based on morphological examination and abiotic tolerances, however, both phylogenetic reconstruction and network structure suggest that *H. dianthus* is a species complex consisting of two phylogenetic lineages (Clades A and B).

Although all specimens morphologically conformed to *H. dianthus*, phylogenetic analysis of COI sequences recovered two clades separated by a genetic distance of 5.6% (Table 2). A standard fragment of COI has been used widely as a barcoding gene for many polychaetes (Barroso et al. 2010; Nygren and Pleijel 2010; Carr et al. 2011; Lobo et al. 2013), although a genetic distance threshold to separate species has not been defined because of the large range of both intraspecific and interspecific distances and overlapping between clades (Kvist 2014). Carr et al. (2011) suggested a 3.8% distance threshold to separate morphologically distinct species based on the COI sequences of 1876 specimens in 333 polychaete species from Canada, with an average of 16.5% interspecific distance compared to an average of 0.38% intraspecific distance. Kvist (2014), however, indicated a much higher divergence, with an average of 29.97% interspecific distance based on 5545 COI sequences of polychaetes collected world-wide. Among cryptic species, genetic distances of COI are slightly lower than those of morphologically distinct species, but still variable among different polychaete families (e.g., 10%–22% in *Eurythoe*; 2.7%–17.8% in Phyllodocidae; 10.2%–14.8% in Polynoidae (Barroso et al. 2010; Nygren and Pleijel 2010; Carr et al. 2011). Among morphologically distinct species of *Hydroides*, interspecific distances varied from 10.4% to 36.9%, and intraspecific distance between 0.4%–0.9% (Sun et al. 2012). In the present study, the genetic distance between clades A and B (5.6%) is below the range detected among morphologically distinct *Hydroides* species, but it is still in the same range as those observed between the other cryptic species of polychaetes mentioned above (Barroso et al. 2010; Nygren and Pleijel 2010; Carr et al. 2011). Moreover, mean genetic distance within each of clade A and B (0.2% and 0.4%) is in the upper range of intraspecific distance observed in other *Hydroides* species. Thus, although we were unable to demonstrate any clear diagnostic morphological characters for *H. dianthus* specimens from both clades (see Fig. 2), the two detected clades can be considered as two potential cryptic species. Since Clade A includes specimens close to the type locality, we recognise it as *H. dianthus sensu stricto*, while clade B is possibly a cryptic lineage of Clade A and therefore referred to as *H.* cf. *dianthus*. Further analyses, beyond the scope of this study, are required to determine the specific status of clade B.

COI barcoding not only helps to discern cryptic invasive species, but together with historical records of introduction, can provide insights into the sources of invasion. Determining the source of invasion facilitates the identification of invasive routes and vectors, and is thus a valid tool for stakeholders aiming at reducing further invasions (Geller et al. 2010). As *H. dianthus* was originally described from New England, the east coast of the United States has always been assumed to be the native range of *H. dianthus* (Zibrowius 1971; Eno et al. 1997; Link et al. 2009). However, Zibrowius and Thorp (1989) mentioned that the species had been collected in the Mediterranean a few years before Verrill described it from Massachusetts which supported a possibility of the Mediterranean native range. The known Pleistocene fossil record does not provide evidence for either the Atlantic or the Mediterranean native range because the species has been reported from both areas (Oldale et al. 1982, Sciuto et al. 2015). Moreover, fossil identifications based on the tube morphology alone are not reliable as tubes of *Hydroides* species are almost indistinguishable (Ippolitov et al., in prep.).With the long history of shipping activity between North America and Europe, the introduced and native status of *H. dianthus* now seems less certain. Our comparisons of genetic diversity of American east coast and Mediterranean populations seem to indicate that the original native range of *H. dianthus* might be the Mediterranean instead of the east coast of the United States, although haplotype biodiversity along the American Coast could have been lost due to metapopulation extinction or selection as shown for some molluscs (e.g., Hoos et al. 2010). Founding populations of introduced species often have lower genetic variation than that of native populations, and contain only samples of the total genetic diversity from their source population due to genetic bottlenecks (Allendorf and Lundquist 2003; Dlugosch and Parker 2008; Geller et al. 2010;, but see Roman and Darling 2007 and Lejeusne et al. 2014 on the effects on multiple introductions). For *H. dianthus sensu stricto*, the highest genetic diversity (nine of ten haplotypes) was observed in samples from the Mediterranean, while only two (H_1 and H_3) occur in those from the United States, which indicated the possibility that the populations in the Mediterranean acted as the source population. The most frequent haplotype in the United States (H_1, 97% of individuals) was also found in the Mediterranean. Loss of genetic diversity was observed in the other introduced populations in Brazil and Asia, in both of which only two haplotypes (H_1 and H_2) were present. The presence of haplotype H_2 in the Brazilian and Asian populations is difficult to explain if the source population is from the United States, where H_2 has not been found. The Mediterranean population, which includes both haplotypes H_1 and H_2, could represent a more plausible source population, with the first introduction to South Carolina and New England pre-dating the nineteenth century.

Unlike the long historical dispersal of the species in North America, Mediterranean and Europe, we propose that *H. dianthus* invaded Asia and Brazil within the last two decades. The species has been collected from bay scallop (*Argopecten irradians* (Lamarck, 1819)) farms since 2000 in China (Sun and Yang 2000, 2015), and was first recorded from Japan in 2008 (Link et al. 2009; Otani and Yamanishi 2010). Specimens collected during this study from a marina in Araruama lagoon in Cabo Frio (Rio de Janeiro) provide the first record of *H. dianthus* in Brazil, even though the undocumented invasion history of *H. dianthus* in Brazil might be longer.

A different genetic pattern of COI emerged in *H.* cf. *dianthus,* where two geographically distant populations (SV and TX) unexpectedly displayed similar genetic diversity, which suggests quite a different invasion history. Records of *H. dianthus* can be dated back to at least 1950 in Texas (Hedgpeth 1950), while its introduction to the Black Sea is certainly a recent event. This species was found in 2009 in the vicinity of the Institute of Biology of the Southern Seas, an institution with a long history of marine biodiversity research in this area dating back to 1871 (Boltachova et al. 2011). Given that the Black Sea is, in fact, an internal sea connected to the Atlantic through the Mediterranean by the narrow Bosphorus and Dardanelles Straits, the obvious source of invasive fauna in the Black Sea is via the Mediterranean (Gomoiu et al. 2002; Ozturk 2002). However, we did not find haplotypes of *H.* cf. *dianthus* in the samples from the Mediterranean. Therefore, our data strongly suggest that *H.* cf. *dianthus* was introduced to the Black Sea directly from an American population. As *H.* cf *dianthus* was only collected from one locality of the USA (Galveston, Texas), additional material from the Gulf of Mexico and further studies are needed to determine the species distribution range and genetic variability of Black Sea specimens before formally describing it as a new species and clarifying its invasion pathways.

Evidence derived from our research suggests that both clades within the *H. dianthus*-complex are expanding their geographic range of distribution. Most recently, *H. dianthus* communities, most likely *H. dianthus sensu stricto*, have been observed in Nova Scotia, Canada (Nicholas Patocka pers. comm.) and Rongcheng, China (Zhijun Dong pers. comm.). The range extension to Canada might be either a result of global warming driven poleward migration (Doney et al. 2012) or other anthropogenic activities, while the new arrival to Rongcheng is more likely due to the spread of individuals from scallop aquaculture farms to natural habitats. Geographic distributions of both clades indicate high tolerance of a wide range of water temperatures and salinities, which would also indicate a high invasion potential (Kupriyanova et al. 2001; Otani and Yamanishi 2010). While they have not been found in the Southern Hemisphere beyond regions in Brazil and Senegal (see Zibrowius 1971), both species of *H. dianthus* complex may extend their ranges southwards to temperate regions of

South America, and eventually to South Africa and Australia due to the heavy shipping routes in these areas (Seebens et al. 2013).

In conclusion, our study for the first time provides barcoding data for the invasive and economically important serpulid *H. dianthus*. The COI barcoding here has proven to be extremely useful in detecting both cryptic speciation and routes of invasions in *Hydroides*. Further studies are needed to determine the native range of this species and to establish adequate monitoring and mitigation policies on a global scale to reduce further potential introductions of *H. dianthus*.

Acknowledgements

The study was funded by Australian Biological Resource Survey (ABRS) grant RF213-19 to EKK. We thank the Department of Biological Sciences at Macquarie University for an International Macquarie University Research Excellence Scholarship (iMQRES) awarded to YS. We are grateful to João Miguel de Matos Nogueira, Orlemir Carrerette, Felipe Ramon De Chiara, and Carlos Diego from the University of São Paulo who helped with our sampling in Brazil; and to Nicholas Patocka and Zhijun Dong who kindly share their observation on *Hydroides dianthus* communities in the field. We also thank Anja Schulze, Aylin Ulman, Eijiroh Nishi, Elena Lisitskaya, James Carlton, Joachim Langeneck, Melih Ertan Çinar, and Xuwen Wu who collected samples around the world and generously donated them for this study.

Compliance with Ethical Standards:

Funding: This study was funded by Australian Biological Resource Survey (ABRS) grant (grant number RF213-19).

Conflict of Interest: All authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed.

References

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17: 24–30

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410

Barroso R, Klautau M, Solé-Cava AM, Paiva PC (2010) *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. Mar Biol 157: 69–80. doi:10.1007/s00227-009-1296-9

Bastida-Zavala JR, Hove HA ten (2002) Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the western Atlantic region. Beaufortia 52: 103–178

Bastida-Zavala JR, Hove HA ten (2003) Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the eastern Pacific Region and Hawaii. Beaufortia 53: 67–110

Bastida-Zavala JR, Salazar-Vallejo SI (2000) Serpúlidos (Polychaeta: Serpulidae) del Caribe noroccidental: *Hydroides* y *Serpula.* Rev Biol Trop 48(4): 841–858

Bastrop R, Jürss K, Sturmbauer C (1998) Cryptic species in a marine polychaete and their independent introduction from North America to Europe. Mol Biol Evol 15(2): 97–103

Boltachova NA, Lisitskaya EV, Lebedovskaya MV (2011) New species *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) from the coastal waters of Crimea (the Black Sea). Mar Ecol J 10(2): 34–38

Bosc LAG (1801) Histoire naturelle des vers: contenant leur description et leurs moeurs, avec figures dessinées d'après nature. Guilleminet, Paris 2.

Breton G (2014) Alien or invasive species in the ports of Le Havre, Antifer and Rouen (Normandy, France) Hydroécol Appl 18: 23–65

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011) A Tri-Oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS ONE 6:e22232

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 4: 331–346.

Dauvin JC, Dewarumez JM, Gentil F (2003) Liste actualisée des espèces d'Annélides Polychètes présentes en Manche. Cah Biol Mar 44(1): 67–95

Dennis AB, Hellberg ME (2010) Ecological partitioning among parapatric cryptic species. Mol Ecol 19: 3206–3225

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17: 431–49

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton, N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4: 11–37

Dürr S, Watson DI (2009) Biofouling and Antifouling in Aquaculture. In: Dürr S, Thomason JC (eds) Biofouling. Chapter 19. Wiley-Blackwell, West Sussex, pp 267–87

Eno CN, Clark RA, Sanderson WG (1997) Non-native marine species in British waters: a review and directory. Joint Nature Conservation Committee, Peterborough

Fofonoff PW, Ruiz GM, Steves B, Carlton JT (2016). NEMESIS National Exotic Marine and Estuarine Species Information System. http://invasions.si.edu/nemesis/. Access Date: 26-May -2016

Geller JB, Darling JA, Carlton JT (2010) Genetic perspectives on marine biological invasions. Annu Rev Mar Sci 2: 367–393

Gomoiu MT, Alexandrov B, Shadrin N, Zaitsev Y (2002) The Black Sea - a recipient, donor and transit area for alien species. In: Leppäkoski E, Gollasch S, Olenin S (eds), Invasive aquatic species of Europe, Distribution, Impacts and Management. Dordrecht, Boston and London, Kluwer Academic Publishers, pp 341–350

Haswell WA (1883) On some new Australian tubicolous annelids. Proc Linn Soc NSW 7: 633–638

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98.

Hedgpeth JW (1950) Annotated list of certain marine invertebrates found on Texas jetties. In: Whitten HL, Rosene HF, Hedgpeth JW (eds) The invertebrate fauna of Texas coast jetties; a preliminary survey. Publications of the Institute of Marine Science, University of Texas, Texas, pp 53–87

Hirata Y, Akashige S (2004) The present situation and problems of oyster culture in Hiroshima Bay. Bull Fish Res Agen 1: 5–12

Hoos PM, Miller A W, Ruiz GM, Vrijenhoek RC, Geller JB (2010) Genetic and historical evidence disagree on likely sources of the Atlantic amethyst gem clam *Gemma gemma* (Totten, 1834) in California. Diversity Distrib 16: 582–592.

Hove HA ten, Wolf PS (1984) Chapter 55. Family Serpulidae Johnston, 1865. In: Uebelacker JM, Johnson PG (eds) Taxonomic Guide to the Polychaetes of the Northern Gulf of Mexico. Final Report to the Minerals Management Service, contract 14-12-001-29091. Barry A Vittor & Ass Inc Mobile, Alabama, pp 1–34

Katsanevakis S, Wallentinus I, Zenetos A, Leppäkoski E, Çinar ME, Oztürk B, Grabowski M, Golani D, Cardoso AC (2014) Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. Aquat Invasions 9(4): 391–423

Kupriyanova EL, Nishi E, ten Hove HA, Rzhavsky AV (2001) A review of life history patterns in Serpulimorph polychaetes: ecological and evolutionary perspectives. Oceanogr Mar Biol 39: 1–101

Kupriyanova EK, Hutchings P, Wong E (2016) A fully-illustrated web-based guide to distinguish native and introduced polychaetes of Australia. Manag Biol Invasion 7 (3): 305–312.

Kvist S (2014) Does a global DNA barcoding gap exist in Annelida? MDN. doi: 10.3109/19401736.2014.984166

Lamarck JBM de (1819) Histoire naturelle des animaux sans vertèbres. Paris: Vol. 6(1) [suite des Conchifères; Les Mollusques]. Published by the Author.

Lejeusne C, Saunier A, Petit N, Beguer M, Otani M, Carlton JT, Rico C, Green AJ (2014) High genetic diversity and absence of founder effects in a worldwide aquatic invader. Sci Rep 4: 1–9

Lewis JA, Watson C, Hove HA ten (2006) Establishment of the Caribbean serpulid tubeworm *Hydroides sanctaecrucis* Kroyer [in] Morch, 1863, in northern Australia. Biol Invasions 8: 665–671

Link H, Nishi E, Tanaka K, Bastida-Zavala R, Kupriyanova EK, Yamakita T (2009) *Hydroides dianthus* (Polychaeta: Serpulidae), and alien species introduced into Tokyo Bay, Japan. Mar Biodivers Rec 2: 1–5

Lobo J, Costa PM, Teixeira MAL, Ferreira MSG, Costa MH, Costa FO (2013) Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. BMC Ecol 13: 34. DOI: 10.1186/1472-6785-13-34

Miller LP, Harley CDG, Denny MW (2009) The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantean*. Funct Ecol 13: 756–767

Minchin D, Cook E, Clark P (2013) Alien species in British brackish and marine waters. Aquat Invasions 8(1): 3–19

Mörch OAL (1863) Revisio critica Serpulidarum. Et bidrag til rørormenes naturhistorie. Naturhistorisk Tidsskrift Henrik Krøyer, København (Ser 3) 1: 347–470

Nishi E, Tanaka K (2008) Serpulid polychaete specimens deposited in Seto Marine Biological Laboratory, Kyoto University, Seto, Wakayama, Japan. Nanki-seibutsu 50: 155–157 [in Japanese]

Nygren A, Pleijel F (2010) From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. Mol Phyl Evol 58: 132–141

Nylander JAA (2004) MrModeltest v2.2. Program Ddistributed by the Author. Uppsala University, Evolutionary Biology Centre: Uppsala, Sweden.

Oldale RN, Valentine PC, Cronin TM, Spiker EC, Blackwelder BW, Belknap DF, Wehmiller JF, Szabo BJ (1982) Stratigraphy, structure, absolute age, and paleontology of the upper Pleistocene deposits at Sankaty Head, Nantucket Island, Massachusetts. Geology 10: 246–252

Okuda S (1934) Some tubicolous annelids from Hokkaido. J Fac Sci Hokkaido Univ (Ser 6), Zool 3: 233–246

Otani M, Yamanishi R (2010) Distribution of the alien species *Hydroides dianthus* (Verrill, 1873) (Polychaeta: Serpulidae) in Osaka Bay, Japan, with comments on the factors limiting its invasion. Plankton Benthos Res 5(2): 62–68

Ozturk B (2002) The Marmara Sea, a link between the Mediterranenan and the Black Sea. In: Leppäkoski E, Gollasch S, Olenin S (eds), Invasive aquatic species of Europe, Distribution, Impacts and Management. Dordrecht, Boston and London, Kluwer Academic Publishers, pp 337–340

Pettengill JB, Wendt DE, Schug MD, Hadfield MG (2007) Biofouling likely serves as a major mode of dispersal for the polychaete tubeworm *Hydroides elegans* as inferred from microsatellite loci. Biofouling 23(3): 161–169. doi: 10.1080/08927010701218952

Qiu J, Qian P (1997) Combined effects of salinity, temperature and food on early development of the polychaete *Hydroides elegans*. Mar Ecol Prog Series 152: 79–88

Rioja E (1941) Estudios anelidológicos, 2. Observaciones de varias especies del género *Hydroides* Gunnerus (sensu Fauvel) de las costas mexicanas del Pacífico. An Inst Biol. Univ Nac Auton Mex Ser 12: 161–175

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. doi:10.1093/bioinformatics/btg180

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22: 454–64

Sciuto F, Rosso A, Sanfilippo R, Maniscalco R (2015) New faunistic data on the Pleistocene environmental evolution of the south-western edge of the Hyblean Plateau (SE Sicily). Carnets de Géologie, Notebooks on Geology 15(5): 41–57

Seebens H, Gastner MT, Blasius B (2013) The risk of marine bioinvasion caused by global shipping. Ecol Lett 16: 782–790

Streftaris N, Zenetos A, Papathanassiou E (2005) Globalisation in marine ecosystems: The story of non-indigenous marine species across European Seas. Oceangr Mar Biol 43: 419–453

Sun R, Yang D (2000) Study on *Hydroides* (Polychaeta: Serpulidae) from waters off China. I. Stud Mar Sin 42: 116–135

Sun R, Yang D (2015) Fauna Sinica of China, Invertebrata Polychaeta (III): Sabellida. Science Press, Beijing

Sun Y, Kupriyanova EK, Qiu JW (2012) CO1 barcoding of *Hydroides*: a road from impossible to difficult. Invertebr Syst 26: 539–547

Sun Y, Wong E, Hove HA ten, Hutchings PA, Williamson JE, Kupriyanova EK (2015) Revision of the genus *Hydroides* (Annelida: Serpulidae) from Australia. Zootaxa 4009 (1): 1–99

Sun Y, Wong E, Tovar-Hernández MA, Williamson JE, Kupriyanova EK (2016) Is *Hydroides brachyacantha* (Serpulidae : Annelida) a widespread species? Invertebr Syst 30(1): 41–59

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725–2729. doi:10.1093/molbev/mst197

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. Cladogram estimation. Genetics 132: 619–633

Trott TJ (2004) Cobscook Bay inventory: a historical checklist of marine invertebrates spanning 162 years. Northeast Nat 11: 261–324

Verrill AE (1873) Results of the recent dredging expeditions on the coast of New England. No. 3. Am J Sci Arts 6: 435–441

Wang J, Huang Z (1993) Fouling polychaetes of Hong Kong and adjacent waters. Asian Mar Biol 10: 1–12

Zenetos A, Gofas S, Verlaque M, Çinar ME, Garcia Raso JE et al (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. Mediterr Mar Sci 11 (2): 318–493

Zhan A, Darling JA, Bock DG, Lacoursière-Roussel A, Maclsaac HJ, Cristescu ME (2012) Complex genetic patterns in closely related colonizing invasive species. Ecol Evol 2 (7): 1331–1346

Zibrowius H (1971) Les espèces Méditerranéennes du genre *Hydroides* (Polychaeta Serpulidae). Remarques sur le prétendu polymorphisme de *Hydroides uncinata*. Téthys 2: 691–746

Zibrowius H (1973) Remarques sur trois espčces de Serpulidae acclimatées en Méditerranée: *Hydroides dianthus* (Verrill, 1873), *Hydroides dirampha* Mörch, 1863, et *Hydroides elegans* (Haswell, 1883). Rapport de la Commission internationale de la Mer Méditerranée 21: 683–686

Zibrowius H (1978) Introduction du polychète Serpulidae japonais *Hydroides ezoensis* sur la côte atlantique française et remarques sur la propagation d'autre espèces de Serpulidae. Téthys 8: 141–150

Zibrowius H, Thorp CH (1989) A review of the alien serpulid and spirorbid polychaetes in the British Isles. Cah Biol Mar 30: 271–285

Table 1. Collection information, haplotype code and Genbank Accession number of specimens used in this study.

Species	Locality code	Locality/state and country	Coordinates	Sample date	Haplotype code	Number of specimens	Registration number of individual	GenBank Accession number of individual
Hydroides dianthus	**AN**	Ancona, Italy	43°37'41.2"N, 13°29'45.9"E	2/8/2010	H_2	1	AM W.48693	
	CT	New London County, Connecticut, USA	41°17'37.3"N, 72°21'48.9"W	6/05/2010	H_1	2	AM W.46419, AM W.47934	KU051470, KU051479
	FL	SPYC Pass-A-Grille, Florida, USA	27°41'56.18"N, 82°44'7.55"W	2012	H_1	1	159291d	KU051455
		Maximo Marina, Florida, USA	27°43'25.85"N, 82°40'57.62"W	2012-2014	H_1	1	163170, 158608d	KU051460, KU051452
		MacDill Air Force Base, Florida, USA	27°51'20.56"N, 82°28'53.54"W	2012	H_1	2	159215d, 159657d	KU051454, KU051457
		St Petersburg, Florida, USA	27°46'8.76"N, 82°37'49.26"W	2014	H_1	1	154667d	KU051449
		Tampa Harbour, Florida, USA	27°53'7.58"N, 82°32'2.29"W	2012	H_1	1	159742d	KU051458
	IZ	Izmir Bay, Izmir, Turkey	38°24'25.0"N 26°59'54.6"E	9/3/2016	H_1; H_2; H17; H18; H19	1; 12; 1; 1; 1	AM W.48787; AM W.48783-AM W.48785, AM W.48786, AM W.48793-AM W.48799; AM W.48790; AM W.48792; AM W.48800	KY123247; KY123235, KY123237- KY123243, KY123245, KY123246, KY123248, KY123249; KY123250; KY123236; KY123244
	LI	Livorno port, Livorno, Italy	43°33'29.1"N 10°18'05.3"E	11/2015	H_2	3	AM W.48700-AM W.48702	
	MA	Woods Hole, Massachusetts, USA	41°31'32.6"N, 70°40'15.5"W	8/08/2013	H_1	4	AM W.42071, AM W.46411, AM W.46413, AM W.46414	KU051465, KU051467, KU051468, KU051469
	OT	Olbia port, Olbia-Tempio, Italy	40°54'46.7"N, 9°32'31.4"E	9/1/2010	H_2; H_20	2; 1	AM W.48689, AM W.48691; AM W.48688	
	QD	Qingdao, China	36°03'20.8"N, 120°25'09.5"E	1/8/2013	H_2	1	AM W.45637	KU051466

Table 1. (continued)

Species	Locality code	Locality/state and country	Coordinates	Sample date	Haplotype code	Number of specimens	Registration number of individual	GenBank Accession number of individual
	RA	Pialassa della Ravenna, Italy	44°30'27.2"N, 12°15'05.0"E	1/3/2013	H_1; H_2	1; 1	AM W.48697; AM W.48698	
	RJ	Cabo Frio, Rio de Janeiro, Brazil	22°52'42.7"S, 42°01'15.6"W	5/07/2015	H_1; H_2	12; 7	AM W.47980, AM W. 47981, AM W.47983, AM W.47984, AM W.47986-AW W.47989, AM W.47994, AM W.47996, AM W.47997, AM W.48001; AM W.47979, AM W.47985, AM W.47990, AM W.47995, AM W.47998-AM W.48000	KU051482-KU051485, KU051487- KU051490, KU051492, KU051494, KU051495, KU051499; KU051481, KU051486, KU051491, KU051493, KU051496, KU051497, KU051498
	RO	Touristic port, Rome, Italy	41°44'14.9"N 12°14'58.6"E	15/7/2015	H_2; H_16	5, 1	AM W.48665-AM W.48669; AM W.48664	
	SV	Sevastopol, Ukraine	44°37'09.5"N, 33°32'03.2"E		H_4; H_7; H_11; H_12; H_13	3; 1; 1; 1; 1	AM W.46421, AM W.48162, AM W.48163; AM W.47945; AM W.48165; AM W.48167; AM W.48170	KU051471, KU051518, KU051519; KU051480; KU051520; KU051521; KU051522
	TX	Galveston, Texas, USA	29°15'36.6"N, 94°54'34.0"W	21/05/2015	H_4; H_5; H_6; H_8; H_9; H_10	12; 1; 1; 2; 1; 2	AM W.47775-AM W.477778, AM W.47780, AM W.47783, AM W.48151, AM W.48153- AM W.48155, AM W.48159; AM W.48161; AM W.47774; AM W.47779; AM W.48152, AM W.48157; AM W.48156; AM W.48158, AM W.48160	KU051473-KU051476, KU051478, KU051506, KU051507, KU051509-KU051511, KU051515; KU051517; KU051472; KU051477; KU051509, KU051513; KU051512; KU051514, KU051516
	TY	Odaiba, Tokyo Bay, Tokyo, Japan	35°38'04.5"N, 139°46'28.6"E	27/12/2015	H_1; H_2	4; 3	AM W.48399, AM W.48401, AM W.48402. AM W.48404; AM W.48396, AM W.48400, AM W.48403	

Table 1. (continued)

Species	**Locality code**	**Locality/state and country**	**Coordinates**	**Sample date**	**Haplotype code**	**Number of specimens**	**Registration number of individual**	**GenBank Accession number of individual**
	VA	Dare Marina, Virginia, USA	37°11'0.40"N, 76°25'23.27"W	2014	H_1	1	158644d	KU051453
		Kiptopeke State Park, Virginia, USA	37° 9'58.89"N, 75°59'20.58"W	2012	H_1	1	159826d	KU051459
		Little Creek Marina, Virginia, USA	36°55'23.79"N, 76°11'10.32"W	2014	H_1; H_3	1; 1	302396d; 302666d	KU051462; KU051463
		Long Bay Pointe, Virginia, USA	36°54'15.39"N, 76° 4'17.58"W	2014	H_1	3	155123d, 155124d, 302676d	KU051450, KU051451, KU051464
		York River Haven, Virginia, USA	37°15'22.42"N, 76°28'45.96"W	2012	H_1	1	159513d	KU051456
	VE	Venetian Arsenal, Venice, Veneto, Italy	45°26'05.6"N, 12°20'59.5"E	25/1/2016	H_1; H_2; H14; H15	1; 3; 1; 1	AM W.48628; AM W.48632, AM W.48634, AM W48638; AM W48629; AM W48637	
	VM	Village Marina, Florida, USA	27°29'44.8"N 80°17'49.9"W	2014	H_1	1	163348	KU051461
Hydroides sanctaecrucis		Darwin, Northern Territory, Australia	12°27'07.9"S, 130°49'10.3"E	9/05/2014		3	AM W.46567, AM W.46568, AM W.46569	KU051505, KU051503, KU051504
		St Petersburg, Florida, USA	27°46'8.76"N, 82°37'49.26"W	2014		3	154666, 154671, 158616d	KU051448, KU051501, KU051502
		Tolo Harbour, Hong Kong, China				1	AM W.40548	JQ885944
		Mazatlan, Mexico				1	AM W.40549	JQ885943
Hydroides elegans		Sydney, New South Wales, Australia				1	AM W.46406	KU051500
Hydroides ezoensis		Vostok Bay, Vladivostok, Russia				1	AM W.40544	JQ885951

Table 2. Genetic distances (based on p-distance) among (below diagonal) and within (italics along diagonal) *H. dianthus* populations from different locations for the COI gene. “n/c” indicates unattainable calculation due to insufficient sequences.

	AN	**CT**	**FL**	**IZ**	**LI**	**MA**	**OT**	**QD**	**RA**	**RJ**	**RO**	**SV**	**TX**	**TY**	**VA**	**VE**	**VM**
AN	*n/c*																
CT	0.003	*0.000*															
FL	0.003	0.000	*0.000*														
IZ	0.000	0.003	0.003	*0.003*													
LI	0.001	0.004	0.004	0.001	*0.000*												
MA	0.003	0.000	0.000	0.004	0.003	*0.000*											
OT	0.001	0.004	0.004	0.002	0.001	0.004	*0.002*										
QD	0.000	0.003	0.003	0.001	0.000	0.003	0.001	*n/c*									
RA	0.001	0.001	0.001	0.002	0.001	0.001	0.002	0.001	*0.003*								
RJ	0.002	0.001	0.001	0.003	0.002	0.001	0.003	0.002	0.001	*0.001*							
RO	0.000	0.003	0.003	0.002	0.000	0.003	0.001	0.000	0.002	0.002	*0.001*						
SV	0.058	0.055	0.055	0.058	0.058	0.055	0.058	0.058	0.056	0.056	0.058	*0.006*					
TX	0.057	0.054	0.054	0.058	0.057	0.054	0.058	0.057	0.055	0.055	0.057	0.004	*0.003*				
TY	0.002	0.001	0.001	0.003	0.002	0.001	0.002	0.002	0.001	0.001	0.002	0.056	0.055	*0.002*			
VA	0.003	0.000	0.000	0.004	0.003	0.000	0.004	0.003	0.002	0.001	0.003	0.055	0.054	0.001	*0.002*		
VE	0.002	0.003	0.003	0.003	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.058	0.057	0.002	0.003	*0.001*	
VM	0.003	0.000	0.000	0.004	0.003	0.000	0.004	0.003	0.001	0.001	0.003	0.055	0.054	0.001	0.000	0.003	*n/c*

Chapter 6

Another exception of conserved gene order in Annelida: Mitogenomics reveals extensive gene rearrangement in the tubeworm genus *Hydroides* (Serpulidae)

Authors:
Yanan Sun[1, 2], Jian-Wen Qiu[3], Jane E. Williamson[2], Elena K. Kupriyanova[1, 2]

[1] *Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia*.
[2] *Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia*.
[3] *Department of Biology, Hong Kong Baptist University, 224 Waterloo Road, Hong Kong, China*.

Abstract

Herein we report sequences of eight mitochondrial genomes of *Hydroides,* including complete ones of *H. elegans* and *H. ezoensis* and six partial ones, which show considerable differences from previously reported annelid mitochondrial genomes. The complete mitochondrial genomes of *Hydroides* spp. consist of 38 genes, including 12 protein coding genes, two rRNA genes, and 24 tRNA genes. In contrast to most bilaterian metazoa, the atp8 gene is missing in *Hydroides*, which is also the first observed absence of atp8 gene ever recorded in annelids. Due to the numerous non-coding regions, mitochondrial genomes of *Hydroides* are larger in size than those of most annelids. The mitochondrial gene order in *Hydroides* shows a completely different pattern from that reported for any other annelid, thus illustrating higher variability of gene order in Sedentaria than previously thought. Species of *Hydroides* are recovered with long branches in phylogenetic analyses, which are probably correlated with nucleotide biases and intensive gene rearrangement.

1. Introduction

Annelida, also known as segmented worms, is a highly diverse group of Lophotrochozoa with over 21000 described species (Weigert et al., 2014). As a group possessing segmentation and a ladder-like nervous system, it has been central in reconstructing pathways of animal evolution (Rivera and Weisblat, 2009; Shain, 2009). In contrast to the traditional classification, which divides annelids into Polychaeta and Clitellata (oligochaetes and leeches), and which is supported by morphological-cladistic analyses, modern phylogenomic analyses classifies annelids into the clades Errantia and Sedentaria, which form the Pleistoannelida, and five additional basal branching families: Sipuncula, Amphinomidae, Chaetopteridae, Oweniidae and Magelonidae (Struck et al., 2011; Weigert et al., 2014; Struck et al., 2015). The tubeworm family Serpulidae is typically recognised by the presence of calcareous tubes as well as a radiolar crown, usually bearing a distal operculum, and is classified along with sessile annelid families in Sedentaria, thus representing a family with a filter-feeding lifestyle (Struck et al., 2011).

Mitochondrial genomes have been widely used in inferring phylogenetic relationships at different taxonomic levels in Bilateria (reviewed in Bernt et al., 2013a). The Metazoan mitochondrial genome is typically conserved in gene content and length because of the important role mitochondria play in cell metabolism. Generally, the metazoan genomes are circular (except in cnidarians (Bridge et al., 1992) and sponges (Lavrov et al., 2013)), 14 to 20 kb in length, and consist of 13 protein coding genes (nad1–6, nad4L, cox I–III, cob, atp6/8), 22 tRNAs (trnX), two rRNAs (rrnS, rrnL), and a control region (CR), which is also recognised as an AT-rich non-coding region (NCR) related to the origin of replication (Boore, 1999; Li et al., 2015; Sahyoun et al., 2014). The mitochondrial genome offers a broad range of characters for analyses with respect to genome architecture, gene arrangement, genetic code and secondary structure of RNA genes (Boore, 1999; Boore and Brown, 2000; Macey et al., 2000; Telford et al., 2000; Bernt et al., 2013a, b). Gene rearrangements, especially such as inversions, transpositions, and tandem duplication random losses (Sahyoun et al., 2014), have been proven as extremely reliable characters in phylogenetic studies of higher taxa because of the low frequency of homoplastic translocations in closely related taxa (Kilpert and Podsiadlowski, 2006; Kilpert et al., 2012).

The gene order within Annelida has been observed to be relatively well conserved, especially in the Pleistoannelida, representing either the putative annelid ground pattern, or a few gene rearrangements from the conserved pattern (Boore, 2004; Jennings and Halanych, 2005; Bleidorn et al., 2006; Vallés and Boore, 2006; Zhong et al., 2008; Mwinyi et al., 2009; Shen et al., 2009; Wu et al., 2009; Golombek et al., 2013; Li et al., 2015). Nevertheless, recent studies have revealed more variable gene order patterns, particularly when lineages from the basal branching families are included (Weigert et al., 2016). Another observed exception to the conserved gene order is the family Syllidae belonging to the clade Errantia, which has a mitochondrial genome gene order completely deviating from the putative ground pattern (Aguado et al., 2015; 2016).

To date, approximately 90 mitochondrial genomes of annelids have become available, representing lineages of Errantia, Sedentaria and their basal branches. However, the vast majority of annelids are still unrepresented. The mitochondrial genomes of Serpulidae, which comprises more than 500 species and represents the suspension feeder lineages in Sedentaria, are not yet available. Hence, in this study eight new mitochondrial genomes of species from *Hydroides*, the largest genus of Serpulidae, were generated using Illumina sequencing. We investigated the mitochondrial genome features of *Hydroides* and compared them with the available mitochondrial genomes of annelids. This study provided new insights into mitochondrial genome rearrangements within annelids.

2. Materials and methods

2.1. Specimen collection and mitochondrial genome sequencing

Specimens of *H. dirampha*, *H. elegans*, *H. ezoensis*, *H. homoceros*, *H. minax*, *H. operculata*, *H. pseudouncinata*, and *H. sanctaecrucis*, were collected for mitochondrial sequencing. Collecting information of specimens of the eight species of *Hydroides* is outlined in Table 1. All specimens were fixed and preserved in 96% ethanol following collection, and deposited in the Australian Museum. Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocols. For *H. elegans* and *H.*

dirampha, sequencing libraries of total genomic DNA preparation were processed by the Beijing Genomics Institute (BGI) in Shenzhen, China, then sequencing of the library was performed as 100 bp paired-end runs on an Illumina Hi-Seq 2000 by BGI. For another six specimens the preparation of sequencing libraries of total genomic DNA were performed by the Australian Genome Research Facility (AGRF) in Melbourne, Australia, and the sequencing of libraries as 100 bp paired-end runs on an Illumina Hi-Seq 2000 by AGRF.

Table 1. Collection information of specimens used for sequencing

AM REGISTRATION	SPECIES	LOCALITY
pending	*Hydroides dirampha*	Hong Kong, China
pending	*Hydroides elegans*	Hong Kong, China
W48019	*Hydroides ezoensis*	Sydney, Australia
K25	*Hydroides homoceros*	Al-Doha, Kuwait
W45420	*Hydroides minax*	Lizard Island, Australia
K24	*Hydroides operculata*	Al-Doha, Kuwait
W47950	*Hydroides pseudouncinata*	Santa Tecla, Italy
W46567	*Hydroides sanctaecrucis*	Darwin, Australia

2.2. Mitochondrial genome assemblies and annotation

De novo assemblies were conducted with CLC Genomics Workbench 7.0 (CLCbio, Arhus, Denmark) using default settings. To identify putative mt contigs, BLASTN searches (Altschul et al., 1997) were performed on contigs produced using the protein and two rRNA sequences from published annelid mitochondrial genomes as database sequences. The coverage of mt genomes was estimated by mapping sequence read back to the contigs comprising the mt genome in CLC workbench. The top-hitting contigs identified by BLASTN recovered the entire mitochondrial genomes of two species: *Hydroides elegans* and *H. ezoensis*. For the remaining six species, however, two or three partial contigs were recovered, respectively.

Annotation of the mitochondrial genomic contigs was conducted with MITOS web server under the mitochondrial code for invertebrate mitochondria (Bernt et al., 2013c), including the protein coding genes and the secondary structure of tRNAs and rRNAs. Gene boundaries generated from automatic annotations were manually examined and adjusted, and the tRNAs identified by MITOS were rechecked via the tRNAscan-SE web server (Lowe and Eddy, 1997; Schattner et al., 2005). Circular representation of the mitochondrial genomes was drawn using CGView (Stothard and Wishart, 2005).

2.3. Gene order and phylogenetic analyses

The pairwise comparisons of the mitochondrial gene order were conducted using CREx (Bernt et al., 2007), which determine the most parsimonious genome rearrangement scenario including transpositions, reverse transpositions, reversals, and tandem-duplication-random-loss (tdrl) events. The analysis was conducted using the common intervals parameter for distance measurement and included the gene order of the protein-coding and rRNA genes, while tRNAs were excluded because they are usually more variable than other genes.

AT and GC skew were determined for the complete mt genomes according to the formulae defined by Perna and Kocher (1995), $\mathrm{AT\ skew} = (\mathrm{A} - \mathrm{T})/(\mathrm{A} + \mathrm{T})$ and $\mathrm{GC\ skew} = (\mathrm{G} - \mathrm{C})/(\mathrm{G} + \mathrm{C})$, where negative values in skewness mean that the coding strand is enriched for T or C, while positive values infer more As and Gs.

Phylogenetic analyses were based on a dataset of 12 protein-coding genes (ATP 8 was excluded). The dataset comprised 30 taxa, including one taxon from each of bryozoans, brachiopods, nemerteans, and phoronids; two mollusc taxa; and 15 annelid taxa representing all families with complete mitochondrial genomes available. Protein sequences of mitochondrial genes were aligned independently and then concatenated using Bioedit v7.2.5 (Hall, 1999). The best fitting evolution model for the protein dataset was determined using the IQ-TREE version 1.3.4 (Nguyen et al., 2015), which suggested the MtZoa +G +F model that was specifically designed for lophotrochozoan mitochondrial genomes (Rota-Stabelli et al., 2009). The maximum likelihood analysis was performed with IQ-TREE version 1.3.4 (Nguyen et al., 2015). Bootstrap support values were generated with a rapid bootstrapping algorithm ultrafast bootstrap (UFBoot) (Minh et al., 2008) for 1000 replicates in IQ-TREE.

3. Results

Two complete mitochondrial genomes were obtained for *H. elegans* and *H. ezoensis* as a single contig of 19,004 bp and 18,363 bp in length, respectively. Partial genome sequences were detected as one to three contigs, with total length of contigs varying from 15,145 to 18,333 bp (Table 2). The circular genomes of *H. elegans* and *H. ezoensis* are illustrated in Fig. 1. Both complete mitochondrial genomes contain 12 protein-coding genes, two rRNA genes, and 24 tRNA genes. Atp8 was not detected in either of the two completed genomes, nor in any of the partial genome contigs. All mitochondrial genes sequenced herein were transcribed from the same strand. A combination of the start codons ATA, ATG and GTT were used, with a bias towards ATG in all species except for *H. elegans*, in which ATA is used as an initiation codon. Most genes ended with the stop codon TAA or TAG, but an incomplete termination codon, either a single T or TA, was observed for nad2 and nad6 in *H. ezoensis*.

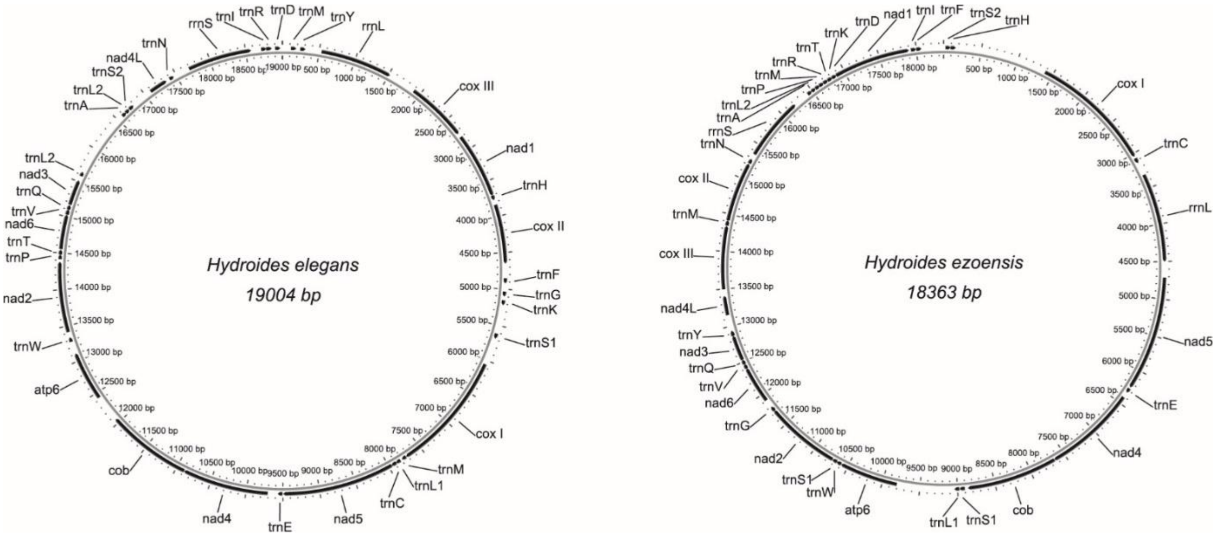


Fig. 1. Circular representation of the mitochondrial genomes of H. elegans *and* H. ezoensis. *Genes are transcribed clockwise. Abbreviations are as explained in the text. Non-coding genes are shown as gaps.*

Two copies of trnM and trnL2 (TAA) were found in *H. elegans*, while two copies of trnM and trnS1 (TCT) were found in *H. ezoensis*. Duplication of tRNAs were also detected in partial genomes, such as two copies of trnaL1 and trnS2 (TCA) in *H. dirampha*, two copies of trnM in *H. homoceros*, and a tandem-duplication of tRNA block trnL2-trnP-trnR-trnT in *H. sanctaecrucis* (Fig. 2).

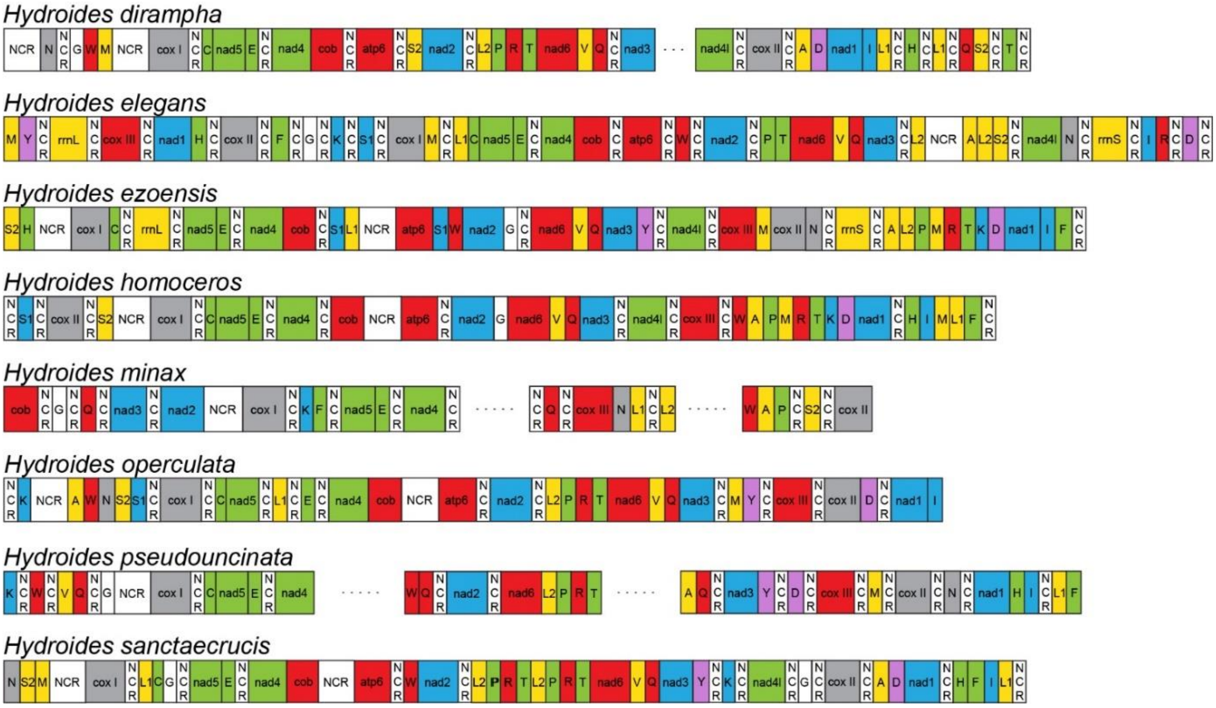


Fig. 2. Gene order of mitochondrial genomes from eight species of Hydroides. *Abbreviations are as explained in the text. Dashed lines with ellipses indicate uncovered regions. Difference colours indicate putative conserved gene clusters of annelids.*

All eight mitochondrial genomes included numerous intergenic regions inserted between genes. In *H. elegans*, 21 non-coding regions were detected, including 14 non-coding regions more than 100 bp. The largest non-coding region of 935 bp was assigned between trnL2 and trnA. In *H. ezoensis*, 20 non-coding regions were assigned, with seven regions longer than 100 bp. Two large non-coding regions of 1,235 bp and 770 bp were assigned between trnH and cox I, and between trnL1 and atp6, respectively. In the six partial genomes, 13 to 17 non-coding regions were detected (Fig. 2). An unassigned region ranging from 117 bp to 153 bp, which did not hit any sequences via BLAST search, was found inserted in the middle of cox II gene in all eight species.

Mitochondrial genomes of *Hydroides* exhibited nucleotide and codon biases. All investigated genomes were AT-rich (range from 54.6% to 63.2%). In all examined species, T was the most common base (35.4 % to 37.1%), followed by G (22.6% to 32.7%), and C was the least common base (11% to 14.2%) on the coding strand. The AT-skew was positive, while GC-skew was negative in all *Hydroides* spp. (Table 2).

The maximum likelihood analyses (ML) recovered *Hydroides* as a monophyletic group with high support value (UFBoot = 100), and place it in a clade with Echiura with low support value (UFBoot = 37) (Fig. 3). The *Hydroides* clade showed an extremely long branch in the tree.

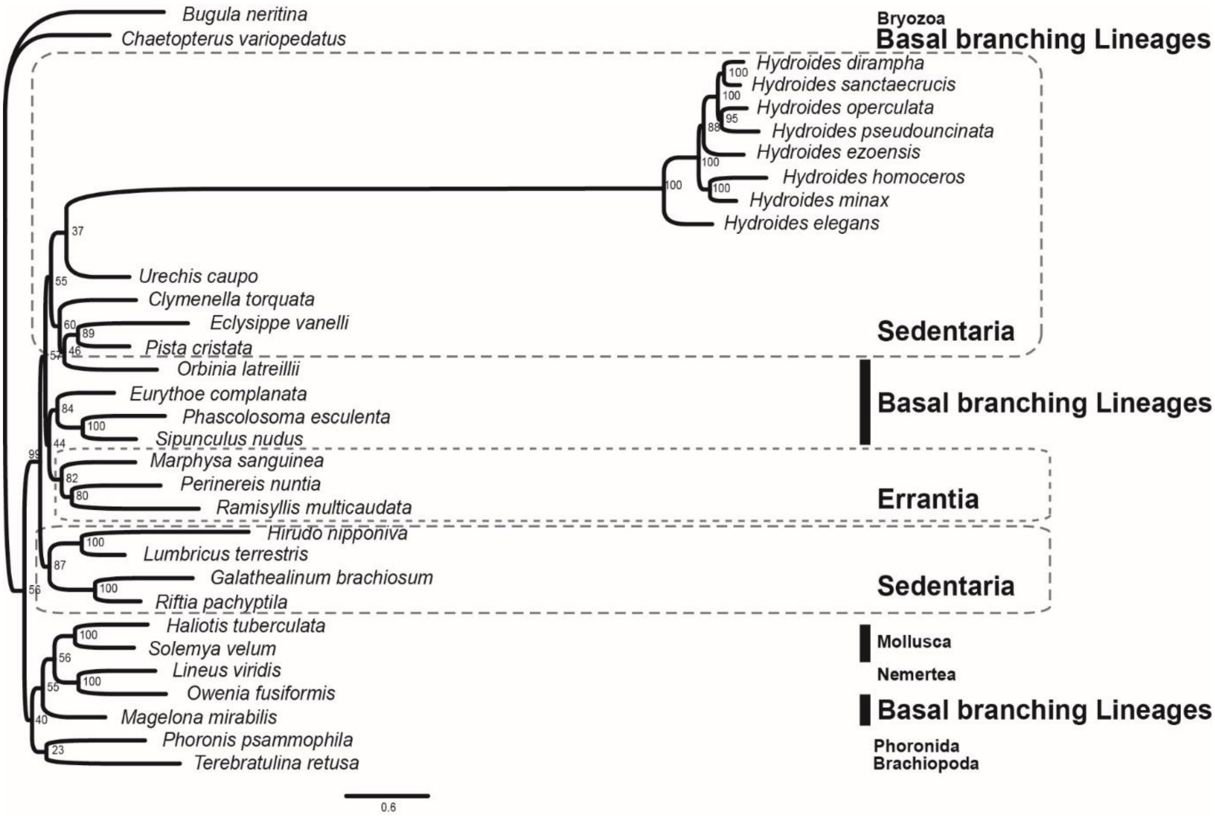


Fig. 3. Maximum likelihood tree based on amino acid sequences of the protein coding genes and rrnL and rrnS, ambiguous regions excluded from all the genes. Bootstrap support values are indicated above nodes.

The gene order of all eight mitochondrial genomes of *Hydroides* differed significantly from each other and showed high variability compared to conserved gene orders from the other annelids reported (Fig. 4). Within *Hydroides*, only two gene blocks, the arrangement of nad6-trnV-trnQ-nad3 and nad5-trnE-nad4-cob, were conserved. The tRNA genes showed a high variability in locations, and they were thus excluded in the rearrangement scenarios reconstruction. The CREx analyses revealed that the difference between *H. elegans* and *H. ezoensis* could be explained by two tdrl events, while the difference between *H. elegans* and the putative annelid ground pattern could be explained by two transpositions, two tdrl, one reverse transposition and one reverse. Differences between *H. ezoensis* and the putative annelid ground pattern was due to two transpositions and three tdrl. The gene order distance obtained from CREx analysis among *Hydroides* and other annelids ranges from 0 to 10 (Table 3).

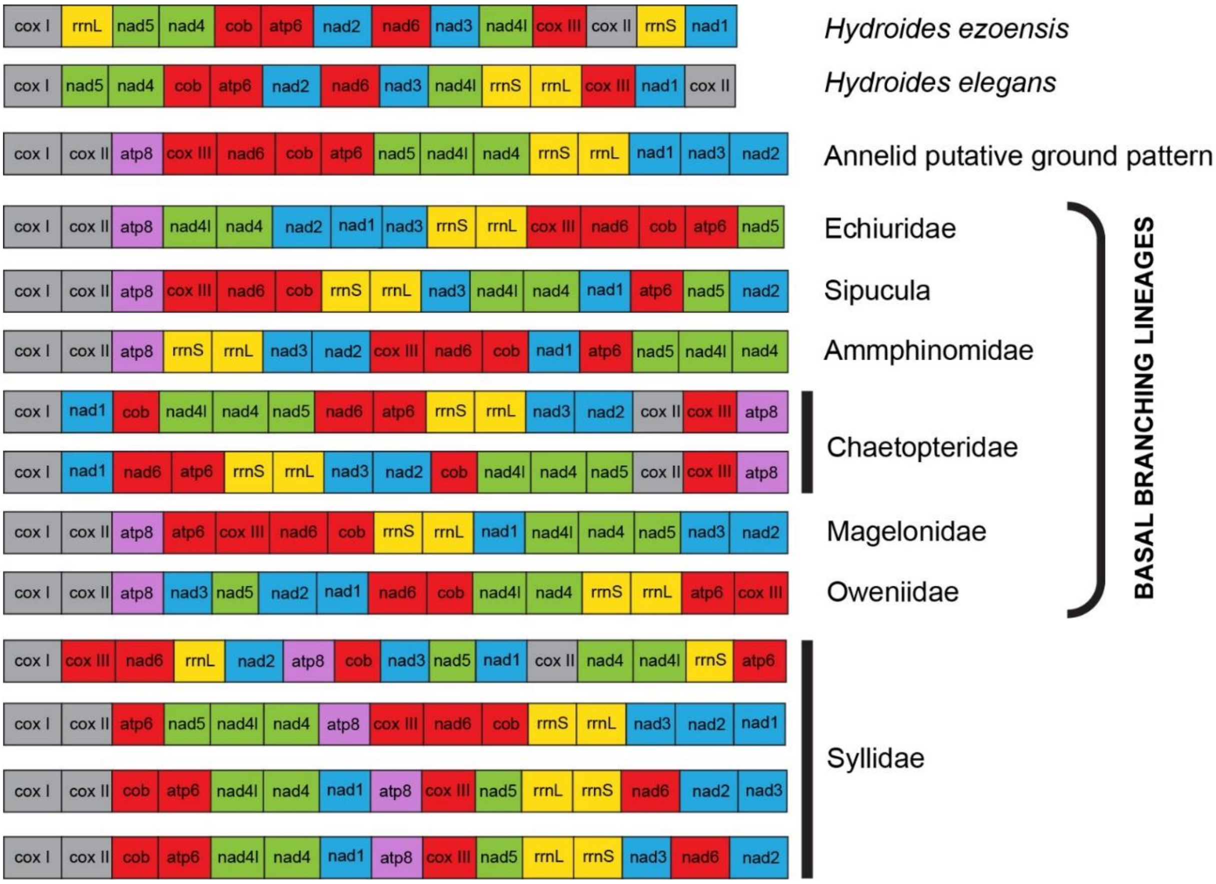


Fig. 4. Comparison of gene orders on Hydroides *and annelids putative ground pattern and other reported annelids. Only protein coding gene and rRNAs included. Genomes are arbitrarily linearized at cox I after Boore and Brown (2000). Different colours indicate putative conserved gene clusters.*

Table 2. Mitochondrial genome length, nucleotide contents and nucleotide skew.

Species	**Number of mt contig(s) recovered**	**Total mt genome length (bp)**	**A (%)**	**T (%)**	**C (%)**	**G (%)**	**A+T (%)**	**C+G (%)**	**AT skew**	**GC skew**
Hydroides dirampha	2	18333	25.14	36.78	13.48	24.6	61.92	38.08	-0.19	0.29
Hydroides elegans	1	19904	19.91	36.18	14.68	29.23	56.09	43.91	-0.29	0.33
Hydroides ezoensis	1	18363	21.89	35.72	14	28.38	57.62	42.38	-0.24	0.34
Hydroides homoceros	1	16301	20.01	35.92	13.37	30.7	55.93	44.07	-0.28	0.39
Hydroides minax	3	15145	18.68	37.09	12.8	31.43	55.77	44.23	-0.33	0.42
Hydroides operculata	1	16443	19.17	35.42	12.72	32.68	54.59	45.41	-0.30	0.44
Hydroides pseudouncinata	3	13233	20.06	38.3	10.98	30.67	58.36	41.64	-0.31	0.47
Hydroides sanctaecrucis	1	15170	26.51	36.66	14.19	22.64	63.17	36.83	-0.16	0.23

Table 3. Matrix of gene order distance measure obtained from CREx analysis. Abbreviation: EZ – *Hydroides ezoensis*, EL – *Hydroides elegans*, AP – Annelid putative ground pattern, A – Amphinomidae, P – Phyllochaetopterus, C – Chaetopteridae, M – Magelonidae, O – Oweniidae, Ra – *Ramisyllis*, Tr – *Trypanobia*, E – Echiuridae, S – Sipuncula, U – Urechis, Ty – *Typosyllis*, St – *Steptosyllis*.

	EZ	EL	AP	A	P	C	M	O	Ra	Tr	E	S	U	Ty	St
Hydroides ezoensis	176	56	6	2	6	6	2	0	8	10	0	4	2	0	2
Hydroides elegans	56	176	10	6	8	12	8	6	12	14	6	10	18	4	8
Annelid putative ground pattern	6	8	204	24	24	16	48	22	32	22	48	42	30	4	50
Amphinomidae	4	4	24	204	22	16	22	16	8	8	22	30	28	4	44
Phyllochaetopterus	6	6	24	22	204	68	10	12	8	8	6	16	6	4	20
Chaetopteridae	6	10	16	16	68	204	10	14	8	8	6	14	6	4	22
Magelonidae	2	6	48	22	10	10	204	26	14	12	82	40	24	6	28
Oweniidae	0	6	22	16	12	14	26	204	6	6	26	18	16	10	10
Ramisyllis	10	14	32	8	8	8	14	6	204	154	14	16	8	2	12
Trypanobia	10	14	22	8	8	8	12	6	154	204	12	22	12	2	12
Echiuridae	0	4	48	22	6	6	82	26	14	12	204	38	42	10	28
Sipuncula	4	8	42	30	16	14	40	18	16	22	38	204	26	4	62
Urechis	2	18	30	28	6	6	24	16	8	12	42	26	204	6	32
Typosyllis	0	4	4	4	4	4	6	10	2	2	10	4	6	204	4
Steptosyllis	2	6	50	44	20	22	28	10	12	12	28	62	32	4	204

4. Discussion

The mitochondrial genome of *Hydroides* is substantially larger than those reported for other polychaetes, with the exception of the deep-sea tubeworm *Siboglinum fiordicum* (19,502 bp; Li et al., 2015), which contains numerous large intergenic non-coding regions. Multiple large (over 50 bp) non-coding regions among genes are generally uncommon in annelids, but have been reported for *Owenia* (see Weigert et al., 2016) and *Typosyllis* (Aguado et al., 2016). Internal spacers with long inverted repeats have been interpreted as an extra origin of replication (Wang et al., 2013; Pons et al., 2014), but in some cases spacers could be remnants of the duplication process produced by tdrl events, slipped strand mispairing or imprecise termination during replication (Bernt et al., 2013a; Aguado et al., 2016). However, BLAST searches did not recover any repeats in internal spacers within the respective mitochondrial genomes of *Hydroides*. Another feature of *Hydroides* mitochondrial genomes that has not been observed in annelids to date is the loss of an atp8 gene. The loss of this gene has been reported in some representatives of Platyhelminthes, Acoelomorpha, Acanthocephala, Chaetognatha, Mollusca and Nematode (Boore et al., 2004; Papillon et al., 2004; Steinauer et al., 2005; Valles and Boore, 2006; Mwinyi et al., 2010). It thus appears that the loss of atp8 is a dispersed case in all major metazoan clades (Bernt et al., 2013a). However, small and fast evolving genes such as atp8 could make the annotation process complex (Bernt et al., 2013a). Thus, we cannot determine if the atp8 was lost during evolution or missed in the annotation process because of methodological restrictions.

Most animal mitochondrial genomes show substantial strand asymmetry in nucleotide composition, which is probably due to mutational constraints during the asymmetry replication process (Perna and Kocher, 1995; Hassanin et al., 2005). There is a strong negative correlation between AT and GC skew observed in bilaterian mitochondrial genomes, represented by two clusters: one with a positive GC skew and a clearly negative AT skew, the other with a predominantly negative GC skew and a positive or moderately negative AT skew (Bernt et al., 2013b). Phylogenetic studies based on more than 650 bilaterian mitochondrial genomes show that phylogenies including long-branching taxa, such as Nematoda, Platyhelminthes, and Tunicata, have been accompanied by a highly positive GC skew and a highly negative AT skew, as well as a reversal of strand bias (Bernt et al., 2013b). Mitochondrial genomes of *Hydroides* show a highly positive GC skew and a negative AT skew, which correlate with the accelerated substitution rate, a high frequency of gene rearrangement, and a long branch in the tree, thus serving as a possible source of misleading phylogenetic signals (Bernt et al., 2013b).

Although the conserved gene order of the annelid mitochondrial genome was suggested to be restricted within Pleistoannelida (Weigert et al., 2016), the recently reported considerable gene rearrangement for Syllinae (Aguado et al., 2016), as well as herein for *Hydroides*, indicate that the mitochondrial gene orders within both Errantia and Sedentaria could be more diverse than expected. This should not be surprising considering that only limited lineages have been investigated in these highly diverse groups. A high degree of gene rearrangement has been found in Tunicata, Nematoda, Mollusca and Arthropoda (Hu et al., 2003; Endo et al., 2005; Akasaki et al., 2006; Iannelli et al., 2007; Rawlings et al., 2010; Stach et al., 2010; Yuan et al., 2012; Luo et al., 2015). The considerable gene rearrangement in *Hydroides*, together with the long branches of *Hydroides* in the phylogenetic tree (Fig. 3), supports the hypothesis that a complete shuffling of a mitochondrial genome correlated with high substitution rates is likely (Bernt et al., 2013b). Anoxic environments, high metabolic rates, short generation times, and bottleneck effects associated with low effective population size, have been speculated to affect substitution rates of mitochondrial genomes, thus resulting in significant gene rearrangements (Martin, 1995; Mueller and Boore, 2005; Min and Hickey, 2008). *Hydroides* are usually not confronted with anoxic conditions. A high metabolic rate, as indicated by a high level of protein expression involved in metabolism (Zhang et al., 2014), might be a partial explanation for the fast evolution of *Hydroides* mitochondrial genomes. Further studies on cellular metabolism and mitochondrial genetic machineries are needed to provide further insights on possible reasons for the more variable gene order in some taxa such as *Hydroides* than in others.

Abbreviations

nad1-6, 4L: NADH dehydrogenase subunits 1-6 and 4L
cox I–III: cytochrome oxidase subunits I–III
cob: cytochrome b
atp6/8: ATP synthase subunit 6/8
rrnL: large ribosomal RNA
rrnS: small ribosomal RNA
NCR: non-coding region. Bp: base pair(s)
mt: mitochondrial
rRNA: ribosomal RNA
tRNA: transfer RNA
Single letters of tRNAs encoding for amino acids: alanine (A), cysteine (C), aspartic acid (D), glutamatic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L1 and L2), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S1 and S2), threonine (T), valine (V), tryptophan (W), tyrosine (Y)

Acknowledgements

The study was funded by Australian Biological Resource Survey (ABRS) grant RF213-19 to EKK. We thank the Department of Biological Sciences at Macquarie University for an International Macquarie University Research Excellence Scholarship (iMQRES) awarded to YS. We are grateful to Francesco Criscione who helped with sample collection in Greece. We also thank Yanjie Zhang from Hong Kong Baptist University and Andre Minoche from Garvan Institute of Medical Research for their contribution to fixing problems during the genome assembly, Kenneth Halanych from Auburn University for his suggestions on genome assembly and his contribution of reference mitochondrial sequences for local BLAST searches, as well as Don Colgan and Andrew King from Australian Museum for their suggestions on assembly software and annotation.

Reference

Aguado, M.T., Glasby, C.J., Schroeder, P., Weigert, A., Bleidorn, C., 2015. The making of a branching annelid: an analysis of complete mitochondrial genome and ribosomal data of *Ramisyllis multicaudata*. Sci. Rep. 5, 12072.

Aguado, M. Teresa, Richter, S, Sontowski, R., Golombek, A., Struck, T.H., Bleidorn, C., 2016. Syllidae mitochondrial gene order is unusually variable for Annelida. Gene 594(1), 89–96.

Akasaki, T., Nikaido, M., Tsuchiya, K., Segawa, S., Hasegawa, M., Okada, N., 2006. Extensive mitochondrial gene arrangements in coleoid cephalopoda and their phylogenetic implications. Mol. Phylogenet. Evol. 38, 648–658.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25, 3389–3402.

Bernt, M., Braband, A., Schierwater, B., Stadler, P.F., 2013a. Genetic aspects of mitochondrial genome evolution. Mol. Phylogenet. Evol. 69, 328–338.

Bernt, M., Bleidorn, C., Braband, A., Dambach, J., Donath, A., Fritzsch, G., Golombek, A., Hadrys, H., Juhling, F., Meusemann, K., Middendorf, M., Misof, B., Perseke, M., Podsiadlowski, L., von Reumont, B., Schierwater, B., Schlegel, M., Schrodl, M., Simon, S., Stadler, P.F., Stoger, I., Struck, T.H., 2013b. A comprehensive analysis of bilaterian mitochondrial genomes and phylogeny. Mol. Phylogenet. Evol. 69, 352–364.

Bernt, M., Donath, A., Juhling, F., Externbrink, F., Florentz, C., Fritzsch, G., Putz, J., Middendorf, M., Stadler, P.F., 2013c. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 69, 313–319.

Bernt, M., Merkle, D., Ramsch, K., Fritzsch, G., Perseke, M., Bernhard, D., Schlegel, M., Stadler, P.F., Middendorf, M., 2007. CREx: inferring genomic rearrangements based on common intervals. Bioinformatics 23, 2957–2958.

Bleidorn, C., Podsiadlowski, L., Bartolomaeus, T., 2006. The complete mitochondrial genome of the orbiniid polychaete *Orbinia latreillii* (Annelida, Orbiniidae) – a novel gene order for Annelida and implications for annelid phylogeny. Gene 370, 96–103.

Bridge, D., Cunningham, C.W., DeSalle, R., Buss, L.W., 1995. Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. Mol. Biol. Evol. 12(4), 679–689.

Boore, J.L., 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27, 1767–1780.

Boore, J.L., 2004. Complete mitochondrial genome sequence of *Urechis caupo*, a representative of the phylum Echiura. BMC Genom. 5, 67.

Boore, J.L., Brown, W.M., 2000. Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate the Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17, 988.

Boore, J.L., Medina, M., Rosenberg, L.A., 2004. Complete sequences of the highly rearranged molluscan mitochondrial genomes of the Scaphopod *Graptacme eborea* and the bivalve *Mytilus edulis*. Mol. Biol. Evol. 21, 1492–1503.

Endo, K., Noguchi, Y., Ueshima, R., Jacobs, H.T., 2005. Novel repetitive structures, deviant protein-encoding sequences and unidentified ORFs in the mitochondrial genome of the brachiopod *Lingula anatina*. J. Mol. Evol. 61, 36–53.

Golombek, A., Tobergte, S., Nesnidal, M.P., Purschke, G., Struck, T.H., 2013. Mitochondrial genomes to the rescue – Diurodrilidae in the myzostomid trap. Mol. Phylogenet. Evol. 68, 312–326.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.

Hassanin, A., Leger, N., Deutsch, J., 2005. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. Syst. Biol. 54, 277–298.

Hu, M., Chilton, N.B., Gasser, R.B., 2003. The mitochondrial genome of *Strongyloides stercoralis* (Nematoda) – idiosyncratic gene order and evolutionary implications. Int. J. Parasitol. 33, 1393–1408.

Iannelli, F., Griggio, F., Pesole, G., Gissi, C., 2007. The mitochondrial genome of *Phallusia mammillata* and *Phallusia fumigata* (Tunicata, Ascidiacea): high genome plasticity at intra-genus level. BMC Evol. Biol. 7, 155.

Jennings, R.M., Halanych, K.M., 2005. Mitochondrial genomes of *Clymenella torquata* (Maldanidae) and *Riftia pachyptila* (Siboglinidae): evidence for conserved gene order in Annelida. Mol. Biol. Evol. 22, 210–222.

Kilpert, F., Held, C., Podsiadlowski, L., 2012. Multiple rearrangements in mitochondrial genomes of Isopoda and phylogenetic implications. Mol. Phylogenet. Evol. 64, 106–117.

Kilpert, F., Podsiadlowski, L., 2006. The complete mitochondrial genome of the common sea slater, *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region features. BMC Genomics 7, 241.

Lavrov, D.V., Pett, W., Voigt, O., Worheide, G., Forget, L., Lang, B.F., Kayal, E., 2013. Mitochondrial DNA of *Clathrina clathrus* (Calcarea, Calcinea): six linear chromosomes, fragmented rRNAs, tRNA editing, and a novel genetic code. Mol. Biol. Evol. 30, 865–880.

Li, Y., Kocot, K.M., Schander, C., Santos, S.R., Thornhill, D.J., Halanych, K.M. 2015. Mitogenomics reveals phylogeny and repeated motifs in control regions of the deep-sea family Siboglinidae (Annelida). Mol. Phylogenet. Evol. 85, 221–229.

Lowe, T.M., Eddy, S.R., 1997. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25, 955–964.

Luo, Y.J., Satoh, N., Endo, K., 2015. Mitochondrial gene order variation in the brachiopod *Lingula anatina* and its implications for mitochondrial evolution in lophotrochozoans. Mar. Genomic. 24, Part 1, 31-40.

Macey, J.R., Schulte, J.A., Larson, A., 2000. Evolution and phylogenetic information content of mitochondrial genomic structural features illustrated with acrodont lizards. Syst. Biol. 49, 257–277.

Martin, A.P., 1995. Metabolic rate and directional nucleotide substitution in animal mitochondrial DNA. Mol. Biol. Evol. 12, 1124–1131.

Min, X.J., Hickey, D.A., 2008. An evolutionary footprint of age-related natural selection in mitochondrial DNA. J. Mol. Evol. 67, 412–417.

Minh, B.Q., Nguyen, M.A.T., von Haeseler A., 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188–1195.

Mueller, R.L., Boore, J.L., 2005. Molecular mechanisms of extensive mitochondrial gene rearrangement in plethodontid salamanders. Mol. Biol. Evol. 22 (10), 2104–2112.

Mwinyi, A., Meyer, A., Bleidorn, C., Lieb, B., Bartolomaeus, T., Podsiadlowski, L., 2009. Mitochondrial genome sequence and gene order of *Sipunculus nudus* give additional support for an inclusion of Sipuncula into Annelida. BMC Genom. 10, 27.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.

Papillon, D., Perez, Y., Caubit, X., Le Parco, Y., 2004. Identification of chaetognaths as protostomes is supported by the analysis of their mitochondrial genome. Mol. Biol. Evol. 21, 2122–2129.

Perna, N., Kocher, T. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J. Mol. Evol. 41, 353–358.

Pons, J., Bauzà-Ribot, M.M., Jaume, D., Juan, C., 2014. Next-generation sequencing, phylogenetic signal and comparative mitogenomic analyses in Metacrangonyctidae (Amphipoda: Crustacea). BMC Genom. 15, 566.

Rawlings, T.A., MacInnis, M.J., Bieler, R., Boore, J.L., Collins, T.M., 2010. Sessile snails, dynamic genomes: gene rearrangements within the mitochondrial genome of a family of caenogastropod molluscs. BMC Genom. 11, 440.

Rivera, A., Weisblat, D., 2009. And Lophotrochozoa makes three: Notch/Hes signalling in annelid segmentation. Dev. Genes Evol. 219, 37–43 (2009).

Rota-Stabelli, O., Yang, Z., Telford, M.J., 2009. MtZoa: A general mitochondrial amino acid substitutions model for animal evolutionary studies. Mol. Phylogenet. Evol. 52, 268–272.

Sahyoun, A.H., Bernt, M., Stadler, P.F. Tout, K. 2014. GC skew and mitochondrial origins of replication. Mitochondrion 17, 56–66.

Schattner, P., Brooks, A.N., Lowe, T.M. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. 33(Web Server issue):686–689.

Shain, D.H. 2009. Annelids in Modern Biology. Wiley-Blackwell, Hoboken, NJ.

Shen, X., Ma, X.Y., Ren, J.F., Zhao, F.Q., 2009. A close phylogenetic relationship between Sipuncula and Annelida evidenced from the complete mitochondrial genome sequence of *Phascolosoma esculenta*. BMC Genom. 10, 136.

Stach, T., Braband, A., Podsiadlowski, L., 2010. Erosion of phylogenetic signal in tunicate mitochondrial genomes on different levels of analysis. Mol. Phylogenet. Evol. 55, 860–870.

Steinauer, M.L., Nickol, B.B., Broughton, R., Orti, G., 2005. First sequenced mitochondrial genome from the phylum Acanthocephala (*Leptorhynchoides thecatus*) and its phylogenetic position within Metazoa. J. Mol. Evol. 60, 706–715.

Stothard, P., Wishart, D.S., 2005. Circular genome visualization and exploration using CGView. Bioinformatics 21, 537–539.

Struck, T.H., Paul, C., Hill, N., Hartmann, S., Hosel, C., Kube, M., Lieb, B., Meyer, A., Tiedemann, R., Purschke, G., Bleidorn, C., 2011. Phylogenomic analyses unravel annelid evolution. Nature 471, 95–98.

Struck, T.H., Golombek, A., Weigert, A., Franke, F.A., Westheide, W., Purschke, G., Bleidorn, C., Halanych, K.M., 2015. The evolution of annelids reveals two adaptive routes to the interstitial realm. Curr. Biol. 25(15), 1993–1999.

Telford, M.J., Herniou, E.A., Russell, R.B., Littlewood, D.T., 2000. Changes in mitochondrial genetic codes as phylogenetic characters: two examples from the flatworms. Proc. Natl. Acad. Sci. USA 97, 11359–11364.

Vallés, Y., Boore, J.L., 2006. Lophotrochozoan mitochondrial genomes. Integr. Comp. Biol. 46, 544–557.

Wang, Y., Huang, X.L., Qiao, G.X., 2013. Comparative analysis of mitochondrial genomes of five aphid species (Hemiptera: Aphididae) and phylogenetic implications. PLoS One 8:e77511.

Weigert, A., Helm, C., Meyer, M., Nickel, B., Arendt, D., Hausdorf, B., Santos, S.R., Halanych, K.M., Purschke, G., Bleidorn, C., Struck, T.H., 2014. Illuminating the base of the annelid tree using transcriptomics. Mol. Biol. Evol. 31, 1391–1401.

Weigert, A., Golombek, A., Gerth, M., Schwarz, F., Struck, T.H., Bleidorn, C., 2016. Evolution of mitochondrial gene order in Annelida. Mol. Phylogenet. Evol. 94, 196–206.

Wu, Z.G., Shen, X., Sun, M.A., Ren, J.F., Wang, Y.J., Huang, Y.L., Liu, B., 2009. Phylogenetic analyses of complete mitochondrial genome of *Urechis unicinctus* (Echiura) support that echiurans are derived annelids. Mol. Phylogenet. Evol. 52, 558–562.

Yuan, Y., Li, Q., Yu, H., Kong, L., 2012. The complete mitochondrial genomes of six heterodont bivalves (Tellinoidea and Solenoidea): Variable gene arrangements and phylogenetic implications. Plos One 7, e32353.

Zhang, Y., Sun, J., Zhang, H., Chandramouli, K.H., Xu, Y., He, L., Ravasi, T., Qian, P.Y., 2014. Proteomic profiling during the pre-competent to competent transition of the biofouling polychaete *Hydroides elegans*. Biofouling 30(8), 921–928.

Zhong, M., Struck, T.H., Halanych, K.M., 2008. Phylogenetic information from three mitochondrial genomes of Terebelliformia (Annelida) worms and duplication of the methionine tRNA. Gene 416, 11–21.

Chapter 7

COI barcoding of *Hydroides*

Author:

Yanan Sun

Abstract

DNA barcoding using a specific region of the mitochondrial cytochrome *c* oxidase I (COI) gene has been proposed as a standard method for species identification and discovery in animals. Amplification of the COI fragment, however, has proven difficult in many taxonomic groups, including serpulid polychaetes, due to a lack of appropriate primers. Worse still, attempts at designing taxon specific primers are always be problematic due to the lack of reference sequences. A case in point is *Hydroides*, which is the largest genus of serpulids, with economically important biofoulers and bioinvaders. Utilising next generation sequencing techniques, genomes of eight species of *Hydroides* were sequenced, making the entire COI sequence available as references for new primer design. Herein, new taxon-specific COI primer sets targeting the barcoding region of *Hydroides* were developed, based on sequences obtained from mitochondrial genomes. Using the new primer sets, 44 of 46 available species of *Hydroides* were successfully amplified and sequenced. This study provides the first barcode reference library for species identification of *Hydroides* on a global scale. Analyses of COI barcoding data confirmed the efficacy of this method for species delimitation in *Hydroides*, especially for detecting potential cryptic species. COI barcoding data, combined with other mitochondrial and nuclear markers, has the potential to elucidate important insights into the phylogeny and taxonomy of this large and ecologically-important group of calcareous tubeworms.

Introduction

DNA barcoding was intended to provide references for species identification through establishing a comprehensive species-specific sequence library for all eukaryotes (Hebert et al., 2003). A 658 bp fragment of mitochondrial cytochrome *c* oxidase subunit I (COI) gene has been claimed as the best barcoding region for animals because it is present in all animal taxa, provides sufficient interspecific variability and can be easily obtained with “universal” PCR primers (Hebert et al., 2003). By covering 175,000 animal species, the online workbench Barcode of Life Datasystem (BOLD) (http://v4.boldsystems.org/) provides valuable COI barcoding resources for the species identification, cryptic species determination, and phylogenetic investigation (Ratnasingham and Hebert, 2007).

Polychaetes are an abundant and speciose group of macrobenthos in marine ecosystems, with 81 families and over 12 000 described species (Hutchings, 1998). Due to the limitations of conventional taxonomy in delimiting morphologically similar species, species with variable reproductive forms, and damaged specimens (Knowlton, 1993; Westheide and Schmidt, 2003; Carr et al., 2011), COI barcoding has been utilized and demonstrated to be effective in distinguishing species and revealing cryptic species of polychaetes (Barroso et al., 2010; Carr et al., 2011; Nygren and Pleijel, 2010; Sun et al., 2012; Lobo et al., 2013). Some groups of polychaetes, however, such as the families Cirratulidae, Nephtyidae, Spionidae, Sabellidae, and Serpulidae (Pleijel et al., 2009; Nygren and Pleijel, 2011; Carr et al., 2011; Sun et al., 2012), are extremely difficult to amplify using the supposedly universal primer set LCO1490/HCO2198, popularly known as "Folmer primers" (Folmer et al., 1994), due to the non-homology between the universal primers and the target DNA region.

As a member of the problematic group Serpulidae, CO1 barcoding of *Hydroides* has travelled a difficult road. As with all other serpulid genera, the earliest attempts to amplify COI fragments in *Hydroides* using Folmer primers completely failed (see Sun et al., 2012: 539–540). The first success was achieved by Sun et al. (2012), who successfully barcoded three species of *Hydroides* using the polychaete-specific primers polyLCO/polyHCO (Carr et al., 2011). Using these three COI sequences as references, the first *Hydroides* specific primers (Hydro-COIF/Hydro-COIR) were designed, and generated COI barcoding fragments from 11 out of 14 tested species (Sun et al., 2012). Attempts to use this primer set on a wider range of species, however, resulted in successful amplification of only 17 of the 45 species of *Hydroides* (this Chapter). Although only a limited number of species were investigated, COI barcoding showed its effectiveness in detecting cryptic species of *Hydroides* (e.g., Chapter 6). Thus, to use this powerful molecular identification tool in *Hydroides*, a more effective primer set is required. Yet new primer design is limited by availability of reference sequences (Yuryev, 2007). Fortunately, next-generation sequencing technologies have made generation of mitochondrial genomes more feasible and less time intensive. Therefore, COI reference sequences of *Hydroides* for new primer design could be readily obtained from genomic data.

The aims of this chapter were to a) develop new taxon-specific primers using entire COI sequences obtained from the mitochondrial genomes in chapter 6 as references, b) test the effectiveness of the new primers on all available specimens of *Hydroides*, and c) investigate the potential utility of COI barcoding for discriminating species of *Hydroides* from across a broad geographic range.

Materials and methods

Sequences of the entire COI gene (1647 bp) of eight *Hydroides* species: *H. dirampha*, *H. elegans*, *H. ezoensis*, *H. homoceros*, *H. minax*, *H. operculata*, *H. pseudouncinata*, and *H. sanctaecrucis* were obtained from the annotated mitochondrial genomes (Chapter 6). Sequences were aligned with ClustalX v2.1 (Larkin et al., 2007) using default settings (15-gap opening penalty and 6.66-gap extension penalty), and subsequently checked by eye using BioEdit v7.0.5.3 (Hall, 1999) to identify the homogeneous regions for new primer sets design.

A total of 284 terminals representing 46 *Hydroides* morphospecies were used for testing the new primer sets. All specimens were deposited in the collection of Australian Museum (AM) in Sydney, Australia. Details of specimens, including collection localities and voucher numbers, are given in Appendix table 1 and Appendix fig. 1. Specimens were removed from their tubes under a dissecting microscope and preserved in 95% ethanol. For each specimen, the posterior end was cut for genome extraction. Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer’s protocol. Polymerase chain reactions (PCR) were performed in a total volume of 20 µl with Invitrogen 10× PCR buffer (2.0 µl), 50 mM $MgCl_2$ (1.5 µl), 10 nM of each primer (0.4 µl), 2.5 µM dNTPs (1.5 µl), Milli-Q H_2O (13.1 µl), Invitrogen Taq DNA Polymerase (0.1 µl) and genomic DNA template (1 µl) in an Eppendorf Mastercycler® pro. Gradient PCRs were performed to detect the best annealing temperature. Thermal cycling was performed with an initial denaturation of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperatures from 47°C to 55°C, 30 s at 72°C, and with a final 2 min extension at 72°C. Successfully amplified products were purified with the ExoSAP-IT PCR clean-up kit and sequenced at Macrogen Inc. (South Korea), using an Applied Biosystems 3730xl DNA Sequencer.

Sequencing results were compared with those available in GenBank (www.ncbi.nlm.nkh.gov/Genbank/index.html) by BLAST search to check whether the correct gene fragments were amplified. A Sequence matrix of all COI sequences generated using ClustalX v2.1 and BioEdit v7.0.5.3 (Hall, 1999) was used for following analyses. The genetic distance based on the aligned COI matrix was calculated using the K2P model (Kimura, 1980) in MEGA 5.0 (Tamura et al., 2011). Bayesian inference (BI) analysis was performed in MrBayes v3.2.6 (Ronquist et al., 2012) on two separate runs of Markov chain for 30 million generations using the best-fit model generated by jModeltest (1^{st} coding positions: GTR+I+G, 2^{nd} coding positions: TIM1+G, 3^{rd} coding positions: GTR+G). The first 25% of trees were discarded as burn-in to generate a maximum clade credibility tree.

Results and discussion

To recover the same barcoding region as used in the public barcoding database, conserved regions were searched around the positions 1490 and 2198 of COI gene (Folmer et al., 1994). The alignment of the COI gene revealed several highly conserved regions that were used as the targets for primer designs.

Altogether, the following four primers listed herein, including one coding-strand and three anti-coding-strand primers, were designed and tested:

HyCOF190: TCNRTNTTKACDGTKACATGCTA

HyCOR688: AAYCTMGTHKWAAARTGHCGATC

HyCOR673: TGHCGATCHRYYAAAAGCATAGT

HyCOR886: ACCCYATYATHCCRATAGARCACAT

In the code names above, Hy refers to *Hydroides*, F and R refer to forward and reverse DNA strands, CO refers to cytochrome oxidase, and the numbers refer to the start position of the primer from the 5’-end of the coding strand. Degenerate positions were represented by the following ambiguity codes: D= A|G|T; H = A|C|T; K= G|T; M = A|C; N = A|G|T|C; R = A|G; W= A|T; Y= C|T.

The three pairs of primers covered barcoding regions of 498 bp, 483 bp, and 696 bp, respectively. The primer set HyCOF190/HyCOR886 resulted in the highest success rate of PCR amplifications (44 of 46 tested species) (Table 1). In total, 218 COI barcoding sequences were obtained from 44 morphospecies representing 47% of currently recognised species in the genus *Hydroides* (chapter 3). Only sequences with less than 1% ambiguous base calls were included. The new primer set of HyCOF190/HyCO673 covered the same barcoding region with that of Hydro-COIF/Hydro-COIR. The main difference of the two primer sets was that the “A” in the 3’ end of Hydro-COIR was removed in HyCO673, due to substitutions of the nucleotide from A to G in the position of some species. This high success rate represents an improvement over the previous primer set (Sun et al., 2012).

BI analyses based on COI barcoding data recovered 56 distinct, well-supported lineages of 44 *Hydroides* morphospecies (Fig. 1). Some species, such as *H. albiceps, H. bandaenisis, H. homoceros, H. longispinosa, H. minax, H. operculata, and H. tuberculata*, were recovered as two or more distinct lineages. The genetic divergences among morphologically distinct species ranged from 12.6% to 37.1% (mean 26.2%), while genetic divergences among totally molecular-based lineages ranged from 5.5% to 37.1% (mean 25.9%), compared to the genetic divergence within lineages that ranged from 0 to 3.6% (mean 1.5%) (Fig. 2). Intermediate divergences ranging from 5% to 12% only occurred among lineages of each of the species that showed subtle differences in morphology, such as *H. albiceps* (10.2%), *H. exaltata* (5.5%–10.4%), *H. operculata* (10%), *H. triversiculosa* (9.3%), and *H. tuberculata* (8.6%–8.8%).

Although the effectiveness of COI barcoding for species identification and detection of cryptic lineages has been already demonstrated for 12 species of *Hydroides* (Sun et al., 2012; Chapter 6), the present results indicate the possibility for the broad application of DNA barcoding in the genus *Hydroides*. In most cases, delineation of clade boundaries from COI barcode data were straightforward. The 17-fold higher mean COI genetic divergence inter- as compared to intra- lineages, and the rarity of intermediate divergences indicated that the COI barcodes had high discrimination power for *Hydroides*. The higher number of genetic lineages than that of morphospecies indicated the effectiveness of COI barcoding in revealing hidden species diversity in *Hydroides* that were overlooked by the current taxonomic system. Further analyses of COI barcoding data combined with the other molecular markers will yield considerable insights in the phylogeny of this large and important group (Chapter 8).

Table 1. Information on new primer sets for *Hydroides*

PRIMER SETS	TAGET REGION LENGTH	TARGET REGION POSITION	BEST ANNEALING TEMPERATURE (°C)	PCR SUCCESSFUL RATE BASED ON SPECIES
HyCOF190 + HyCOR688	498	190–688	51	80%
HyCOF190 + HyCOR673	483	190–673	51	50%
HyCOF190 + HyCOR886	696	190–886	51	95%

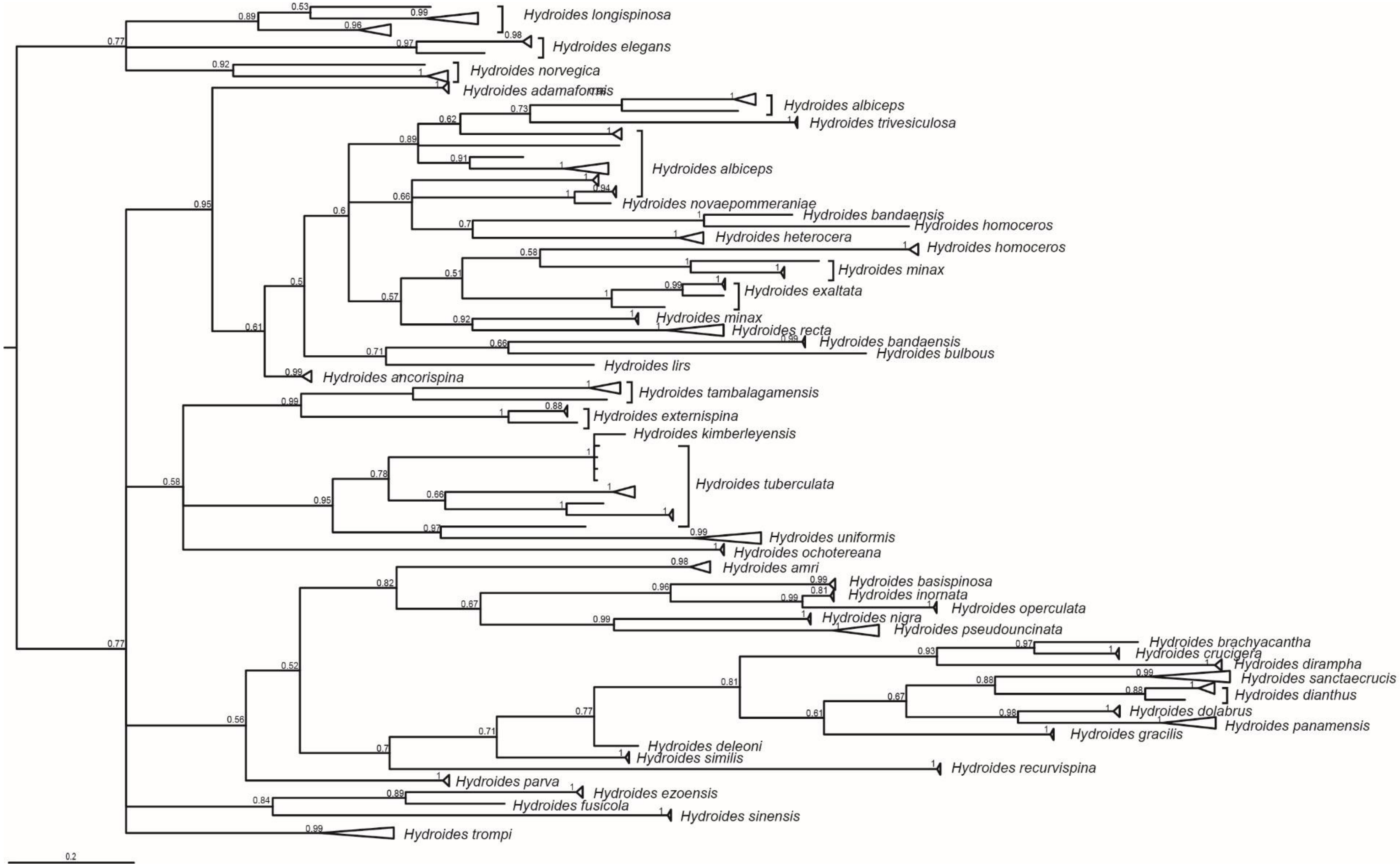


Fig. 1 Bayesian majority ruled consensus tree of 44 morphospecies of Hydroides *based on COI barcoding sequences. Values represent posterior probabilities.*

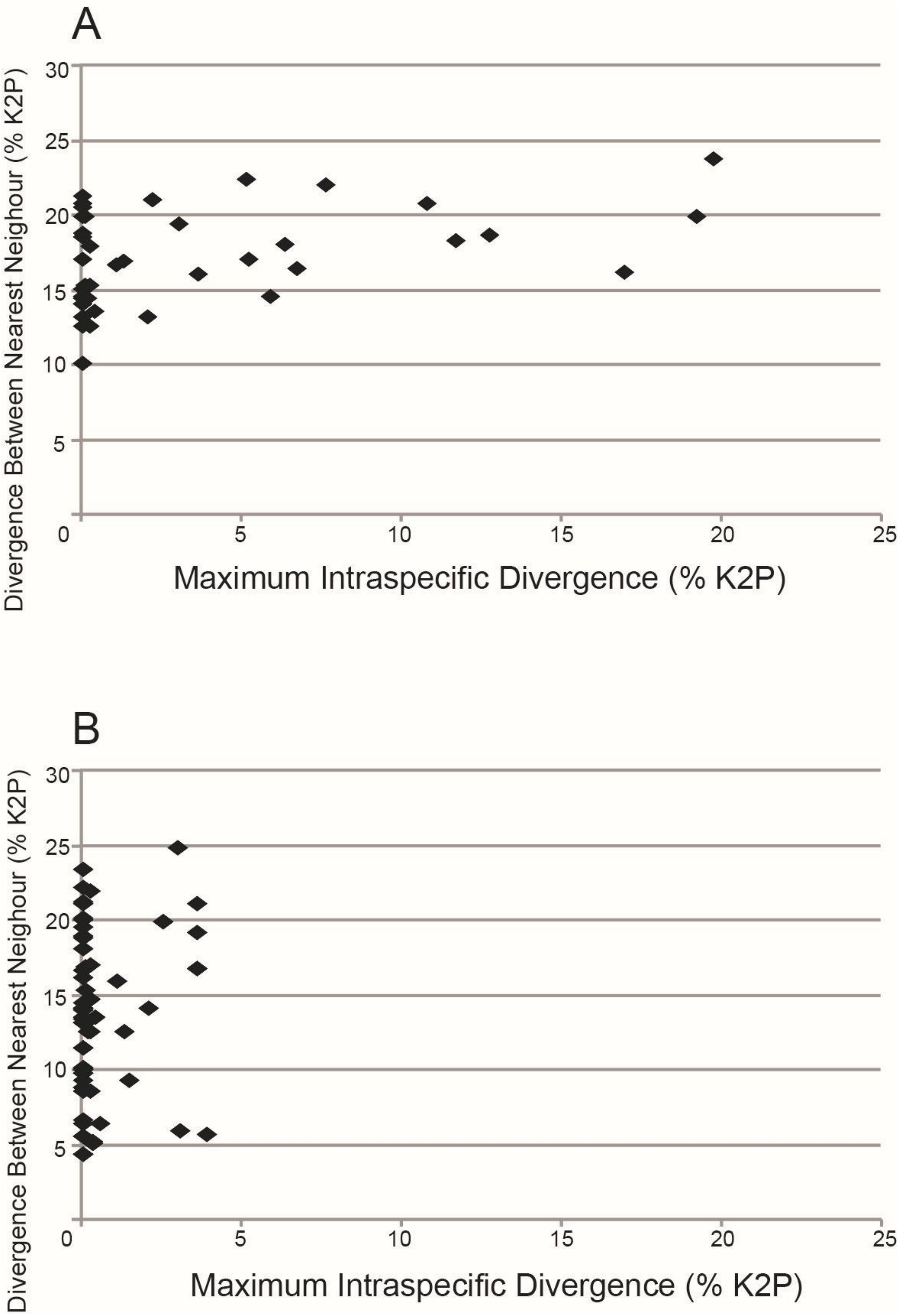


Fig. 2 Comparison of COI (K2P) distances of inter- and intra- groups. (A) Comparison of COI (K2P) distances of morphospecies of Hydroides*, (B) Comparison of COI (K2P) distances of genetic lineages recovered by phylogenetic analyses.*

References

Barroso, R., Klautau, M., Solé-Cava, A. M., and Paiva, P. C. (2010). *Eurythoe complanata* (Polychaeta: Amphinomidae), the ‘cosmopolitan’ fireworm, consists of at least three cryptic species. *Marine Biology* **157**, 69–80.

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., and Hebert, P. H. N. (2011). A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* **6**, e22232.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hebert, P. D. N., Cywinska, A., Ball, S. L., and deWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings. *Biological Sciences* **270**, 313–321.

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Knowlton, N. (1993). Sibling species in the sea. *Annual Review of Ecology, Evolution, and Systematics* **24**, 189–216.

Lobo, J., Costa, P. M., Teixeira, M. A. L., Ferreira, M. S. G., Costa, M. H., and Costa, F. O. (2013). Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecology* **13**, 34.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Nygren, A., and Pleijel, F. (2011). Fromone to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. *Molecular Phylogenetics and Evolution* **58**, 132–141.

Pleijel, F., Rouse, G., and Nygren, A. (2009). Five colour morphs and three new species of *Gyptis* (Hesionidae, Annelida) under a jetty in Edithburgh, South Australia. *Zoologica Scripta* **38**, 89–99.

Ratnasngham, S., and Hebert, P. D. (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.

Ronquist, F.,Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A.,Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P., (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* **61**, 539–542.

Sun, Y., Kupriyanova, E. K., and Qiu, J. W. (2012). COI barcoding of *Hydroides*: a road from impossible to difficult *Invertebrate Systematics* **26**, 539–547.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S., (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Westheide, W., and Schmidt, H. (2003). Cosmopolitan versus cryptic meiofaunal polychaete species: An approach to a molecular taxonomy. *Helgoland Marine Research* **57**, 1–6.

Yuryev, A. (2007). *PCR Primer Design (Methods in Molecular Biology)*. Humana Press, Totowa, New Jersey, 431 pp.

Chapter 8

Phylogenetics and biogeography of the globally important calcareous tubeworm genus *Hydroides* Gunnerus, 1768 (Serpulidae, Polychaeta) inferred with multiple gene markers

Authors:
Yanan Sun[1, 2, *], Eunice Wong[3], Jane E. Williamson[2], Pat Hutchings[1],
Elena K. Kupriyanova[1, 2]

[1] *Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia*.
[2] *Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia*.
[3] *Department of Biology, University of Queensland, St Lucia QLD 4072, Australia*

Abstract

Hydroides is a diverse group of calcareous tubeworm. Despite considerable research on several species of *Hydroides* as models in ecology and biofouling studies, our understanding of their phylogenetic and biogeographic relationships is still limited. Herein, by examining combined genetic markers of both mitochondrial (COI, cyt b) and nuclear genes (18S, 28S and ITS) for 284 individuals of 44 morphospecies in *Hydroides,* we investigated the phylogenetic and biogeographic relationships within the genus worldwide. Phylogenetic analyses resulted in a well-supported phylogeny that indicated high genetic diversity within *Hydroides*, and revealed potential cryptic species and species complexes. Phylogenetic relationships within *Hydroides* were more concordant with geographical distributions than morphological expectations. The molecular divergence estimates suggested possible origin and subsequent diversification of ancestral *Hydroides* in the western Tethys Sea and a possible shift of the historical centre of biodiversity from the Indo-Mediterranean region to the central Indo-Pacific during the last 50 Million years. Further studies on population genetics of species consisting of multiple lineages would provide a better understanding on the status of potential cryptic species. Furthermore, paleogeographic studies based on *Hydroides* fossil tubes would provide evidence to test the biogeographic hypothesis.

1. Introduction

Serpulidae is a group of marine segmented worms that can be easily recognised by their self-secreted calcareous tubes and colourful radiolar crown (Rouse and Pleijel, 2001). A modified radiole called the operculum, serving as a tube plug when the worm withdraws into its tube, is generally present in Serpulidae and has been traditionally used as one of the most important characteristics in morphological classification (ten Hove and Kupriyanova, 2009). *Hydroides* Gunnerus, 1768, is the most speciose genus in Serpulidae with 105 nominal species (Read et al., 2016) and contains species with a two-tiered operculum consisting of the basal funnel and the distal spinous structure (crown of chitinous spines) called the verticil, autapomorphic for the genus. *Hydroides* has commonly been recorded in subtidal habitats of tropical and subtropical regions worldwide as well as in temperate and cold waters, where they adhere to various hard substrata such as rocks, coral skeletons, mooring buoys, ship hulls and other artificial substrates (Bastida-Zavala and ten Hove, 2003; Bastida-Zavala, 2008; ten Hove and Kupriyanova, 2009). *Hydroides* includes a number of important biofouling species and bioinvaders such as *Hydroides elegans* (Haswell, 1883), *Hydroides dianthus* (Verrill, 1873), and *H. dirampha* (Mörch, 1863). These biofoulers have been intensively studied in terms of their reproduction, larval development, growth and settlement (reviewed by Kupriyanova et al., 2001; Lau et al., 2002; Pettengill et al., 2007; Qian et al., 2007; Huggett et al., 2009; Shikuma et al., 2016). For many other species, however, little is known beyond the basic taxonomic description.

1.1. Taxonomic status of genus Hydroides

Since the genus *Hydroides* was erected by Gunnerus (1768) with *H. norvegica* Gunnerus, 1768 as the type species, numerous generic and subgeneric names were erected to further distinguish the species with a two-tiered operculum based on the various morphologies of the verticil. Phillippi (1844) erected *Eupomatus* to separate species without lateral processes on their verticil spines, and this name was subsequently widely used to group up to 23 species (Hartman, 1959). Mörch (1863), however, considered that the presence or lack of lateral processes on verticil spines is only of sub-generic value, and he erected the subgenus *Eucarphus* for the species with spines having lateral processes. Grube (1878) described two new species of serpulids with a two-tiered operculum under genus *Serpula*, namely, *Serpula minax* and *Serpula furcifera*. Later, Bush (1905) erected the genus *Glossopsis* for *Serpula minax* Grube, 1878, to recognize the asymmetrical arrangement of the verticil spines, and genus *Schizocraspedon* for *Serpula furcifera* Grube, 1878, according to the similar bifid shape of both funnel radii and verticil spines of the operculum. *Eucarphus*, *Glossopsis*, *Schizocraspedon* were considered as redundant and only two generic names *Hydroides* and *Eupomatus* were retained to subdivide the group (Pixell, 1913; Rioja, 1958; Hartman, 1959; Jones, 1962). Other taxonomists recognized *Hydroides* as a single genus (Okuda, 1934; Dew, 1959; Pillai, 1960, 1961, 1971; Straughan, 1967a, b). Uchida (1978) proposed the genus name *Protohydroides* for *H. elegans* based on the apparent absence of the verticil in some specimens and on the presence of collar chaetae with more than three teeth. However, these two characteristics were considered indistinguishable when compared with the closely related genus *Serpula*; and the genus *Protohydroides* is currently considered invalid (ten Hove, 1984). Thus, the consensus that *Hydroides* is a single genus prevailed. Not surprisingly, with a long and complex list of synonyms, members of the genus *Hydroides* show high diversity in morphology that may confound reconstructing relationships among them (Bastida-Zavala and ten Hove, 2002).

1.2. Phylogenetic relationship within Hydroides

Jones (1962) proposed the first phylogenetic scheme within *Hydroides,* and divided 21 species of the *Eupomatus* group into four groups according to the morphology of the verticil spines: i.e., species with verticil spines of different size, species with verticil spines in asymmetrical arrangement, species with straight verticil spines, as well as species with verticil spines with T-shaped tips. As *Eupomatus* was later synonymised with *Hydroides,* Pillai (1972) proposed a new phylogenetic scheme for 42 species of *Hydroides* worldwide. He divided these species into two major categories, and then placed them into 12 groups (Fig. 1A).

Neither of the two proposed phylogenetic schemes above, however, were inferred by formal phylogenetic analyses. Moreover, with the synonymy of *Hydroides* and *Eupomatus*, exponentially increasing numbers of discovered species of *Hydroides* in subsequent years, as well as synonymization of species of *Hydroides* in subsequent taxonomic revisions (e.g., at least four species in Pillai's scheme, *H. lunulifera*, *H. okudai*, *H. spinalateralis*, *H. spinosus*, have been synonymised under another names; Read et al., 2016), the phylogenetic schemes proposed based on morphological characteristics are still contentious.

Molecular phylogenetic analyses of multiple species of *Hydroides* were first performed by Kupriyanova et al. (2008), followed by Sun et al. (2012) and Tovar-Hernández et al. (2015). Based on two nuclear (18S and 28S rRNA) and one mitochondrial (cytochrome b, cyt b) genes of eight species of *Hydroides*, Kupriyanova et al. (2008) was the first to confirm the monophyly of *Hydroides* and suggested a close relationship existed between the Pacific species *H. novaepommeraniae*, *H. minax* and *H. trivesiculosa* (Fig. 1B). Sun et al. (2012) grouped together the Mexican Pacific species *H. brachyacantha*, *H. recurvispina* and *H. sanctaecrucis*, based on the mitochondrial barcoding gene cytochrome *c* oxidase subunit I (COI) of ten species. Tovar-Hernández et al. (2015) corrected the identification of *H. recurvispina* by Sun et al. (2012) to *H. dolabrus* and added *H. panamensis* to the *H. brachyacantha*-*H. dolabrus*-*H. sanctaecrucis* group. The close relationship among *H. elegans* and *H. norvegica*, *H. ezoensis* and *H. fusicola*, *H. pseudouncinata* and *H. operculata* was also recovered by Tovar-Hernández et al. (2015) (Fig. 1C). However, the relationships among these groups remain unclear due to the low support of phylogenetic topologies. In addition, taxon sampling of all these studies was limited to only 18 of 105 known species. The phylogenetic positions of most species within *Hydroides* remained uncertain.

Given that none of the molecular phylogenetic analyses above supported the morphology based phylogenetic groups proposed by either Jones (1962) or Pillai (1972), the relationship between the morphological characteristics and the phylogeny of *Hydroides* remained unclear. Thus, an up-to-date and integrative phylogenetic study based on broad taxon sampling to understand the phylogenetic relationships within *Hydroides* is undertaken here.

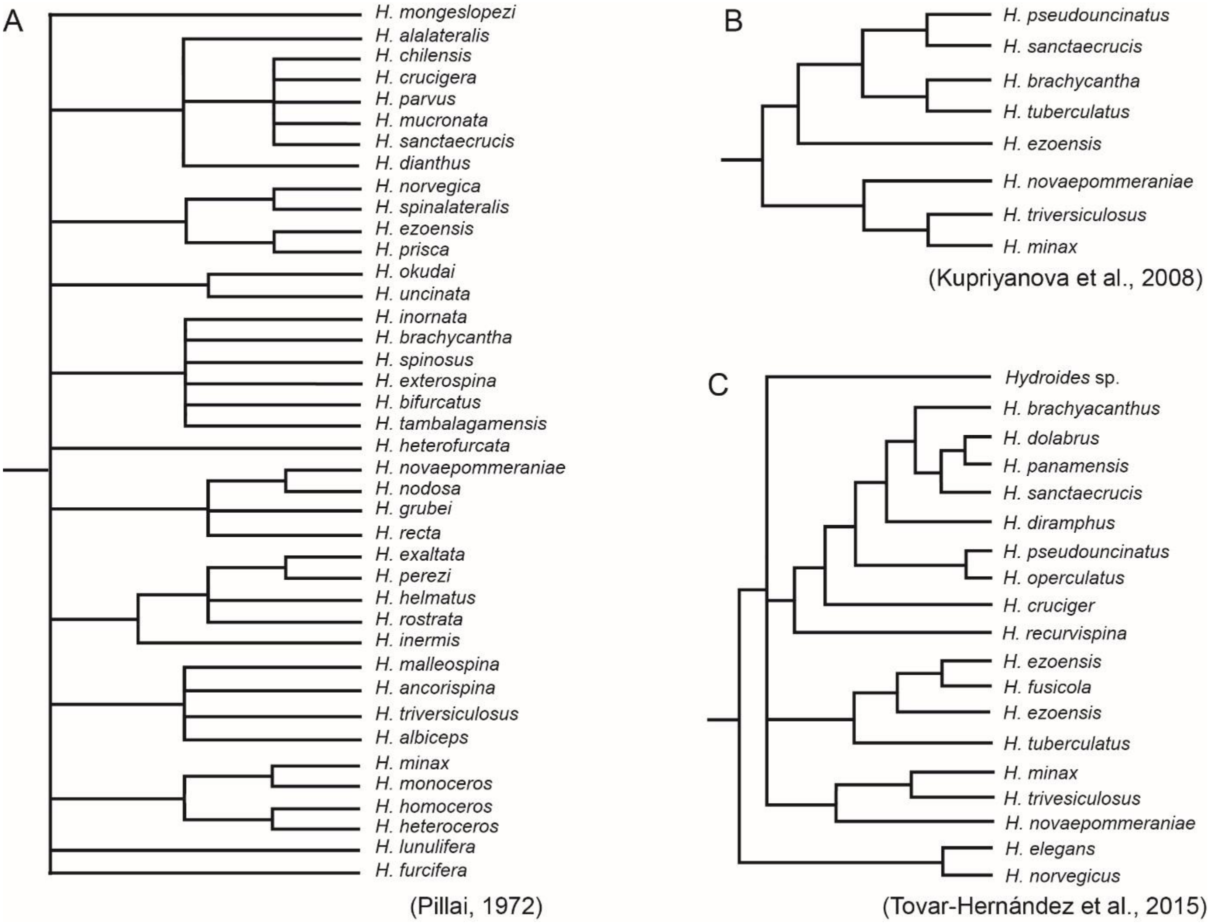


Fig. 1 Phylogenetic relationships indicated in previous studies.

1.3. Geographical distribution of Hydroides

Most species of *Hydroides* have been described from the Indo-Pacific, followed by the tropical east Pacific and Caribbean region (Bastida-Zavala and ten Hove, 2002). By linking morphological characteristics of species of *Hydroides* and their native distribution ranges, Pillai (1972) proposed that *Hydroides* can be separated into two main geographical groups: one inhabiting tropical and sub-tropical regions off the western Atlantic coasts, including species bearing a symmetrical verticil; the other from the eastern Atlantic, the Mediterranean Sea, and the Indo-West Pacific region with Indo-West-Pacific as the center of origin, consisting of species bearing an asymmetrical verticil. However, Pillai’s hypothesis remains contentious because species with both symmetrical and asymmetrical verticils have been described from both of the main geographic regions since 1972.

As a genus with species that can easily disperse via anthropogenic transport, the distribution and native ranges of reported “widespread” species such as *H. brachyacantha, H. dianthus*, *H. elegans* also now seems uncertain (Sun et al., 2016; Chapter 5). Recent phylogenetic studies demonstrated a geographic pattern where species of *Hydroides* from the eastern Pacific and western Atlantic showed closer relationships than those collected from other regions (Sun et al., 2012; Tovar-Hernández et al., 2015). However, this geographic pattern is based on limited sampling of taxon, and the geographic patterns of other species in *Hydroides* remain unclear.

In this study, a phylogenetic analyses based on a large multi-locus dataset of five markers (three nuclear and two mitochondrial) using species of *Hydroides* sampled worldwide was performed. The aims of this study were to 1) generate a well-supported, comprehensive species-level phylogeny that can aid in species delimitation and cryptic species detection; 2) estimate the divergence time for the major clades of *Hydroides*; and 3) reconstruct the geographic patterns of dispersal within the group by linking the cladogenic history with the biogeological framework.

2. Material and Methods

2.1. Taxon sampling and DNA sequencing

A total of 284 specimens of *Hydroides* were sampled from 48 locations representative of the current known species distribution range all over the world with the exception of Africa (Appendix fig. 1). All specimens were deposited in the collection of Australian Museum (AM) in Sydney, Australia. Details of specimens, including collection localities, voucher numbers are given in Appendix table 1. Specimens were removed from their tubes under a dissecting microscope and preserved in 95% ethanol. For each specimen, the posterior end was cut for DNA extraction. Eight species from closely related genera, *Crucigera* Benedict, 1887 and *Serpula* Linnaeus, 1758, one species of *Spirobranchus* de Blainville, 1818, and one species of *Galeolaria* Lamarck, 1818, were selected as outgroup species (Appendix table 1).

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer’s protocol. Polymerase chain reactions (PCR) were performed to amplify ~900 bp of 18S, ~1000 bp of 28S, ~500 bp of the internal transcribed spacer 2 (ITS2) region, ~700 bp of COI, ~400 bp of cyt b. Amplifications were performed in a total volume of 20 µl with Invitrogen 10x PCR buffer (2.0 µl), 50 mM $MgCl_2$ (1.5 µl), 10 nM of each primer (0.4 µl), 2.5 µM dNTPs (1.5 µl), Milli-Q H_2O (13.1 µl), Invitrogen Taq DNA Polymerase (0.1 µl) and genomic DNA template (1 µl) in an Eppendorf Mastercycler® pro. Thermal cycling was performed with an initial denaturation for 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at gene-specific annealing temperatures (shown in Table 1), and 30 s at 72°C, with a final 2 min extension at 72°C. The primers and annealing temperatures used in PCR are given in Table 1. Successfully amplified products were purified with the USB ExoSAP-IT PCR clean-up kit, and bidirectionally sequenced at Macrogen Inc. (South Korea), using an Applied Biosystems 3730 xl DNA Sequencer. Sequences will be submitted to GenBank.

2.2. *Sequence alignments and phylogeny reconstruction*

COI sequences obtained in chapter 7 were added to the combined dataset for the phylogenetic and biogeographic analyses. Sequences of the 18S, 28S, ITS2, COI, and cyt b gene fragments, together with sequences of outgroup species downloaded from GenBank were aligned separately using ClustalX v2.1 (Larkin et al., 2007) with default settings (15 gap opening penalty and 6.66 gap extension penalty) and subsequently manually corrected for obvious misalignments by eye and combined using BioEdit v7.0.5.3 (Hall, 1999). In the combined dataset, each nuclear gene and each of the three codon positions of COI and cyt b were separated as different partitions. For each partition, best-fit models of nucleotide substitution were identified using the Bayesian information criterion (BIC) as implemented in jModeltest v2.1.9 (Darriba et al., 2012). The best-fit models for each partition are show in Table 1.

The final lengths of 18S, 28S, ITS2, COI, and cyt b sequences used in phylogenetic analyses are shown in Table 1. Phylogenetic trees were constructed based on the combined dataset with both maximum-likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Huelsenbeck and Ronquist, 2001) methods and using the result from jModeltest for each partition. ML analysis was performed in IQ-TREE v1.4.4 (Nguyen et al., 2015) with 1000 ultrafast bootstrap replications (Minh et al., 2013). BI analysis was performed in MrBayes v3.2.6 (Ronquist et al., 2012) on two separate runs of Markov chain for 50 million generations. The first 25% of trees were discarded as burn-in to generate a maximum clade credibility tree.

2.3. *Divergence time estimates*

Divergence times were estimated using BEAST v2.4.3 (Bouckaert et al., 2014). Taxa used for divergence time estimate were selected according to the phylogenetic tree generated from MrBayes analysis. Outgroup species *Galeolaria caespitosa* and *Spirobranchus corniculatus* were excluded. For the species with high genetic distance among locations, one taxon from each location was selected. For other species collected from one location only or those with identical gene sequences among different locations, one taxon from each species was randomly selected. The combined dataset was repartitioned to three partitions: 18S + 28S, ITS2, COI + cyt b. The GTR + Gamma substitution model was used for each of the three partitions according to the result of jModeltest on each partition. Divergence times were estimated using an uncorrelated lognormal relaxed molecular clock with the Yule process to describe cladogenesis. The Markov chain was run twice for 50 million generations each, sampling every 5000 generations. Tracer v. 1.6 (Rambaut et al., 2014) was used to check the convergence of the chains, posterior distribution of all parameters and their associated statistics, including estimated sample sizes (ESS) and 95% highest posterior density (HPD) intervals. The TreeAnnotator v. 1.5.4 was used to summarize the set of post burn-in trees and produce a maximum clade credibility chronogram.

Although serpulids inhabiting calcareous tubes have the best fossil records among the mostly soft-bodied annelids, it is difficult to assign tube fossils to Recent species without ultrastructural examination, especially in *Hydroides*, due to the high similarity of the tube external macrostructure among species within the group (Ippolitov et al., 2014). The taxonomic status of the *Serpula-Hydroides* group was also problematic until recently (ten Hove and Kupriyanova, 2009), as it was represented by a synonymy of species and new species descriptions. The identities of most fossil tubes that were assigned to species of the *Serpula-Hydroides* group in earlier studies are ambiguous (Ippolitov et al., 2014) and cannot serve as calibration points. Thus, only two calculation points were included, based on the review of tube fossils of the *Serpula-Hydroides* group by Ippolitov et al. (2014). Coiled tubes with slowly growing loops are a flattened upper side are considered nearest to *Hydroides* (Rovereto, 1904; Lommerzheim, 1981; Ippolitov et al., 2014), of which the most ancient fossil has been dated to middle Paleogene (~ 61 Ma; Lommerzheim, 1981). Recent studies of ultrastructure of tube fossils indicate that tubes with complex highly-ordered ultrastructure similar to those of Recent taxa of the *Serpula-Hydroides* group appeared in the Cenozoic (66 Ma) (Vinn, 2007; Vinn et al., 2008; Ippolitov et al., 2014). Thus, two calculation points were used in here. One time constraint is on the node of the *Hydroides* group, using an exponential distribution prior with an offset of 61 Ma according to the most ancient fossil record from Lommerzheim (1981). The other is a rooting constraint of the *Serpula-Hydroides* group, using a normal prior with a mean of 66 Ma and soft 5% and 95% bounds according to the appearance age estimated by Ippolitov et al. (2014).

2.4. *Inference of biogeographic history*

Possible ancestral distributions were estimated in RASP v.2.1b (Yu et al., 2015) using the Lagrange dispersal, extinction and cladogenesis (DEC) model (Ree et al., 2005; Ree and Smith, 2008). Dispersal range was restricted to occur only between adjacent regions. The maximum areas allowed per node reconstruction were reduced to three, which helps to mitigate the tendency for DEC analyses to infer widespread ancestors inaccurately (Kodandaramaiah, 2010). The chronogram constructed using BEAST was used in the ancestral range reconstruction analysis. Geographic ranges of the lineages were allocated to seven regions according to the biogeographic scheme defined by Spalding et al. (2007) and the reported distribution of *Hydroides*: (A) East Pacific, (B) West Atlantic, (C) East Atlantic and Mediterranean, (D) West Indo-Pacific, (E) Central Indo-Pacific, (F) Temperate Northern

Pacific, (G) Temperate Australasia (Fig. 5). For widely reported invasive species, only the native distributions of these species were included in the analyses. *Hydroides elegans* was excluded from the historical biogeographic analyses because its native distribution range remains unclear. RASP allows constraints to be placed on each model to reflect past geographical events and configurations. Thus, the phylogeny was stratified into three different time slices: T1 occurred between the root age of 62 and 30 Mya, for which we decreased the following: the probability of dispersal from East Pacific (A) to West Pacific (E) from 1 to 0.05 to reflect the East Pacific barrier; the probability of dispersal between the western and eastern Atlantic (B and C) from 1 to 0.5 to reflect the expansion of the Atlantic; and the probability of dispersal among temperate Australasia (G) and other regions and within temperate Australasia to 0 to reflect the connection of Australasia with Antarctica. T2 occurred from 30 Mya to 18 Mya, for which the probabilities of dispersal between temperate Australasia (G) and tropical Pacific (E) and within temperate Australasia were increased to 1 to reflect the formation of the Great Australian Bight. T3 occurred from 18 Mya onwards, for which we decreased the possibility of dispersal between the eastern Atlantic (C) and Indian Ocean (D) from 1 to 0.05 to reflect the closure of the Tethys seaway (Ree and Sanmartín, 2009). Decreasing probabilities of dispersal between the eastern Pacific and western Atlantic, which reflect the closure of the Isthmus of Panama around 3.1 Mya, were not included because no sister lineages spanning this boundary that dated later occurred, according to the divergence time estimates.

Table 1. Details of primers used for PCR of each gene and the best-fit models used in phylogenetic analyses.

Gene	Primer name	Reference	Annealing Temperature (°C) used in PCR	Fragment length used in phylogenetic analyses	Best-fit Model used in phylogenetic analyses
18S	TimA/1100R2	Nóren and Jordelius, 1999	60	935	TIM2e+I+G
28S	LSUD1F/D2ar	Osborn et al., 2007	61	983	TPM2u+I+G
COI	HyCOF190/HyCOR886	Chapter 7	51	581	1st coding position: GTR+I+G 2nd coding position: TIM+G 3rd coding position: GTR+G
cyt b	424-f/COBr825	Boore and Brown, 2000/ Burnette et al., 2005	51	301	1st coding position: TN+G 2nd coding position: TPM2u+G 3rd coding position: TPM2u+G
ITS	ITS3/ITS4	White et al., 1990	55	602	TPM2+G

3. Results

3.1 Phylogenetic analyses

Both BI (Fig. 2) and ML (Fig. 3) phylogenetic analyses of the concatenated dataset resulted in well-supported phylograms. All results supported the monophyly of *Hydroides* (posterior probability (PP) = 1.00, bootstrap proportion (BP) = 100), and suggested a close relationship with its sister group including representatives of the genera *Serpula* and *Crucigera*. Five distinct clades of *Hydroides* could be recognized (A–E in Fig. 2) in the BI phylogram. Of the five clades, three (clades A, B, D) were well-supported by both BI and ML analyses (PP: 0.92–1.00; BP: 96–100) and showed nearly identical topologies within each clade. Clades A and B were grouped as a well-supported sister group consisting of specimens collected from the Indo-Pacific region in both analyses (PP = 0.99; BP = 97). Clade D grouped all specimens collected from the eastern Pacific and western Atlantic species (subclade F in Fig. 2), Mediterranean (Subclade G in Fig. 2), and contained two Indo-Pacific taxa: *H. amri* and *H. operculata*-complex. Clade C, including a subclade of *H. ezoensis*+*H. fusicola*+*H. sinensis* (subclade H in Fig. 2), *H. ochotereana* and *H. trompi*, was moderately supported by BI analyses, whereas, the three subclades were not grouped together in ML analyses. ML analyses recovered clade F and *H. trompi* as a sister group to clade D with high support value (BP: 98) and recovered *H. ochotereana* at the base position relative to all other *Hydroides* clades except for *H. norvegica*. ML analyses also nested the subclade *H. elegans*+*H. longispinosa* of clade E (PP = 1) in clade D.

3.2 Divergence time estimations

All effective sample size (ESS) values for the divergence time analyses were well above 200 after 50 million generations, which indicated that the parameter space had been sufficiently sampled (Drummond and Rambaut, 2007). The chronogram estimated that the ancestor of clade E (Fig. 4) diverged from other clades around 62.4 Mya (61–65.1 Mya, 95% HPD), during the Mid-Paleocene. The main eastern Pacific and Atlantic clade (Clade D) appeared approximately 42.7 Mya (32.6–51.7 Mya, 95% HPD). Divergence between species from the temperate northern Pacific and Indo-Pacific occurred approximately43.5 Mya (34–53 Mya, 95% HPD), with the subsequent divergence of the two main Indo-Pacific branches (splits of clades A and B) occurring around 39 Mya (29.7–47.9 Mya, 95% HPD), during the Mid-Eocene (Fig. 4). The most significant diversification within *Hydroides* occurred during the Miocene (~ 5–23 Mya).

Fig. 2 BI tree reconstructed based on a combined dataset of five molecular markers.

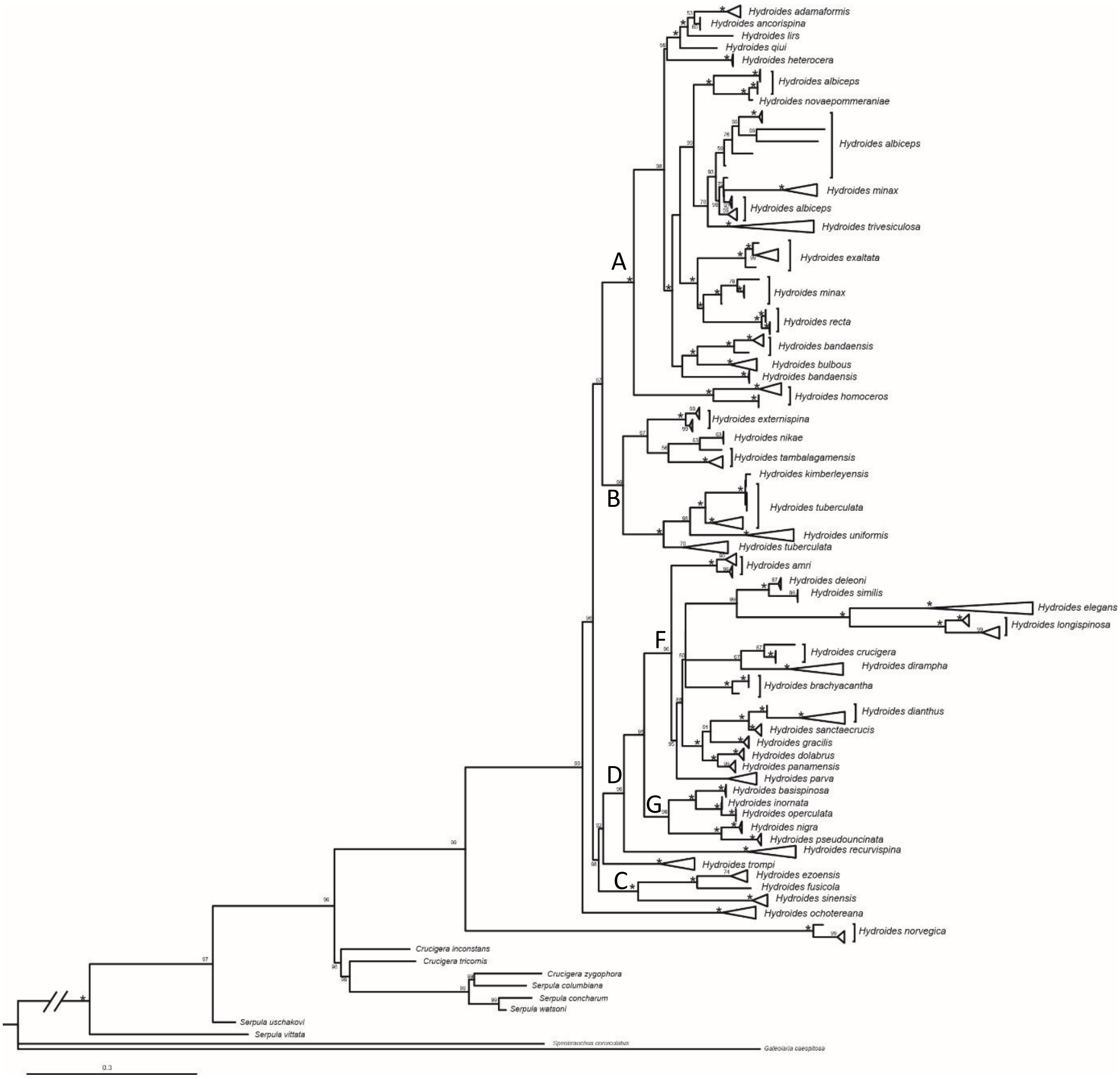


Fig. 3 ML tree reconstructed based on a combined dataset of five molecular markers.

3.3 Inference of biogeographic history

The biogeographical analyses estimated that the ancestor of *Hydroides* showed a wide distribution in the eastern Atlantic and Indian Ocean during the Palaeocene (clade L; Fig 4). Further dispersal events occurred during the early Eocene (clade J; Fig. 5), including the eastward dispersal to the tropical western and northern Pacific (clade I; Fig. 5), as well as the dispersal westwards to eastern Pacific via Atlantic (clade D; Fig. 5). Subsequent diversifications of clades C and H were restricted in the northern Pacific and Indo-Pacific, respectively. Within an Indo-Pacific (clade H), the reconstruction showed the within-region origin of clade A and B, of which, clade A showed a widespread range in both the western and central Indo-Pacific, while clade B was restricted to the central Indo-Pacific and dispersed to the temperate Australasia by the late Neogene.

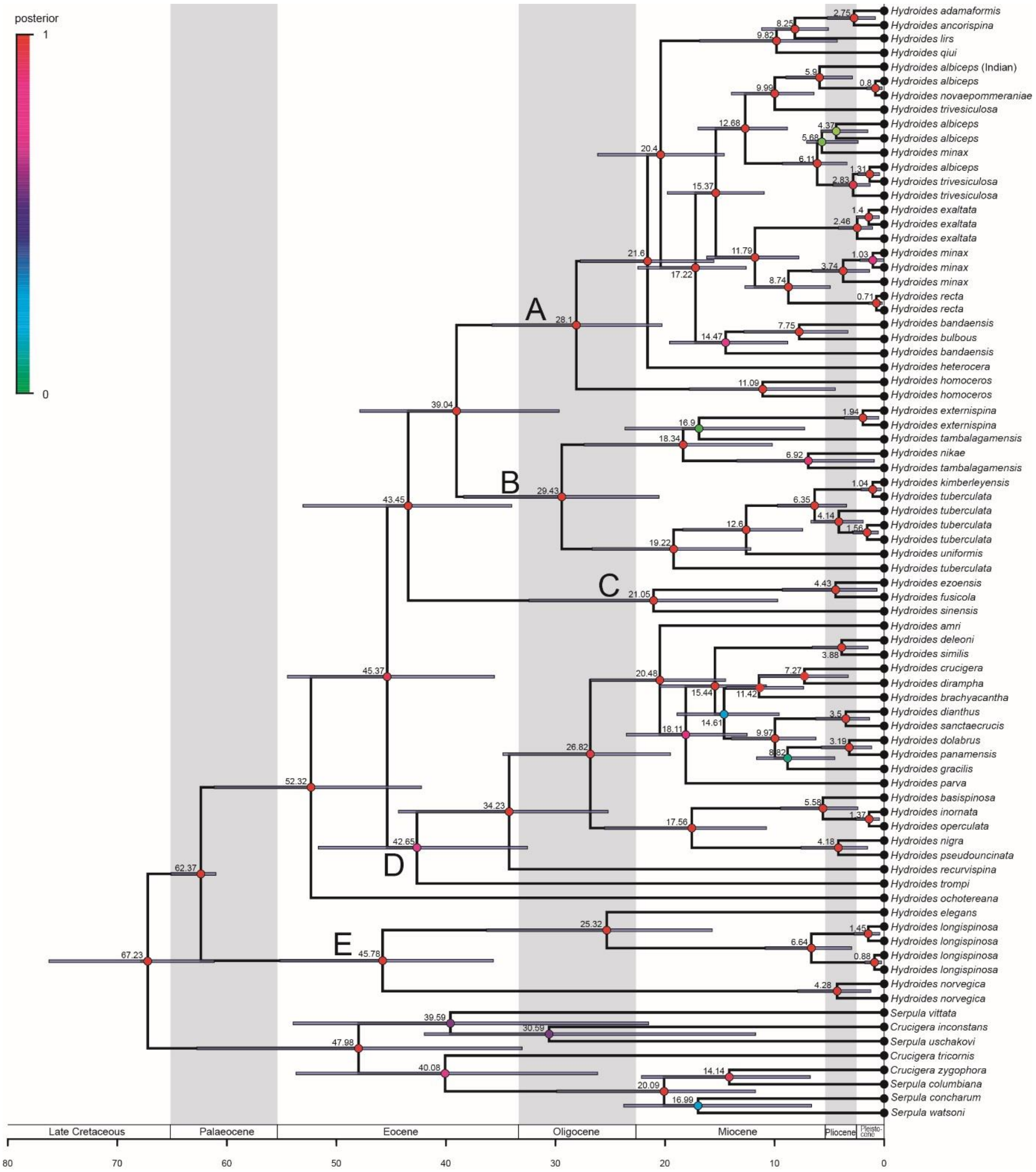


Fig. 4 Chronogram of Hydroides *produced from the BEAST analysis. Maximum clade credibility tree with mean nodal ages and 95% highest posterior density (HPD) intervals are indicated by bars. The time-scale in Mya and geological time periods are shown at the bottom. The colour bar indicates the posterior probability on the node.*

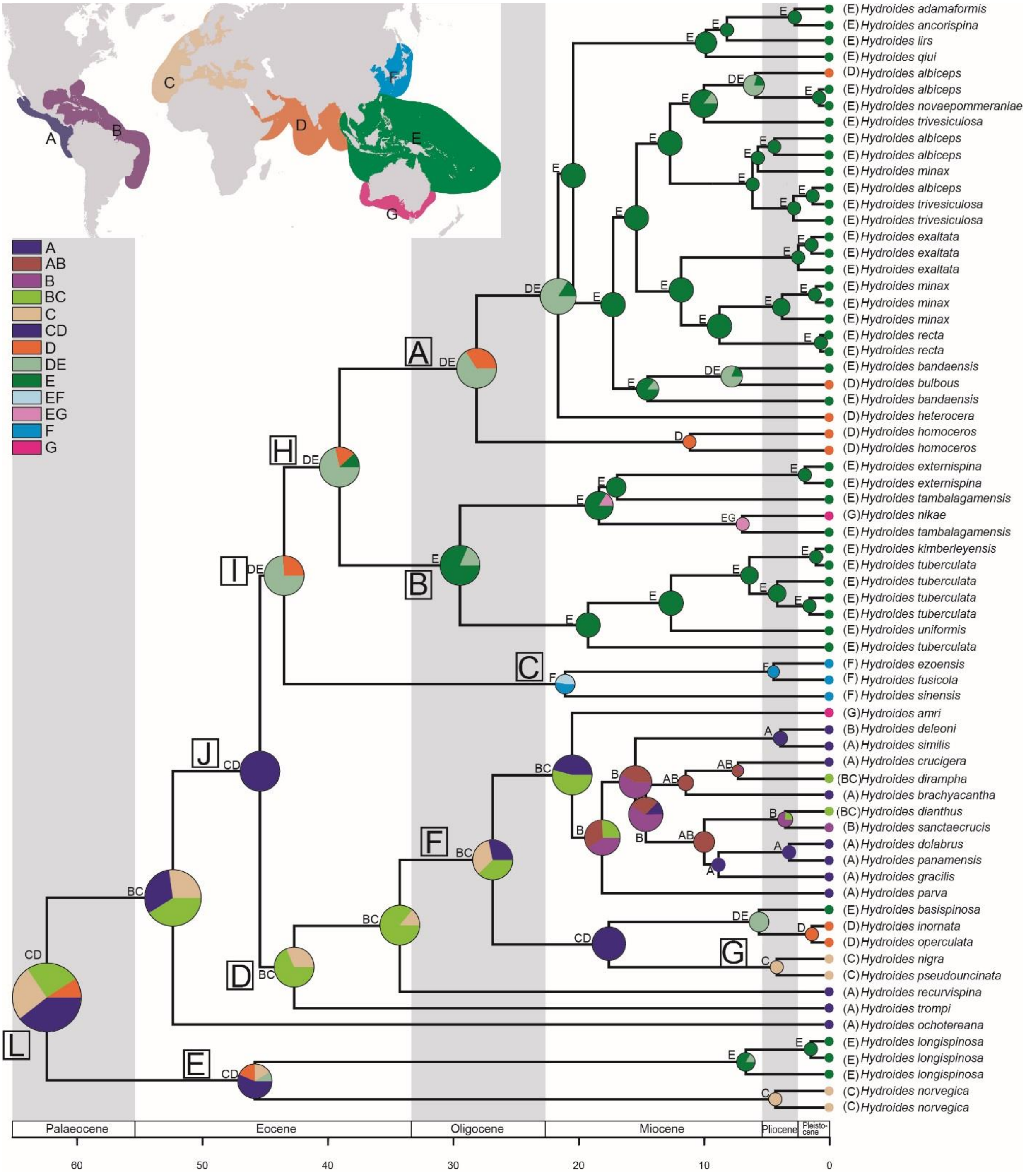


Fig. 5 Biogeographical reconstruction of ancestral ranges in the Hydroides *using DEC with time-slicing. Map on top-left corner illustrated the Biogeographic regions used in this study. A: East Pacific, B: West Atlantic, C: East Atlantic and Mediterranean, D: West Indo-Pacific, E: Central Indo-Pacific, F: Temperate Northern Pacific, G: Temperate Australasia. Pie charts and letters at nodes represent the probabilities of the most likely ancestral ranges. Letters to the left of the species names indicate current biogeographical distributions.*

4. Discussion

4.1 Phylogenetic analysis, COI barcoding, and taxonomy implications

The analyses in this study were based on more extensive sampling of taxa and loci than any previous study of *Hydroides*, and has thus resulted in the most comprehensive and best-resolved phylogeny within the genus to date. The present results agree with those of previous studies (Kupriyanova et al., 2008; Tovar-Hernández et al., 2015) that *Hydroides* is monophyletic and nested within the *Serpula-Crucigera* clade. Within *Hydroides*, analyses based on a combined dataset supported the close relationship among Mexican *H. dolabrus*, *H. panamensis*, and *H. sanctaecrucis* suggested by Tovar-Hernández et al. (2015). Compared to the 43 morphospecies used in phylogenetic analyses, the new molecular phylogeny suggests 70 distinct lineages in total by recovering multiple lineages within each of eight morphospecies *H. albiceps*, *H. bandaenisis, H. exaltata*, *H. homoceros, H. longispinosa*, *H. minax*, *H. operculata,* and *H. tuberculata,* which indicates a high genetic diversity in the Indo-Pacific region.

Of the multi-lineage morphospecies above, three genetic lineages within *H. exaltata*, *H. longispinosa*, and *H. operculata* (Fig. 6 A, B, C), as well as two genetic lineages within *H. homoceros*, were detected. Lineages of each species were recovered as a single monophyletic groups, with genetic divergences of COI among lineages ranging from 9.8% to 30.9% in *H exaltata*, 15.9% to 16.9% in *H. longispinosa*, and 10% to 19.4% in *H. operculata*. The last taxon, *Hydroides operculata*, has a long and convoluted taxonomic history. It was originally described from East Africa (Somali, Gulf of Aden) and is characterized by a verticil of incurved spines without additional spinules (Treadwell, 1929). After descriptions of other morphologically similar species from remote regions such as *H. inornata* Pillai, 1960 from Sri Lanka, *H. basispinosa* Straughan, 1967b and *H. gradata* Straughan, 1967b from Australia, taxonomists started to synonymize these species under *H. operculata* (Zibrowius, 1971; Pillai, 1971, 1972; Ishaq and Mustaquim, 1996), and used the name "*H. operculata*" to record any morphologically similar specimens from all over the world (Ben-Eliahu, 1991; Ben-Eliahu and ten Hove, 1992; Çinar, 2006; Kubal et al., 2012; Sun et al., 2012; Sun et al., 2015). Sun and Yang (2015) were the exception, and used "*H. inornata*" for specimens from southern China. They also suggested that Sun et al. (2012) incorrectly synonymized *H. inornata* from Hong Kong with *H. operculata*. As a result, *H. operculata* has been recorded with a distribution in eastern and southern Africa, the Mediterranean Sea, India, Pakistan, Sri Lanka, and Australia (Sun et al., 2016). Phylogenetic results recovered three well-supported lineages that represented specimens from 1) Kuwait, 2) Maharashtra (India) and Hong Kong, and 3) Mission Beach (Queensland, Australia) (Fig. 6C), thus rejecting the morphology-based synonymy of *H. inornata*, *H. basispinosa* and *H. gradata* under *H. operculata*. The low genetic diversity (0.1%) among specimens from Australia confirmed the synonymy of *H. basispinosa* and *H. gradata*. By referring to the type localities of each of the historical species,

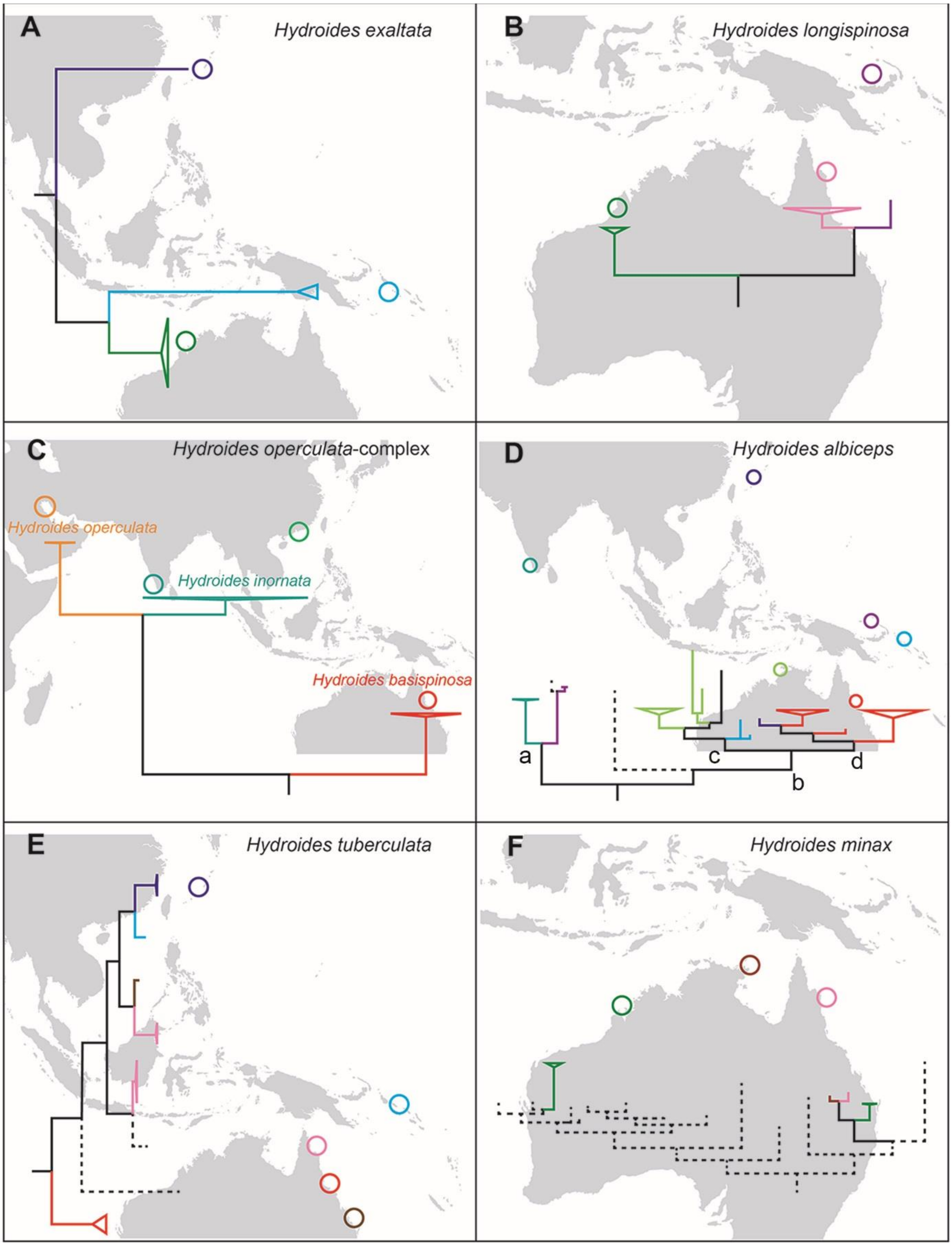


Fig. 6 Widespread species with multiple lineages reconstructed from phylogenetic analysis. Topologies are generated from the phylogenetic tree in Fig. 2. Colour circles indicates the different sampling locations. The colour of the circles is consistent to that of the lineages.

our phylogenetic results suggested that specimens from the Arabian Gulf (Kuwait) should be assigned to *H. operculata*, that specimens from India and Hong Kong should be assigned to *H. inornata*, and specimens from Australia should be assigned to *H. basispinosa* of which the type specimens were better preserved that those of *H. gradata*. Our phylogenetic results also confirmed the record of *H. inornata* from southern China by Sun and Yang (2015). Due to the absence of specimens, the status of *H. operculata* from the South Africa and the Mediterranean is still uncertain. Further studies of specimens from these regions are required to give a better understanding of this widely distributed species complex.

The multiple lineages of *H. albiceps* were recovered as a paraphyletic group, as well as that of *H. tuberculata*. *Hydroides albiceps,* which can be distinguished from other species of *Hydroides* by the operculum having a large vesicular spine and several bottle-shaped smaller spines, has been recorded as having a wide distribution in Indo-Pacific region, from the Red Sea to Polynesia (Sun et al., 2015). Our results indicated a strikingly high genetic diversity within this species by recovering seven distinct genetic lineages, representing specimens from Augustus Island (Western Australia), Mission Beach (Queensland, Australia), Darwin (Northern Territory, Australia), Maharashtra (India), Okinawa (Japan), Papua New Guinea, and Solomon Islands, respectively (Fig. 6D). Pair-wise genetic distances for COI among the eight groups ranged from 10% to 21.3%, compared to within group genetic distances ranging from 0 to 5.7%. Two main clades were recovered from the eight lineages (Fig. 2, clade a, b; Fig. 6, clade a, b). The lineages from Maharashtra and Papua New Guinea were grouped together in both BI and ML analyses (Fig. 2, 5D, clade a; PP: 1.00; BP: 100), and recovered as the sister group to other six genetic lineages. The other clade (Fig. 2, 5D, clade b) consisted of a subclade of lineages from Okinawa, Augustus Island and Mission Beach (Fig. 2, 5D, clade c; PP: 0.99; BP: 98), and specimens from the Solomon Islands and Darwin with good support (Fig. 2, 5D, clade d; PP: 0.99; BP: 98). The species *H. trivesiculosa*, which is morphologically similar to *H. albiceps,* was nested within the two main clades (Fig. 2, clade a, b) with low support values. Although previous taxonomic studies of *H. albiceps* indicated variations in number, shape and relative size of the spines among specimens from different locations (Imajima, 1976; Fiege and Sun, 1999; Sun et al., 2015), the overlap of these morphological variations made it difficult to distinguish specimens from different locations. Our phylogenetic results, however, indicated that there are at least four highly divergent lineages of *H. albiceps* morphotypes that constitute potential cryptic species. Apart from *H. albiceps, H. tuberculata* was recovered as a paraphyletic group with the incursion of *H. kimberleyensis* and *H. uniformis*. Within *H. tuberculate*, the main clade grouped specimens collected from Okinawa (Japan), Broomfield Reef (Queensland, Australia), Solomon Islands, and Lizard Island (Queensland, Australia). Specimens collected from Lizard Island were split into two paraphyletic lineages, excepted the specimens that grouped in the main clade, the other specimens from Lizard Islanded were grouped with *H. kimberleyensis,* which indicates possible presence of sympatric cryptic diversity in *H. tuberculata* morphotype. Specimens collected from Mission Beach were grouped outside of the clade consisting other specimens of *H. tuberculate*, *H. kumberleyensis* and *H. uniformis* (collected from Vanuatu).

Two genetic lineages of *H. bandaensis* representing specimens collected from Papua New Guinea and Solomon Islands did not form a clade, and had *H. bulbosa* nested within. Four genetic lineages were detected in *H. minax*. Of three of the lineages represented, one specimen was collected from Lizard Island, one from Bremer Island (Northern Territory, Australia), and two from the five specimens collected from Augustus Island (WA) were recovered as a monophyletic group with the genetic divergences of COI ranging from 11.5% to 29.4%. The other three specimens from Augustus Island were nested in the *H. albiceps* clade (Fig. 2).

The multiple lineages detected in several morphospecies indicate the potential presence of new species awaiting formal description. However, we were unable to demonstrate clear distinguishing morphological characters to separate these genetic lineages. Previous studies indicated that cryptic species of polychaetes discovered through genetic approaches regularly correlated with variation in traits such as protracted reproductive isolation, difference in life history characteristics, or ecological differences (Manchenko and Radashevsky, 1993; Sato and Masuda, 1997; Rice et al., 2008; Carr et al., 2011). Thus, further studies investigating a diverse range of traits may facilitate new cryptic species description that supports the genetic identification.

4.2 Operculum evolution of Hydroides

The hypothesis of the opercular transformation series in serpulids was demonstrated by the shift in opercular structure from a simple a soft swollen tip of the radiole, to a complex operculum such as the two-tiered operculum in *Hydroides*. The hypothesis has been considered as a general evolutionary trend in Serpulidae (ten Hove 1984). The two-tiered operculum of *Hydroides* is suggested to consist of the lower funnel, which is homologous with the single funnel-shaped operculum of *Serpula* and the upper verticil representing an apomorphic feature in *Hydroides* (Uchida, 1978; Kupriyanova et al., 2008). However, our dating estimates indicated that the opercular structure within *Hydroides* does not show a transformation series from simple to complex. The ancestor of species such as *H. norvegica*, *H. elegans* and *H. longsipinosa,* having the most complex opercula with both multiple lateral and internal spinules on the verticil spines, diverged from all the other species in the Mid-Palaeocene. The second divergence from the remaining species is *H. ochotereana* in the early Eocene, with only multiple lateral spinules on the verticil spines. Any subsequent diversification of the operculum structure seems to be localized. Species with thick, swollen, or bulbous verticil spines (Fig. 4, clades M, L and N) originated in the Indo-Pacific, while species that originated in the east Pacific commonly displayed elongated verticil spines with sharp tips (Fig. 4, clade D). *H. ezoensis* and *H. sinensis* contained only internal spinules on the verticil spines and occurred in the temperate northern Pacific. The reconstruction of divergence times indicated that most opercular diversification occurred during the Miocene (5–23 Mya). The lineages formed from the Pliocene onwards showed subtle morphological differences (e.g., multiple genetic lineages of morphospecies discussed above) or overlapped in the extend of variation with respect to morphology (e.g., *H. dolabrus* and *H. panamensis*). In some marine organisms, the high genetic diversity as found in the central Indo-Pacific and originated in the Pleistocene tended to be accompanied by a low level of morphological contrast (Roy et al., 2001; Briggs; 2006), suggesting that the morphological stasis may be involved (Knowlton, 2000). The diversity and distribution of the present species in the central Indo-Pacific could corresponds to sea level changes during Pliocene and Pleistocene (Briggs, 2006; Knowlton, 2000; Pellissier et al., 2014).

4.3 Historical biogeography

Results from historical geographic reconstruction indicate that the most probable range of the most recent common ancestor of *Hydroides* was in the western Atlantic and Indian Ocean, which was a tropical Indo-Mediterranean region in the Mid-Palaeocene (Briggs, 2006) or so called the west Tethyan region (Renema et al., 2008). The tropical Indo-Mediterranean region has been suggested as a source of marine organisms with broad diversity in the Indo-Pacific region, such as coral reef fishes and the giant clams (Bellwood and Meyer, 2009; Herrera et al., 2016). Our results are consistent with the record of the oldest fossil of *Hydroides* from the Mediterranean (Lommerzheim, 1981). The results also indicated isolation between the Indo-Pacific clade (Fig. 5, clade I) and the eastern tropical Pacific and Atlantic clade (Fig. 5, clade D) since the Mid-Eocene with few dispersal events post isolation. In each of the two major clades, the peak of lineage diversification occurred during the Miocene, which is concordant with the common appearance of tube fossils of the *Hydroides* morphotype at that time (Ippolitov et al., 2014). In clade I (Fig. 5), the initial cladogenetic event separated the ancestor of *H. ezoensis* + *H. fusicola* + *H. sinensis* (Fig. 5, clade C) in the temperate Northern Pacific from the tropical and subtropical species in the same clade. The reconstruction identified the central Indo-Pacific region as a centre of palaeodiversity of molecular lineages in clade H from the early Miocene, evidenced by the highly localized cladogenesis. Also, the central Indo-Pacific region appeared to act as a source for the nearby Indian Ocean and temperate Australia from the Miocene via dispersal and subsequent vicariant separation of *H. albiceps* and *H. nikae*. In the east Pacific and Atlantic clade (Fig. 5, clade D), the split between the western Atlantic and the Mediterranean groups occurred in the late Oligocene. The ancestor of *H. operculata* was reconstructed with a wide distribution in the Mediterranean and Indian Ocean during the early Miocene, with diversification occurring after the closure of the Tethys seaway (Ree and Sanmartín, 2009). The continuous range dispersal and diversification of the ancestor of *H. operculata* in Indo-Pacific region led to the speciation of *H. basispinosa* in Australia, *H. operculata* in Kuwait, and *H. inornata* in the west coast of India.

Although the Indian Ocean has been suggested as part of the ancestral range of *Hydroides*, our reconstruction observed limited origins of current molecular lineages in the Indian Ocean before the Miocene. Instead, our results indicated the Indian Ocean as a recipient and the central Indo-Pacific as the major donor. The pattern in the Indian Ocean and the central Indo-Pacific reflects a shift in historical biodiversity hotspots in marine organisms during the past 50 million years (Renema et al., 2008). The west Tethys Sea, which served as a biodiversity hotspot for many marine organisms (Ellison et al., 1999; Harzhauser et al., 2007; Renema, 2007), was dramatically affected by tectonic rearrangement and global cooling during the Oligocene and early Miocene (20 Ma, Hallam, 1994; Cowman and Bellwood, 2013), which led to large extinctions from the west Tethys and an eastward shift of west Tethyan fauna (Kay, 1996; Harzhauser et al., 2007; Renema et al., 2008). Meanwhile, the central Indo-Pacific region has shown a high capacity to maintain and support ancestral lineages (Cowman and Bellwood, 2011; Cowman et al., 2013), and hosted the initial divergences from Miocene onwards, which led to the emerging of the central Indo-Pacific as a centre for diversity.

5. Conclusions

This is the first study to include a nearly comprehensive representation of *Hydroides* lineages worldwide. Although not all described species of the genus could be sampled, this study demonstrate that phylogenetic relationships within *Hydroides* are more concordant with geographical than morphological expectations. The presence of high genetic diversity accompanied by morphologically similar species in the central Indo-Pacific region calls for investigation of traits in addition to morphological characteristics such as mode reproduction, particular life history characteristic, or defining ecological differences in the further new species description. Our dating and historical biogeographic reconstruction results suggest possible origin and subsequent diversification of ancestral *Hydroides* in the western Tethys Sea and a subsequent shift of centers of biodiversity from the Indo-Mediterranean region to the central Indo-Pacific during last 50 Million years. Further paleogeographic studies based on *Hydroides* fossil tubes would provide a test for this biogeographic hypothesis.

Acknowledgement

The study was funded by Australian Biological Resource Survey (ABRS) grant RF213-19 to EKK. We thank the Department of Biological Sciences at Macquarie University for an International Macquarie University Research Excellence Scholarship (iMQRES) awarded to YS, and the Lizard Island Reef Research Foundation grant to Anne Hoggett, Pat Hutchings, and EKK for sampling at Lizard Island, Great Barrier Reef. We are grateful to Chris Glasby from the Museum & Art Gallery Northern Territory, João Miguel de Matos Nogueira, Orlemir Carrerette, Felipe Ramon De Chiara, and Carlos Diego from the University of São Paulo in Brazil who helped with our sampling. We thank Anja Schulze, Aylin Ulman, Eijiroh Nishi, Elena Lisitskaya, Erica Keppel, Giuseppe Suaria, James Carlton, Jixing Sui, Joachim Langeneck, María A. Tovar-Hernández, Maria Capa, Melih Ertan Çinar, Priti Kubal, Tulio F. Villalobos-Guerrero, and Xuwen Wu who collected samples around the world and generously donated them for this study. We also thank Simon Ho for his advise on analyses using BEAST, and Alexei Ippolitov for his comments on fossil records of *Hydroides*.

References

Bastida-Zavala, J.R., 2008. Serpulids (Annelida: Polychaeta) from the Eastern Pacific, including a short mention of Hawaiian serpulids. Zootaxa 1722, 1–61.

Bastida-Zavala, J.R., Hove, H.A. ten, 2002. Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the western Atlantic region. Beaufortia 52, 103–178.

Bastida-Zavala, J.R., Hove, H.A. ten, 2003. Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the eastern Pacific Region and Hawaii. Beaufortia 53, 67–110.

Bellwood, D.R., Meyer, C.P., 2009. Searching for heat in a marine biodiversity hotspot. J. Biogeogr. 36, 569–576.

Ben-Eliahu, M.N., 1991. Red Sea serpulids (Polychaeta) in the eastern Mediterranean. In: Petersen, M. E., Kirkegaard, J. B., (Eds.), Proceedings of 2nd International Polychaeta Conference, Copenhagen, 1986. Systematics, Biology and Morphology of World Polychaeta. Ophelia Suppl. 5, 515–528.

Ben-Eliahu, M.N., Hove, H.A. ten, 1992. Serpulids, Annelida: Polychaeta, along the Mediterranean coast of Israel—New population build-ups of Lessepsian migrants. Israel. J. Zool. 38(1), 35–53.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, MA., Rambaut, A., & Drummond, A. J., 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput. Biol. 10(4), e1003537.

Briggs, J.C., 2006. Proximate sources of marine biodiversity. J. Biogeogr. 33, 1–10.

Bush, K.J., 1905. Tubicolous annelids of the tribes Sabellides and Serpulides from the Pacific Ocean. Harriman Alaska Expedition 12, 169–346.

Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T.A., Hebert, P.D.N., 2011. A Tri-Oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS ONE 6, e22232.

Çinar, M.E., 2006. Serpulid species (Polychaeta: Serpulidae) from the Levantine coast of Turkey (eastern Mediterranean), with special emphasis on alien species. Aquat. Invasions 1(4), 223–240.

Cowman, P.F., Bellwood, D.R. 2011. Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. J. Evol. Biol. 24, 2543–2562.

Cowman, P.F., Bellwood, D.R., Rocha, L.A., 2013. The historical biogeography of coral reef fishes: global patterns of origination and dispersal. J. Biogeogr. 40, 209– 224.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8), 772.

Dew, B., 1959. Serpulidae (Polychaeta) from Australia. Rec. Aust. Mus. 25, 19–56.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Ellison, A.M., Farnsworth, E.J., Merkt, R.E., 1999. Origins of mangrove ecosystems and the mangrove biodiversity anomaly.Glob. Ecol. Biogeogr. 8, 95–115.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Fiege, D., Sun, R., 1999. Polychaeta from Hainan Island, South China Sea Part I: Serpulidae. Senckenb. Biol. 79, 109–141.

Grube, A.E. 1878. Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen. Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 25(8), 1–300.

Gunnerus, J.E., 1768. Om nogle Norske Coraller. Kongel. Norske Vidensk. Selsk. Skr. (Trondheim) 4, 38–73.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic. Acids. Symp. Ser. 41, 95–98.

Hallam, A., 1994. An outline of Phanerozoic biogeography. Oxford University Press, Oxford.

Hartman, O. 1959. Catalogue of the polychaetous annelids of the world. Part II. Allan Hancock Found. Publ. Occas. Pap. 23(2), 354–628.

Harzhauser, M., Kroh, A., Mandic, O., Piller, W.E., Göhlicha, U., Reuter, M., Berning, B., 2007. Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene Tethyan Seaway. Zool. Anz. 246, 241–256.

Haswell, W.A. 1883. On some new Australian tubicolous annelids. Proc. Linn. Soc. NSW 7, 633–638.

Herrera, N.D., ten Poorten, J.J., Bieler, R., Mikkelsen, P.M., Strong, E.E., Jablonski, D., Steppan, S.J., 2015. Molecular phylogenetics and historical biogeography amid shifting continents in the cockles and giant clams (Bivalvia: Cardiidae). Mol. Phylogenet. Evol. 93, 94–106.

Hove, H.A. ten 1984. Towards a phylogeny in serpulids (Annelida; Polychaeta). In: Hutchings, P.A., (Ed.). Proceeding of the First International Polychaete Conference, Sydney. Linnaean Society of New South Wales, Sydney, pp. 181–196.

Hove, H.A. ten, Kupriyanova, E.K., 2009. Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs. Zootaxa 2036, 1–126.

Huelsenbeck, J.P., Ronquist, F.R., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huggett, M.J., Nedved, B.T., Hadfield, M.G., 2009. Effects of initial surface wettability on biofilm formation and subsequent settlement of *Hydroides elegans*. 25(5), 387–99.

Imajima, M., 1976. Serpulid polychaetes from Tanega-shima, southwest Japan. Mem. Natl. Sci. Mus. Tokyo 9, 123–143.

Ippolitov, A.P., Vinn, O., Kupriyanova, E.K., 2015. Written in stone: history of serpulid polychaetes through time. Mem. Mus. Vic. 71, 123–159.

Ishaq, S., Mustaquim, J., 1996. Polychaetous annelids (order Sabellida) from the Karachi coast, Pakistan. Pakistan J. Mar. Sci. 5(2), 161–197.

Jones, M.L., 1962. On some polychaetous annelids from Jamaica, the West Indies. Bull. Am. Mus. Nat. Hist. 124(5), 169–212.

Kay, E.A., 1996. Evolutionary radiations in the Cypraeidae. In: Taylor, J.D. (Ed.), Origin and Evolutionary Radiation of the Mollusca. Oxford University Press, Oxford, pp. 211–220.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420, 73–90.

Kodandaramaiah, U., 2010. Use of dispersal-vicariance analysis in biogeography a critique. J. Biogeogr. 37, 3–11.

Kubal, P., Sukumaran, S., Vijapure, T., 2012. Record of bi-operculate fouling serpulid polychaete, *Hydroides operculatus* (Treadwell, 1929) from Diu, west coast of India. Indian J. Geo-Mar. Sci., 41(4), 381–384.

Kupriyanova, E.K., Bastida-Zavala, R., Halt, M.N., Lee, M., and Rouse, G.W., 2008. Phylogeny of the *Serpula-Crucigera-Hydroides* clade (Serpulidae; Annelida) using molecular and morphological data: implications for operculum evolution. Invertebr. Syst. 22, 425-437.

Kupriyanova, E.K., Nishi, E., Hove, H.A. ten, Rzhavsky, A.V., 2001. Life-history patterns in Serpulimorph polychaetes: ecological and evolutionary perspectives. Oceangr. Mar. Biol. 39, 1–101.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Lau, S.C.K., Mak, K.K.W., Chen, F., Qian, P.Y., 2002. Bioactivity of bacterial strains from marine biofilms in Hong Kong waters for the induction of larval settlement in the marine polychaete *Hydroides elegans*. Mar. Ecol. Prog. Ser. 226, 301–310.

Lewis, J.A., Watson, C., Hove, H.A. ten, 2006. Establishment of the Caribbean serpulid tubeworm *Hydroides sanctaecrucis* Krøyer in Mörch, 1863, in northern Australia. Biol. Invasions. 8, 665–671.

Link. H., Nishi, E., Tanaka, K., Bastida-Zavala, R., Kupriyanova, E.K., Yamakita, T., 2009. *Hydroides dianthus* (Polychaeta: Serpulidae), and alien species introduced into Tokyo Bay, Japan. Mar. Biodivers. Rec. 2, 1–5.

Lommerzheim, A., 1981. Paläozäne Serpulidae und Spirorbidae (Polychaeta) von den Emperor Seamounts, NW-Pazifik. Zitteliana 7, 31–54.

Manchenko, G.P., Radashevsky, V.I. 1993. Genetic differences between two sibling species of the *Polydora ciliata* complex (Polychaeta: Spionidae). Biochem. Syst. Ecol. 21, 543–548.

Minh, B.Q., Nguyen, M.A.T., von Haeseler A., 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188–1195.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.

Okuda, S., 1934. Some tubicolous annelids from Hokkaido. Journal of the Faculty of Science, Hokkaido University, Series 6, Zoology 3(4), 233–246.

Pellissier, L., Leprieur, F., Parravicini, V., Cowman, P.F., Kulbicki, M., Litsios, G., Olsen, S.M., Wisz, M.S., Bellwood, D.R., Mouillot, D., 2014. Quaternary coral reef refugia preserved fish diversity. Science 344(6187), 1016–1019.

Pettengill, J.B., Wendt, D.E., Schug, M.D., Hadfield, M.G., 2007. Biofouling likely serves as a major mode of dispersal for the polychaete tubeworm *Hydroides elegans* as inferred from microsatellite loci. Biofouling 23, 161–169.

Philippi, A., 1844. Einige Bemerkungen über die Gattung Serpula, nebst Aufzählung der von mir im Mittelmeer mit dem Their beobachteten Arten. Archiv für Naturgeschichte, Berlin, 10, 186–198. Ann. Mag. Nat. Hist. 14, 153–162.

Pillai, T.G., 1960. Some marine and brackish water serpulid Polychaeta from Ceylon, including new genera and species. Ceylon J. Sci. Biol. Sci. 3(1), 1–40.

Pillai, T.G., 1961. Annelida Polychaeta of Tambalagam Lake, Ceylon. Ceylon J. Sci. Biol. Sci. 4(1), 1–40.

Pillai, T.G., 1971. Studies on a collection of marine and brackish-water polychaete annelids of the family Serpulidae from Ceylon. Ceylon J. Sci. Biol. Sci. 9(2), 88–130.

Pillai, T.G., 1972. A review and revision of the systematics of the genera *Hydroides* and *Eupomatus* together with an account of their phylogeny and zoogeography. Ceylon J. Sci. Biol. Sci. 10(1), 7–31.

Pixell, H.L.M., 1913. Polychaeta of the Indian Ocean, together with some species from the Cape Verde Islands. The Serpulidae, with a classification of the genera *Hydroides* and *Eupomatus*. Trans. Linn. Soc. Lond. 2 (16), 69–92.

Qian, P.Y., Lau, S.C.K., Dahm, H.U., Dobretsov, S., Harder, T., 2007. Marine biofilms as mediators of colonization by marine macroorganisms: Implications for antifouling and aquaculture. Mar. Biotechnol. 9, 399–410.

Read, G., Hove, H.A. ten, Sun, Y., Kupriyanova, E.K., 2017. *Hydroides* Gunnerus, 1768 (Annelida: Serpulidae) is feminine: a checklist of updated names. Zookeys in press

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.

Ree, R.H., Sanmartín, I., 2009. Prospects and challenges for parametric models in historical biogeographical inference. J. Biogeogr. 36, 1211–1220.

Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.

Renema, W., 2007. Fauna development of larger benthic foraminifera in the Cenozoic of Southeast Asia. In: Renema, W. (Ed.), Biogeography, Time and Place: Distributions, Barriers and Islands. Springer, Dordrecht, pp. 179–215.

Renema, W., Bellwood, D.R., Braga, J.C., Bromfield, K., Hall, R., Johnson, K.G., Lunt, P., Meyer, C.P., McMonagle, L.B., Morley, R.J., O’Dea, A., Todd, J.A., Wesselingh, F.P., Wilson, M.E.J., Pandolfi, J.M., 2008. Hopping hotspots: Global shifts in marine biodiversity. Science 321, 654–657.

Rice, S.A., Stephen, K., Rice, K.A., 2008. The *Polydora cornuta* complex (Annelida: Polychaeta) contains populations that are reproductively isolated and genetically distinct. Invert. Biol. 127, 45–64.

Rioja, E., 1958. Estudios Anelidologicos. XXI. Observaciones acerca de algunas especies de serpulidos de los generos *Hydroides* y *Eupomatus* de las costas Mexicanas del Golfo de Mexico. An. Inst. Biol. Mex. 28(1/2), 247–266.

Ronquist, F.,Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A.,Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Syst. Biol. 61(3), 539–542.

Rouse, G.W., Pleijel, F., 2001. Polychaetes. Oxford University Press, Oxford, 354 pp.

Rovereto, G., 1904. Studi monografici sugli Anellidi fossili, 1, Terziario. Palaeontogr. ital. 10, 1–73.

Roy, K., Balch, D.P., Hellberg, M.E., 2001. Spatial patterns of morphological diversity across the Indo-Pacific: analyses using strombid gastropods. Proc. R. Soc. Lond. [Biol]. 268, 2503–2508.

Sato, M., Masuda, Y., 1997. Genetic differentiation in two sibling species of the brackish-water polychaete *Hediste japonica* complex (Nereididae). Mar. Biol. 130, 163–170.

Shikuma, N.J., Antoshechkin, I., Medeiros, J.M., Pilhofer, M., Newman, D.K., 2016. Stepwise metamorphosis of the tubeworm *Hydroides elegans* is mediated by a bacterial inducer and MAPK signalling. Proc. Natl. Acad. Sci. U.S.A. 113(36), 10097–10102.

Spalding, M.D., Allen, G.R., Davidson, N., Ferdana, Z.A., Finlayson, M., Fox, H.E., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. Bioscience 57, 573–583.

Straughan, D., 1967a. Marine Serpulidae (Annelida: Polychaeta) of eastern Queensland and New South Wales. Aust. J. Zool. 15(1), 201–261.

Straughan, D., 1967b. Some Serpulidae (Annelida: Polychaeta) from Heron Island, Queensland. University of Queensland Papers [Great Barrier Reef Committee, Heron Island Research Station] 1(2), 27–45.

Sun, R., Yang, D., 2015. Fauna Sinica of China, Invertebrata Polychaeta (III): Sabellida. Science Press, Beijing.

Sun, Y., Kupriyanova, E.K., Qiu, J.W., 2012. CO1 barcoding of *Hydroides*: a road from impossible to difficult. Invertebr. Syst. 26, 539–547.

Sun, Y., Wong, E., Hove, H.A. ten, Hutchings, P.A., Williamson, J.E., Kupriyanova, E.K. 2015. Revision of the genus *Hydroides* (Annelida: Serpulidae) from Australia. Zootaxa 4009 (1), 1–99.

Sun, Y., Wong, E., Tovar-Hernández, M.A., Williamson, J.E., Kupriyanova, E.K., 2016. Is *Hydroides brachyacantha* (Serpulidae: Annelida) a widespread species? Invertebr. Syst. 30(1), 41–59.

Tovar-Hernández, M.A., Villalobos-Guerrero, T.F., Kupriyanova, E.K., Sun, Y., 2015. A new fouling *Hydroides* (Annelida, Sabellida, Serpulidae) from southern Gulf of California. J. Mar. Biol. Assoc. U. K. 96(3), 693–705.

Treadwell, A.L., 1929. New species of polychaetous annelids in the collections of the American Museum of Natural History from Porto Rico, Florida, Lower California, and British Somaliland. Am. Mus. Novit. 392, 1–13.

Uchida, H., 1978. Serpulid tube worms (Polychaeta, Sedentaria) from Japan with the systematic review of the group. Bull. Mar. Park Res. Sta. 2, 1–98.

Vinn, O., 2007. Taxonomic implications and fossilization of tube ultrastructure of some Cenozoic serpulids (Annelida, Polychaeta) from Europe. Neues Jahrb. Geol. Paläontol. 244, 115–128.

Vinn, O., Hove, H.A. ten, Mutvei, H., Kirsimäe, K., 2008. Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida). Zool. J. Linnean Soc. 154, 633–650.

WoRMS, 2016. *Hydroides* Gunnerus, 1768. In: Read, G., Fauchald, K., (Eds.) World Polychaeta database. Accessed through: http://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=129566 on 2016-10-23.

Yu, Y., Harris, A.J., Blair, C., He, X.J., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49.

Zibrowius, H., 1971. Les espèces Méditerranéennes du genre *Hydroides* (Polychaeta Serpulidae). Remarques sur le prétendu polymorphisme de *Hydroides uncinata*. Téthys 2, 691–746.

Chapter 9

General summary and conclusions

Author:
Yanan Sun

Integrating morphological and molecular approaches, this thesis provides the most comprehensive revision of the genus *Hydroides*, one of the most economically important but poorly known groups of marine invertebrates that includes notorious foulers and common bioinvaders.

A taxonomic revision of *Hydroides* based on morphology, both specifically for Australia (Chapter 2) and globally (Chapter 3) was completed. This represents the first ever dedicated study of *Hydroides* from Australia (Chapter 2) and was based both on the historical collections from Australian museums (Australian Museum, Museum Victoria, South Australian Museum, Western Australian Museum, Queensland Museum, and Museum and Art Gallery of Northern Territory) and newly collected material from New South Wales, Victoria, Queensland, Northern Territory, and Western Australia. As a result, full descriptions and illustrations of 25 species considered valid in Australia were compiled, including three newly described species: *H. amri* n. sp., *H. glasbyi* n. sp. and *H. qiui* n. sp., and two new records of *H. furcifera* and *H. multispinosa* for Australia. In addition, based on examination of the type specimens of the 98 species from collections of 21 museums from all over the world, as well as the comparison between the morphology of the type specimens and the original description for each species, diagnoses and original high-quality photographs illustrating all species of *Hydroides* were completed (Chapter 3). As a part of the synopsis, for the first time ever a comprehensive and fully-illustrated taxonomic key to all species of *Hydroides* was developed in Chapter 3. In addition, Chapter 3 included updates of all *Hydroides* specific names to the feminine form. Such an update was needed because the gender of the genus name *Hydroides* was corrected to its original feminine form according to the International Code of Zoological Nomenclature (ICZN, 1999), but frequently overlooked by taxonomists since that change (Read et al., 2017).

Adding the molecular approach to this study helped improve the understanding of the taxonomy and phylogeny of *Hydroides*. Over the past few decades, the use of genetic data in taxonomy has led to the frequent detection of cryptic species in a wide variety of taxa, dramatically increasing estimates of species diversity (Bickford et al., 2007; Nygren, 2014). By integrating morphological examination and molecular analyses, the status of two putatively widespread taxa *H. brachyacantha* (Chapter 4) and *H. dianthus* (Chapter 5), both of which have been reported as important fouling and invasive species, was investigated. The present study showed that both taxa as traditionally understood were species complexes. The *H. brachyacantha*-complex, with at least three species now recognized, consists of two morphologically distinct species: *H. brachyacantha sensu stricto* from Mexico, and *H. amri* newly described from Australia (Chapter 2), as well as one cryptic species close to *H. amri* from Australia, *H. nikae* (described in Chapter 4). Also the widely distributed species *H.*

dianthus was confirmed as a global invader, and additionally revealed to be a complex of two cryptic invasive species. These studies corroborate the general statement that the wide distribution of marine invertebrates usually reflects an incomplete understanding of the biodiversity and biogeography at species level, and the widespread species most likely consist of a number of undiscovered cryptic or even morphologically distinct species (Geller et al. 2010). Thus, any “well-known” globally-distributed invasive species should be treated as suspect unless proven otherwise.

The results of Chapter 5 further confirmed that COI barcoding is extremely useful in detecting cryptic speciation in *Hydroides*. However, COI barcoding has not been widely applied for species identification and delimitation of *Hydroides* due to a lack of suitable primers and reference sequences for primer design. To obtain reference sequences for new designing primers, genomes of eight species of *Hydroides* were sequenced using the Illumina HiSeq (Chapter 6). Analyses of the mitochondrial gene sequences from the whole genome data revealed surprising genomic features in *Hydroides*, such as 1) missing atp8 gene, which is the first absence of atp8 observed in annelids to date; 2) unusually numerous non-coding regions, which resulted in a larger size of mitochondrial genomes of *Hydroides* than those of most annelids; 3) significant gene rearrangements that led to the gene order in *Hydroides* mitochondrial genomes being completely different from that of any other reported annelid mitochondrial genomes. The phylogenetic analyses based on mitochondrial protein coding genes of eight *Hydroides* species and 24 species representing the main clades of Lophotrochozoa recovered all species of *Hydroides* as a monophyletic group placed at the end of a long branch. A strong nucleotide usage bias of G and T observed in *Hydroides* probably correlated to the gene rearrangements and the long branch of the *Hydroides* clade recovered in phylogenetic analyses.

Referring to the eight entire COI gene sequences extracted from the sequenced mitochondrial genomes, new taxon-specific COI primer sets targeting the barcoding region of *Hydroides* were developed (Chapter 7). Using the new primer sets, the barcoding regions in 44 of 46 available species of *Hydroides* were successfully amplified and sequenced. The high success rate of amplification indicated that the new primers work on all species of *Hydroides* and the well-known problems of barcoding in *Hydroides* have now been resolved as a result of this work. The results of Chapter 7 not only provide the first barcode reference library for species identification of *Hydroides* on a global scale, but also confirmed the efficacy of this method for species delimitation in *Hydroides*, especially for detecting potential cryptic species. Integrated analyses of mitochondrial (COI, cyt b) and nuclear (18S, 28S and ITS) markers for 284 individuals of 46 morphospecies of *Hydroides* collected from 48 locations in 16 countries, further supported the genetic relationships within *Hydroides* revealed by COI barcoding data (Chapter 8). The phylogenetic analyses resulted in a well-supported phylogeny which demonstrated high genetic diversity within *Hydroides*, and revealed potential cryptic species and species complexes. The phylogenetic relationships within *Hydroides* were in greater concordance with geography rather than patterns expected by morphology. The molecular divergence estimates suggested a possible origin and subsequent diversification of the ancestor of *Hydroides* in the western Tethys Sea and the subsequent shift of the centre of diversity from the Indo-Mediterranean region to the central Indo-Pacific during last 50 million years.

In summary, studies in the thesis significantly advance our knowledge of the largest and most economically important genus of calcareous tubeworms in Australia and worldwide, provide a valuable resource for species identification, facilitate discovery of undescribed native species, and help distinguish native species from exotics.

Limitations of the study

Due to obvious restrictions of sampling overseas, this thesis mainly is biased towards the Australian regions. In taxonomic revision of the entire genus (Chapter 3), as many type specimens as possible were illustrated, but there are still species of which type specimens are lost, unavailable for observation, or perhaps never designated. Thus, diagnoses of these species were based on original descriptions rather than on studies of the type material. The lack of type specimens and incomplete or obscure descriptions in the historical literature made the possible synonymization of some species difficult. For instance, whereas two Indo-Pacific species, *H. externispina* and *H. ralumiana*, could not be separated according to their respective (very cursory) original descriptions, these two species could not be synonymised due to the missing type specimens of *H. ralumiana*. A similar situation was observed for widely-distributed *H. sanctaecrucis* and the poorly known species *H. vizagensis* from India with the type specimens of the latter species missing. In molecular analyses of Chapter 5, without sufficient specimens from each location, further population genetic studies to test the hypothesis of the dispersal routes of this taxon were hindered. In Chapters 7 and 8 all species from Africa, most species reported from the East Atlantic and South China Sea, as well as more than half of species reported from the Caribbean region were not included in the phylogenetic analyses due to unavailability of specimens from the regions above, which prevented the comprehensive study of genetic diversity in Atlantic, Mediterranean and Indian Ocean.

Another limitation of this study was the paucity of fossil data on *Hydroides*. Because of this, only limited calibration points were available to reconstruct the divergence time among species of *Hydroides*. Although among the mostly soft-bodied annelids, serpulids have the best fossil record in the form of the calcareous tubes, but it is difficult to link tube fossils to current species without ultrastructural examinations. This is especially true for *Hydroides* due to high similarity of the external tube morphology among species of the group. Fortunately, distinguishing tube fossils at genus level is relatively reliable, so the dates of the nearest fossil representing each genus of *Hydroides* and *Serpula* were used in this study to calculate the divergence time. However, because using only two calculation nodes might lead to a bias in age estimates (Zheng and Wiens, 2015), the divergence time estimated in this study should be further verified in future studies.

Future directions

It should be aware that the gender of genus *Hydroides* has been reverted to the original feminine from 2000, according to the 4th edition of International Code of Zoological Nomenclature (ICZN), thus an update on species names is needed in all further studies or reports. Further integrative studies combing traditional morphological and modern genomic approaches are needed for species of *Hydroides*. For the described species for which the holotype was missing, a neotype should be designated, accompanied with a full description and barcoding sequences if possible. Further taxonomic revisions of the entire genus should focus on revealing the intraspecific morphological diversity of each species, which would substantially improve current knowledge on the status of morphospecies in *Hydroides*.

As demonstrated in Chapters 4 and 5, the present widespread species or species-complexes of *Hydroides* are highly likely to consist of undiscovered cryptic species and even morphologically distinct species. Thus, any future reports of "*H. brachyacantha*" from outside Mexico should be treated with caution. Further molecular studies on nominal "*H. brachyacantha*" reported from Brazil, Hawaii, India, Japan, the Mediterranean and Micronesia are needed to improve our knowledge on the status of this species complex. For the known invasive *H. dianthus*, further studies are needed to determine the genetic variability within the species complex before the genetic lineages from Texas and the Black Sea can be formally described as a new species.

Further studies are also needed to investigate the status of species consisting of multiple genetic lineages revealed in Chapter 8, some of which, such as *Hydroides operculata* and *H. homoceros,* have been reported as important invasive species (Streftaris and Zenetos, 2006). Population genetic studies based on a large number of samples from across a wide range for each of morphospecies, coupled with morphological examinations, would improve our knowledge on the status of the species with high genetic diversity, reveal potential cryptic species and assist in the understanding of their geographic distribution patterns and native ranges.

Further paleogeographic studies on *Hydroides* tube fossils, especially using fossil records from Africa, would provide a test for the biogeographic hypothesis suggested in Chapter 8. Studies on comparing ultrastructure of both tube fossils and tubes of the current species of *Hydroides* would help assigning fossil tubes to current species, thus improving the divergence time analyses by providing additional calculation nodes.

Finally, continued monitoring and management of the routes responsible for introductions of invasive species of *Hydroides* is needed.

References

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., and Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**, 148–155.

Geller, J.B., Darling, J.A., and Carlton, J.T. (2010). Genetic perspectives on marine biological invasions. *The Annual Review of Marine Science* **2**, 367–393

ICZN (1999). International Code of Zoological Nomenclature Fourth edition adopted by the International Union of Biological Sciences. The International Trust for Zoological Nomenclature, London. 306 pp.

Nygren, A. (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta* **43**, 172–183

Read, G.B., ten Hove, H.A. Sun, Y., and Kupriyanova, E.K. (2017). *Hydroides* Gunnerus, 1768 (Annelida: Serpulidae) is feminine: a nomenclatural checklist of updated names. *Zookeys* in press

Streftaris, A., and Zenetos, N. (2006). Alien marine species in the Mediterranean – the 100 ‘worst invasives’ and their impacts. *Mediterranean Marine Science* **7**, 87–118.

Zheng, Y., and Wiens, J.J. (2015). Do missing data influence the accuracy of divergence-time estimation with BEAST? *Molecular Phylogenetics and Evolution* **85**, 41–49.

Appendix

Appendix table 1. Collection information of specimens used in Chapter 7 and 8.

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W47046	*Hydroides adamaformis*	Clerke Reef, Western Australia	Australia	17°00'30.6"S, 119°12'35.8"E	3/10/2014	+	+	+		+
W48807	*Hydroides albiceps*	Heron Island, Queensland	Australia	23°26'39.3"S, 151°54'46.4"E	7/04/2016		+			
W48845	*Hydroides albiceps*	Ratnagiri, Maharashtra	India	17°04'41.11"N, 73°17'23.03" E	30/03/2016	+	+	+	+	+
W48842	*Hydroides albiceps*	Sindhudurg, Maharashtra	India	16°22'15.26"N, 73°22'17.58"E	28/03/2016	+	+	+	+	+
W48843	*Hydroides albiceps*	Sindhudurg, Maharashtr	India	16°22'15.26"N, 73°22'17.58"E	28/03/2016	+	+	+	+	
W48844	*Hydroides albiceps*	Sindhudurg, Maharashtra	India	16°22'15.26"N, 73°22'17.58"E	28/03/2016	+	+	+	+	
W46551	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	16/05/2014	+	+	+	+	+
W46553	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	16/05/2014	+	+	+	+	+
W46555	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	16/05/2014	+	+	+	+	+
W46565	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	17/05/2014	+	+	+		
W46583	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	16/05/2014	+		+	+	+
W46589	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	17/05/2014	+	+	+		
W46590	*Hydroides albiceps*	Darwin, Northern Territory	Australia	12°33'15.36"S, 130°52'34.01"E	16/05/2014	+	+	+	+	+
W.47928	*Hydroides albiceps*	Lissenung Island Resort House Reef, Kavieng	Papua New Guinea	2°39'22.95"S, 150°44'20.20"E	16/07/2015	+	+	+	+	+
W.47929	*Hydroides albiceps*	Lissenung Island Resort House Reef, Kavieng	Papua New Guinea	2°39'22.95"S, 150°44'20.20"E	16/07/2015	+	+	+	+	+
W.48267	*Hydroides albiceps*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	11/10/2015	+	+	+		+
W.48268	*Hydroides albiceps*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	9/10/2015	+		+		+
W.48705	*Hydroides albiceps*	Bise, Motobu, Okinawa	Japan	26°42'41.2"N 127°52'50.4"E	5/03/2016	+	+	+		
W.48706	*Hydroides albiceps*	Bise, Motobu, Okinawa	Japan	26°42'41.2"N 127°52'50.4"E	5/03/2016	+	+	+		+
W.48341	*Hydroides albiceps*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	16/11/2015	+		+		+
W.48342	*Hydroides albiceps*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	16/11/2015	+	+	+		+
W.48343	*Hydroides albiceps*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	16/11/2015	+				+
W.48353	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°57'06.8"S 146°08'44.7"E	16/11/2015	+		+		+
W.48354	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°57'06.8"S 146°08'44.7"E	16/11/2015	+	+	+		+
W.48357	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°57'06.8"S 146°08'44.7"E	16/11/2015	+	+			+
W.48276	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	8/11/2015	+			+	
W.48279	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	9/11/2015	+	+	+		+
W.48280	*Hydroides albiceps*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	9/11/2015	+	+	+		+
W46426	*Hydroides amrii*	Forster, New South Wales	Australia	32°10'50"S, 152°30'42"E	3/05/2014	+		+	+	+
W46591	*Hydroides amrii*	Bass Point, New South Wales	Australia	34°35'50"S, 150°54'00"E	23/05/2014	+		+	+	+
W46684	*Hydroides amrii*	Port Phillip, Victoria	Australia	37°58'9.48"S, 145°0'34.92"E	18/06/2014	+			+	+
W46687	*Hydroides amrii*	Port Phillip, Victoria	Australia	37°58'9.48"S, 145°0'34.92"E	18/06/2014	+			+	+
W.47474	*Hydroides amrii*	Port Lincoln, South Australia	Australia	34°44'22.3"S, 135°52'03.3"E	6/03/2015	+				+
W.47475	*Hydroides amrii*	Port Lincoln, South Australia	Australia	34°44'22.3"S, 135°52'03.3"E	6/03/2015	+				+
W.48365	*Hydroides amri*	Bremer Bay, Western Australia	Australia	34°24'02.7"S, 119°24'51.7"E	22/11/2015	+	+	+	+	+
W.48366	*Hydroides amri*	Bremer Bay, Western Australia	Australia	34°24'02.7"S, 119°24'51.7"E	22/11/2015	+	+	+		+
W.48367	*Hydroides amri*	Bremer Bay, Western Australia	Australia	34°24'02.7"S, 119°24'51.7"E	22/11/2015	+	+	+		+

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W.48368	*Hydroides amri*	Esperance, Western Australia	Australia	33°53'13.7"S, 121°55'55.2"E	21/11/2015	+	+	+		+
W.48371	*Hydroides amri*	Bremer Bay, Western Australia	Australia	34°24'02.7"S, 119°24'51.7"E	22/11/2015	+		+		
W.47027	*Hydroides ancorispina*	Clerke Reef, Western Australia	Australia	17°00'30.6"S, 119°12'35.8"E	3/10/2014	+	+	+	+	+
W.47035	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+	+	+	+	
W.47060	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+		+	+	+
W.47061	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+		+		
W.47062	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+		+	+	+
W.47066	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+	+	+	+	+
W.47067	*Hydroides ancorispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	3/10/2014	+		+	+	
W.47931	*Hydroides bandaensis*	Lissenung Island Resort House Reef, Kavieng	Papua New Guinea	2°39'22.95"S, 150°44'20.20"E	01/07/2015	+	+	+	+	+
W.47932	*Hydroides bandaensis*	Lissenung Island Resort House Reef, Kavieng	Papua New Guinea	2°39'22.95"S, 150°44'20.20"E	01/07/2015	+		+	+	+
W.48269	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	11/10/2015	+	+	+		+
W.48270	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+	+		+
W.48271	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+	+		
W.48272	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+	+		+
W.48273	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+			+
W.48274	*Hydroides bandaensis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+	+		+
W.48315	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48317	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48318	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48324	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48327	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48328	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+		+	+	+
W45577	*Hydroides brachyacantha*	Marina Mazatlán, Sinaloa	Mexico	23°16'47.4"N,106°27'39.9"W	3/11/2011	+		+	+	+
W46899	*Hydroides brachyacantha*	Gulf of California, Sinaloa	Mexico	23° 10'55.81"N, 106°25'28.3"W	18/08/2014	+			+	+
W46903	*Hydroides brachyacantha*	Gulf of California, Sinaloa	Mexico	23° 10'55.81"N, 106°25'28.3"W	18/08/2014	+			+	+
W.47022	*Hydroides brachyacantha*	Gulf of California, Sinaloa	Mexico	23° 10'55.81"N, 106°25'28.3"W	18/08/2014	+			+	+
W.47971	*Hydroides brachyacantha*	Sao Francisco Beach, São Sebastião	Brazil	23°47'30.8"S, 45°33'50.5"W	1/7/2015	+				+
K11	*Hydroides bulbosus*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+		+	+	+
K12	*Hydroides bulbosus*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+		+	+	+
K14	*Hydroides bulbosus*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+	+	+	+	
W.47052	*Hydroides externispina*	Clerke Reef, Western Australia	Australia	17°19'03.1"S, 119°18'43.8"E	12/10/2014	+	+	+		
W.47054	*Hydroides externispina*	Imperieuse Reef, Western Australia	Australia	17°30'32.9"S 118°57'47.4"E	6/10/2014	+	+	+		
W.47063	*Hydroides externispina*	Imperieuse Reef, Western Australia	Australia	17°30'32.9"S 118°57'47.4"E	6/10/2014	+	+	+	+	
W.47073	*Hydroides externispina*	Clerke Reef, Western Australia	Australia	17°19'03.1"S, 119°18'43.8"E	12/10/2014	+	+		+	
W45608	*Hydroides crucigera*	Palmira Topolobampo, Sinaloa	Mexico	25°35'59"N, 109°03'29"W	8/08/2011	+	+		+	
W.48200	*Hydroides crucigera*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W.48201	*Hydroides crucigera*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+		+		+
W.48202	*Hydroides crucigera*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+			+
W.48203	*Hydroides crucigera*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+
W.47674	*Hydroides deleoni*	Gulf of California, Sinoloa	Mexico	23°16'47.4"N, 106°27'39.9"W	16/08/2014	+	+		+	+
154666d	*Hydroides dianthus*	St. Petersburg, Florida	USA	27°46'12.7"N 82°37'55.1"W	2014	+	+	+	+	+
W42071	*Hydroides dianthus*	Woods Hole, Massachusetts	USA	41°31'N, 70°41'W		+	+	+		+
W46421	*Hydroides dianthus*	Sevastopol	Ukraine	44°37'09.5"N, 33°32'03.2"E		+	+	+	+	
W42402	*Hydroides dirampha*	Sydney Harbour, New South Wales	Australia	33°51'46"S, 151°11'0"E	5/03/2009	+	+	+		
W.47948	*Hydroides dirampha*	São Sebastião	Brazil	23°49'41"S, 45°25'22"W	3/7/2015	+		+	+	+
W.48204	*Hydroides dirampha*	Colon Container Terminal	Panama	9°22'40.9"N, 79°53'30.3"W	2014	+		+	+	+
W.48205	*Hydroides dirampha*	Cristobal, Colon	Panama	9°21'01.2"N, 79°54'36.1"W	2014	+	+	+		+
W.48206	*Hydroides dirampha*	Colon Container Terminal	Panama	9°22'40.9"N, 79°53'30.3"W	2014	+	+			+
W.48207	*Hydroides dirampha*	Colon Container Terminal	Panama	9°22'40.9"N, 79°53'30.3"W	2014	+	+	+		+
W.48362	*Hydroides dirampha*	Koombana Bay, Bunburry	Australia	33°19'4.4"N, 115°39'5.9"W	26/11/2015	+	+	+		+
W46904	*Hydroides dolabrus*	Gulf of California, Sinaloa	Mexico	23°16'47.4"N, 106°27'39.9"W	17/04/2014	+	+			
W46906	*Hydroides dolabrus*	Gulf of California, Sinaloa	Mexico	23°16'47.4"N, 106°27'39.9"W	17/04/2014	+	+		+	+
W46406	*Hydroides elegans*	Sydney	Australia	33°52'22.3"S, 151°11'27.8"E		+	+	+	+	
W.47946	*Hydroides elegans*	Cabo Frio, Rio de Janeiro	Brazil	22°53'55.0"S, 42°05'50.7"W	5/7/2015	+	+	+	+	
W.48208	*Hydroides elegans*	Shelter Bay Marina	Panama	9°22'10.2"N 79°57'00.3"W	2014	+	+	+		
W.48210	*Hydroides elegans*	Shelter Bay Marina	Panama	9°22'10.2"N 79°57'00.3"W	2014	+	+	+		
W.48216	*Hydroides elegans*	Marina del Rey, California	USA	33°58'19.7"N, 118°26'57.9"W		+	+	+	+	
W.48217	*Hydroides elegans*	Marina del Rey, California	USA	33°58'19.7"N, 118°26'57.9"W		+	+	+		
W.48363	*Hydroides elegans*	Bunburry, Western Australia	Australia	33°14'56.2"S, 115°41'01.1"E	26/11/2015	+	+	+		
W.48640	*Hydroides elegans*	Venetian Arsenal, Venice	Italy	45°26'06.8"N 12°21'01.6"E			+			
W46080	*Hydroides exaltata*	east side of Ashmore reef, Western Australia	Australia	12° 11' 40" S , 123° 3' 0" E	1/10/2013		+	+		
W.47033	*Hydroides exaltata*	Mermaid Reef, Western Australia	Australia	17°04'21.4"S 119°37'37.5"E	2/10/2014	+	+	+	+	+
W.47034	*Hydroides exaltata*	Mermaid Reef, Western Australia	Australia	17°04'21.4"S 119°37'37.5"E	2/10/2014	+	+	+		+
W.47036	*Hydroides exaltata*	Mermaid Reef, Western Australia	Australia	17°04'21.4"S 119°37'37.5"E	12/10/2014	+	+	+	+	+
W.47037	*Hydroides exaltata*	Mermaid Reef, Western Australia	Australia	17°03'46.3"S, 119°38'54.4"E	1/10/2014	+	+	+	+	+
W.48265	*Hydroides exaltata*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	9/10/2015	+		+		+
W.48266	*Hydroides exaltata*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	8/10/2015	+	+	+		+
W.48361	*Hydroides exaltata*	Bremer Bay, Western Australia	Australia	34°24'02.7"S, 119°24'51.7"E	22/11/2015	+	+	+		
W.48710	*Hydroides exaltata*	Bise, Motobu, Okinawa	Japan	26°42'41.2"N 127°52'50.4"E	5/3/2016	+	+	+	+	+
W45056	*Hydroides externispina*	Lizard Island, Queensland	Australia	14°40' 52.9" S, 145°28' 12.4" E	19/08/2013	+		+		
W45086	*Hydroides externispina*	Lizard Island, Queensland	Australia	14°42' 13.3" S, 145°27'37.2" E	21/08/2013	+	+	+		
W.47051	*Hydroides externispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	9/10/2014	+	+	+	+	
W.47059	*Hydroides externispina*	Imperieuse Reef, Western Australia	Australia	17°32'09.5"S, 118°58'23.7"E	9/10/2014	+	+	+	+	+

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W45609	*Hydroides ezoensis*	Qingdao, Shandong	China	36°03'22.1"N, 120°20'16.6"E	31/07/2013	+		+	+	
W.48405	*Hydroides ezoensis*	Tokyo Bay, Odaiba	Japan	35°37'15.9"N, 139°45'52.9"E	27/12/2015		+			
W.48406	*Hydroides ezoensis*	Tokyo Bay, Odaiba	Japan	35°37'15.9"N, 139°45'52.9"E	27/12/2015		+			
W.48407	*Hydroides ezoensis*	Tokyo Bay, Odaiba	Japan	35°37'15.9"N, 139°45'52.9"E	27/12/2015		+			
W.48408	*Hydroides ezoensis*	Tokyo Bay, Odaiba	Japan	35°37'15.9"N, 139°45'52.9"E	27/12/2015		+			
W45632	*Hydroides fusicola*	Qingdao, Shandong	China	36°03'22.1"N, 120°20'16.6"E	4/02/2014		+		+	
W48616	*Hydroides gracilis*	San Francisco Bay, California	USA	37°42'11.3"N, 122°17'51.9"W	2015		+			
W.48242	*Hydroides gracilis*	Port Hueneme, California	USA	34°08'54.2"N 119°12'35.1"W		+	+	+		+
W.48243	*Hydroides gracilis*	Port Hueneme, California	USA	34°08'54.2"N 119°12'35.1"W		+	+	+		+
W.48244	*Hydroides gracilis*	Port Hueneme, California	USA	34°08'54.2"N 119°12'35.1"W		+	+	+		+
W.48247	*Hydroides gracilis*	Port Hueneme, California	USA	34°08'54.2"N 119°12'35.1"W		+	+	+		+
W.48251	*Hydroides gracilis*	Port Hueneme, California	USA	34°08'54.2"N 119°12'35.1"W		+	+	+	+	+
K10	*Hydroides heterocera*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+	+	+	+	
K8	*Hydroides heterocerus*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+	+	+	+	+
K9	*Hydroides heterocerus*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+	+	+	+	+
Indian1	*Hydroides homoceros*					+	+	+	+	+
Indian2	*Hydroides homoceros*					+	+	+	+	+
Indian3	*Hydroides homoceros*					+	+	+	+	+
Indian4	*Hydroides homoceros*					+	+	+	+	+
K5	*Hydroides homoceros*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+		+	+	+
K6	*Hydroides homoceros*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015	+		+	+	+
W48847	*Hydroides inornata*	Ratnagiri, Maharashtra	India	17°04'41.1"N, 73°17'23" E	30/03/2016	+		+	+	+
W48848	*Hydroides inornata*	Ratnagiri, Maharashtra	India	17°04'41.1"N, 73°17'23" E	30/03/2016	+	+	+	+	+
W48849	*Hydroides inornata*	Ratnagiri, Maharashtra	India	17°04'41.1"N, 73°17'23" E	30/03/2016	+	+	+	+	+
W48850	*Hydroides inornata*	Ratnagiri, Maharashtra	India	17°04'41.1"N, 73°17'23" E	30/03/2016	+	+	+	+	+
W48851	*Hydroides inornata*	Ratnagiri, Maharashtra	India	17°04'41.1"N, 73°17'23" E	30/03/2016	+	+		+	
W46072	*Hydroides kimberleyensis*	Long Reef, Western Australia	Australia	13°51'24"S, 125°49'29"E	21/10/2010	+	+	+	+	+
W44226	*Hydroides longispinosa*	Lizard Island, Queensland	Australia	14°41'12.1"S, 145°26'37.5"E	16/08/2013	+	+	+		
W45085	*Hydroides longispinosa*	Lizard Island, Queensland	Australia	14°42'13.3"S, 145°27'37.2"E	21/08/2013	+	+	+		
W45096	*Hydroides longispinosa*	Macgillivray Reef, Queensland	Australia	14°39' 23.8" S, 145°29'33.6" E	22/08/2013	+		+		
W.47041	*Hydroides longispinosa*	Imperieuse Reef, Western Australia	Australia	17°32'52.5"S, 118°58'25.8"E	7/10/2014	+	+	+		+
W.47049	*Hydroides longispinosa*	Imperieuse Reef, Western Australia	Australia	17°32'52.5"S, 118°58'25.8"E	7/10/2014	+	+	+	+	
W.47930	*Hydroides longispinosa*	Nusa Blowholes, Kavieng	Papua New Guinea	2°34'05.1"S, 150°46'25.4"E	01/07/2015	+	+	+	+	
W43967	*Hydroides minax*	Lizard Island group, Queensland	Australia	14°41'51.5" S, 145°27'49.5"E	14/08/2013	+	+	+	+	
W44056	*Hydroides minax*	Lizard Island group, Queensland	Australia	14°41'51.5"S, 145°27'49.5"E	14/08/2013	+	+	+	+	
W44228	*Hydroides minax*	Lizard Island group, Queensland	Australia	14°41'51.5"S, 145°27'49.5"E	16/08/2013	+	+	+	+	

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W46074	*Hydroides minax*	Cassini Island, Western Australia	Australia	13°56'33"S, 125°37'18"E	15/10/2010		+			
W46075	*Hydroides minax*	Long Reef, Western Australia	Australia	13°49'11"S, 125°50'2"E	21/10/2010	+	+			+
W46105	*Hydroides minax*	Eugene Mcdermott Shoal, Western Australia	Australia	13°4'36"S, 124°35'0"E	6/10/2013	+	+			
W46110	*Hydroides minax*	south side of Ashmore reef, Western Australia	Australia	12°17'36"S, 123°7'25"E	28/09/2013	+	+	+		
W46111	*Hydroides minax*	east side of Ashmore reef, Western Australia	Australia	12°11'40"S, 123°3'0"E	01/10/2013		+	+		
W46585	*Hydroides minax*	Gove moog (export wharf), Northern Territory	Australia	12°12'2"S, 136° 40'1"E	26/03/2014	+		+		
W42073	*Hydroides nigra*	Palma de Mallorca	Spain	39° 30' N, 3° E			+	+		
W.48678	*Hydroides nigra*	Sa Porrasa, Mallorca	Spain	39°30'10.4"N 2°32'49.9"E	8/02/2016	+	+	+		+
W46916	*Hydroides nikae*	Hallet Cove Beach, South Australia	Australia	35°04'37"S, 138°30'07"E	14/9/2014	+			+	+
W46923	*Hydroides nikae*	Hallet Cove Beach, South Australia	Australia	35°04'37"S, 138°30'07"E	14/9/2014	+			+	+
W46935	*Hydroides nikae*	Hallet Cove Beach, South Australia	Australia	35°04'37"S, 138°30'07"E	14/9/2014	+			+	+
W46078	*Hydroides norvegica*	Tromsø Sund	Norway	69°39'34"N, 18°57'19"E	03/04/2014		+	+		
W46079	*Hydroides norvegica*	Tromsø Sund	Norway	69°39'34"N, 18°57'19"E	03/04/2012	+	+	+	+	+
W46895	*Hydroides norvegica*	Mølnbukt, Agdenes	Norway	63°38'32.7"N, 9°36'34.0"E	25/06/2014		+			
W46896	*Hydroides norvegica*	Mølnbukt, Agdenes	Norway	63°38'32.7"N, 9°36'34.0"E	25/06/2014	+	+			+
W46897	*Hydroides norvegica*	Mølnbukt, Agdenes	Norway	63°38'32.7"N, 9°36'34.0"E	25/06/2014		+		+	+
W46898	*Hydroides norvegica*	Flakkbukta, Trondheimsfjorden	Norway	63°26'41.0"N, 10°10'15.8"E	17/01/2013	+	+			+
W.48387	*Hydroides norvegica*	Adriatic Sea	Italy	42°05'10.6"N, 17°22'45.0"E	25/01/2016		+			
W.48390	*Hydroides norvegica*	Adriatic Sea	Italy	42°05'10.6"N, 17°22'45.0"E	25/01/2016		+		+	
W.48393	*Hydroides norvegica*	Adriatic Sea	Italy	42°05'10.6"N, 17°22'45.0"E	25/01/2016		+		+	
W45071	*Hydroides novaepommeroniae*	Lizard Island, Queensland	Australia	14°40' 46.0" S, 145°26' 49.3" E	20/08/2013	+	+	+		
W.48654	*Hydroides ochoterena*	Puerto Angel, Oaxaca	Mexico	15°39'56.1"N, 96°29'31.6"W		+	+	+		+
W.48656	*Hydroides ochoterena*	La Boquilla, Oaxaca	Mexico	15°40'55.7"N, 96°27'52.5"W	05/11/2015	+	+	+		+
W.48657	*Hydroides ochoterena*	Acapuleo, Playa Manzanilla, Guerrero	Mexico	16°44'00.1"N, 99°50'03.4"W	10/11/2015	+	+	+		+
W.48658	*Hydroides ochoterena*	Acapuleo, Playa Manzanilla, Guerrero	Mexico	16°44'00.1"N, 99°50'03.4"W	10/11/2015	+	+	+		+
W.48659	*Hydroides ochoterena*	Acapuleo, Playa Manzanilla, Guerrero	Mexico	16°44'00.1"N, 99°50'03.4"W	10/11/2015	+	+	+	+	
K2	*Hydroides operculatq*	Al-Doha	Kuwait	29°24'32.9"N, 48°19'27.8"E	5/02/2015		+	+	+	+
W46605	*Hydroides operculatq*	Kuwait	Kuwait	29°20'35"N, 48°4'11"E		+	+		+	+
W.48310	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48311	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48312	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+		+
W.48313	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.48314	*Hydroides basispinosa*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+	+	+
W.47427	*Hydroides panamensis*	Cuajiniquil, Guanacaste	Costa Rica	9°58'27.35"N, 84°49'52.71"W	25/11/2012		+	+	+	+
W.47428	*Hydroides panamensis*	Cuajiniquil, Guanacaste	Costa Rica	9°58'27.35"N, 84°49'52.71"W	25/11/2012	+	+	+	+	+
W.48220	*Hydroides panamensis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+		+		+
W.48221	*Hydroides panamensis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+

Appendix table 1. (continued)

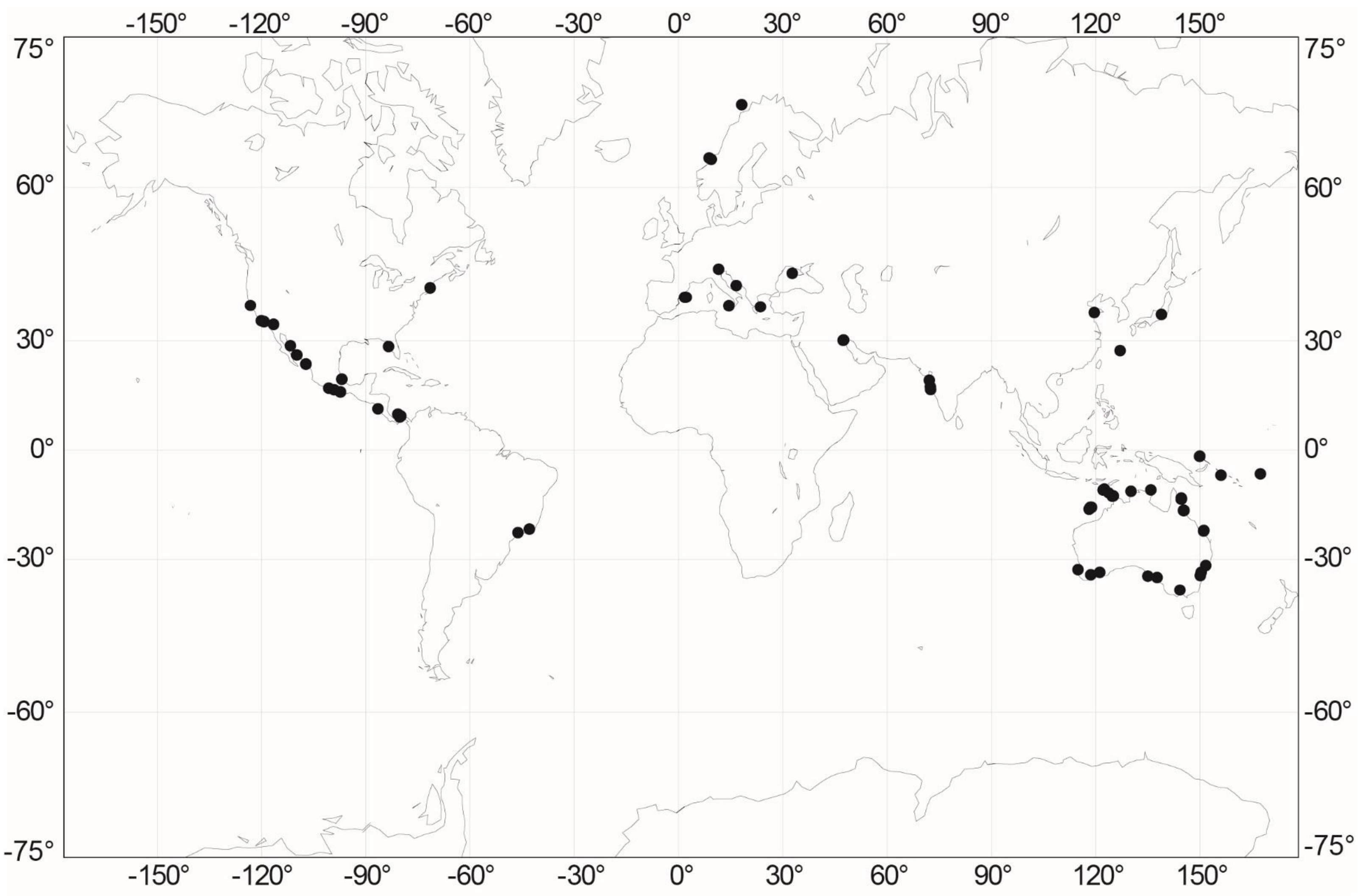
AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W.48222	*Hydroides panamensis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+
W.48226	*Hydroides panamensis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+
W.48229	*Hydroides panamensis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014		+	+		+
W43682	*Hydroides parva*	Veracruz port, Gulf of Mexico	Mexico	19°12'16.6"N, 96°6'46.6"W	8/12/2012	+	+			
W.47673	*Hydroides parva*	Veracruz, Gulf of California	Mexico	19°11'17.5"N, 96°7'19.3"W	7/12/2012	+	+		+	+
W.48230	*Hydroides parva*	Shelter Bay, Colon	Panama	9°26'49.6"N, 79°57'35.6"W	2014	+	+	+		+
W.48232	*Hydroides parva*	Cristobal, Colon	Panama	9°21'01.2"N, 79°54'36.1"W	2014	+	+	+		+
W.48233	*Hydroides parva*	Cristobal, Colon	Panama	9°21'01.2"N, 79°54'36.1"W	2014	+	+	+		+
W.48234	*Hydroides parva*	Colon Container Terminal	Panama	9°22'40.9"N, 79°53'30.3"W	2014	+	+	+		+
W.48235	*Hydroides parva*	Shelter Bay, Colon	Panama	9°26'49.6"N, 79°57'35.6"W	2014	+	+	+		+
W.48645	*Hydroides parva*	Marina Acuano, Veracruz	Mexico	19°11'14.3"N, 96°07'13.5"W	28/10/2015	+	+	+	+	
W.48646	*Hydroides parva*	Marina Acuano, Veracruz	Mexico	19°11'14.3"N, 96°07'13.5"W	28/10/2015	+	+	+	+	+
W42080	*Hydroides pseudouncinata*	Palma de Mallorca	Spain	39°30' N, 3°E			+	+		
W47500	*Hydroides pseudouncinata*	Greece	Greece		2008	+	+	+	+	+
W.47950	*Hydroides pseudouncinata*	Santa Tecla, Sicily	Italy	37°38'19.1"N, 15°10'56.0"E	28/06/2015	+		+		+
W.48679	*Hydroides pseudouncinata*	Mallorca	Spain	39°32'02.3"N, 2°35'35.3"E	08/02/2016	+	+	+	+	+
W46561	*Hydroides qiui*	Darwin, Northern Territory	Australia	12° 28'S, 130° 50' E	13/05/2014	+		+	+	
W46557	*Hydroides recta*	Darwin, Northern Territory	Australia	12°26'08.8"S, 130°49'55.7" E	17/05/2014	+	+	+	+	+
W46558	*Hydroides recta*	Darwin, Northern Territory	Australia	12°33'15.4"S, 130°52'34.0"E	4/06/2014	+	+	+	+	
W46587	*Hydroides recta*	Darwin, Northern Territory	Australia	12°33'15.4"S, 130°52'34.0"E	16/05/2014	+	+	+	+	+
W.48329	*Hydroides recta*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+		
W.48330	*Hydroides recta*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015	+	+	+		+
W.48331	*Hydroides recta*	Mission Beach, Queensland	Australia	17°51'59.7"S, 146°06'40.2"E	7/11/2015		+	+		+
W46941	*Hydroides recurvispina*	Cuajiniquil, Guanacaste	Costa Rica	10°56'56"N, 85°42'13"W	25/11/2012	+	+			
W.48236	*Hydroides recurvispina*	Panama Pacific side	Panama			+	+	+		
W48854	*Hydroides sanctaecrusis*	Uran, Maharashtra	India	18°53'10.1"N, 72°54'27.4"E	11/03/2016	+	+	+		+
W46567	*Hydroides sanctaecrucis*	Darwin, Northern Territory	Australia	12°27'6"S, 130°49'12"E	9/05/2014	+	+	+		+
W46568	*Hydroides sanctaecrucis*	Darwin, Northern Territory	Australia	12°27'5"S, 130°49'27"E	9/05/2014	+	+	+		+
W46569	*Hydroides sanctaecrucis*	Darwin, Northern Territory	Australia	12°27'6"S, 130°49'12"E	9/05/2014		+	+		+
W.48237	*Hydroides sanctaecrucis*	Cristobal, Colon	Panama	9°21'01.2"N, 79°54'36.1"W	2014	+	+	+		+
W.48238	*Hydroides sanctaecrucis*	Cristobal, Colon	Panama	9°21'01.2"N, 79°54'36.1"W	2014	+	+	+		+
W.48239	*Hydroides sanctaecrucis*	Colon Container Terminal	Panama	9°22'40.9"N, 79°53'30.3"W	2014	+	+	+		+
W.48240	*Hydroides sanctaecrucis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+
W.48241	*Hydroides sanctaecrucis*	Panama Bay	Panama	8°39'27.1"N, 79°26'28.8"W	2014	+	+	+		+
W45605	*Hydroides similis*	Guaymas, Sonora	Mexico	27°54'2"N, 110°51'15"W	11/08/2011	+	+			+
W45606	*Hydroides similis*	Marina La Paz, Baja California Sur	Mexico	24°9'19"N, 110°19'38"W	14/08/2011		+			
W45633	*Hydroides sinensis*	Qingdao, Shandong	China	36°3'22"N, 120°20'16"E	1/08/2013	+	+	+	+	+

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W45634	*Hydroides sinensis*	Qingdao, Shandong	China	36°3'22"N, 120°20'16"E	1/08/2013	+	+	+	+	
W42379	*Hydroides tambalagamensis*	Heron Island, Queensland	Australia	23°28'20"S, 151°57'1"E	18/11/2010		+	+		
W44052	*Hydroides tambalagamensis*	Lizard Island, Queensland	Australia	14°41'51.5"S, 145°27'49.5"E	14/08/2013	+	+	+		
W45058	*Hydroides tambalangamensis*	Lizard Island, Queensland	Australia	14°40'52.9"S, 145°28'12.4"E	19/08/2013	+	+	+	+	
W45064	*Hydroides tambalangamensis*	Lizard Island, Queensland	Australia	14°38'45.9"S,145°27'12.9"E	20/08/2013	+	+	+	+	
W46082	*Hydroides tambalangamensis*	south side of Ashmore Reef, Western Australia	Australia	12°16'29"S, 122°58'52"E	30/09/2013	+	+			
W46539	*Hydroides tambalagamensis*	Heron Island lagoon, Queensland	Australia	23°27'15.4"S, 151°52'1.6"E	10/09/2008	+		+	+	
W46542	*Hydroides tambalagamensis*	Heron Island, Queensland	Australia	23°27'15.4"S, 151°52'1.6"E	23/11/2009	+		+	+	
W.48355	*Hydroides tambalagamensis*	Dunk Island, Queensland	Australia	17°56'51"S, 146°08'23"E	10/11/2015	+	+	+		+
W43964	*Hydroides trivesiculosa*	Big Vicki's reef, Queensland	Australia	14°41'10"S, 145°26'32"E	13/08/2013		+	+		
W43968	*Hydroides trivesiculosa*	Lizard Island group, Queensland	Australia	14°41'51"S, 145°27'49"E	14/08/2013	+	+	+	+	
W44234	*Hydroides trivesiculosa*	Lizard Island, Queensland	Australia	14°40'46"S, 145°26'49"E	17/08/2013	+	+	+	+	
W45418	*Hydroides trivesiculosa*	Lizard Island, Queensland	Australia	14°39'07"S, 145°27'2"E	24/08/2013	+	+	+		
W45423	*Hydroides trivesiculosa*	Lizard Island, Queensland	Australia	14°39'07"S, 145°27'2"E	24/08/2013	+	+	+	+	
W46108	*Hydroides trivesiculosa*	Ashmore Reef, Western Australia	Australia	12°12'51"S, 123°1'12"E	26/09/2013	+	+			
W46536	*Hydroides trivesiculosa*	Hicks Reef, Queensland	Australia	14°28'49"S, 145°29'14"E	14/02/2009	+		+		
W46538	*Hydroides trivesiculosa*	North side of Broomfield Reef, Queensland	Australia	23°15'29"S, 151°57'11"E	30/08/2008	+		+	+	
W.47026	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°33'18"S, 118°57'54"E	7/10/2014	+		+		
W.47028	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°33'18"S, 118°57'54"E	7/10/2014	+	+	+	+	+
W.47029	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°33'18"S, 118°57'54"E	7/10/2014	+	+	+	+	
W.47040	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°33'18"S, 118°57'54"E	7/10/2014	+		+	+	
W.47045	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°32'9"S, 118°58'23"E	6/10/2014	+	+	+	+	
W.47050	*Hydroides trivesiculosa*	Mermaid Reef, Western Australia	Australia	17°4'21"S, 119°37'37"E	2/10/2014	+	+	+	+	
W.47068	*Hydroides trivesiculosa*	Imperieuse Reef, Western Australia	Australia	17°32'9"S, 118°58'23"E	6/10/2014	+	+	+		
W.47683	*Hydroides trompi*	Gulf of California, Sinoloa	Mexico	23°16'47.4"N, 106°27'39.9"W	3/11/2012		+			
W.48653	*Hydroides trompi*	Tijeras, Oaxaca	Mexico	16°14'1"N, 98°17'3.6"W	5/11/2015	+	+	+	+	+
W44230	*Hydroides tuberculata*	Vicki's Reef, Queensland	Australia	14°41'17"S, 145°26'35"E	16/08/2013		+	+		
W45068	*Hydroides tuberculata*	Lizard Island, Queensland	Australia	14°38'46"S, 145°27'13"E	20/08/2013	+	+	+	+	
W45079	*Hydroides tuberculata*	Lizard Island, Queensland	Australia	14°40'46"S, 145°26'49"E	20/08/2013	+	+			
W45414	*Hydroides tuberculata*	Lizard Island, Queensland	Australia	14°39'7"S, 145°27'2"E	24/08/2013	+	+	+	+	
W45419	*Hydroides tuberculata*	Lizard Island, Queensland	Australia	14°39'7"S, 145°27'2"E	24/08/2013	+	+	+	+	
W45425	*Hydroides tuberculata*	Lizard Island, Queensland	Australia	14°39'7"S, 145°27'2"E	24/08/2013	+	+	+	+	
W46084	*Hydroides tuberculata*	Hibernia Reef, Western Australia	Australia	11°59'17"S, 123°20'9"E	4/10/2013		+			
W46112	*Hydroides tuberculata*	Hibernia Reef, Western Australia	Australia	11°58'26"S, 123°19'19"E	5/10/2013	+		+		
W46429	*Hydroides tuberculata*	Linnet Reef, Queensland	Australia	14°46'50"S, 145°20'58"E	13/02/2009		+			
W46537	*Hydroides tuberculata*	North side of Broomfield Reef, Queensland	Australia	23°15'29"S, 151°57'11"E	30/08/2008	+		+		
W.47031	*Hydroides tuberculata*	Mermaid Reef, Western Australia	Australia	17°4'21"S, 119°37'37"E	02/10/2014	+	+	+	+	

Appendix table 1. (continued)

AM REGISTRATION	SPECIES	LOCALITY	COUNTRY	COORDINATES	DATE	18S	COI	28S	cyt b	ITS
W.48261	*Hydroides tuberculata*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	10/10/2015	+	+	+		+
W.48277	*Hydroides tuberculata*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	9/11/2015	+	+	+		+
W.48278	*Hydroides tuberculata*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	9/11/2015	+		+		+
W.48298	*Hydroides tuberculata*	Dunk Island, Queensland	Australia	17°56'50.9"S, 146°08'23.1"E	9/11/2015	+	+			+
W.48707	*Hydroides tuberculata*	Bise, Motobu, Okinawa	Japan	26°42'41.2"N, 127°52'50.4"E	5/03/2016	+	+	+	+	+
W.48709	*Hydroides tuberculata*	Bise, Motobu, Okinawa	Japan	26°42'41.2"N, 127°52'50.4"E	5/03/2016	+	+	+		+
W.47091	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014		+		+	+
W.47092	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014		+		+	+
W.47093	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014	+		+		+
W.47094	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014	+	+	+	+	+
W.47095	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014	+	+	+		+
W.47096	*Hydroides uniformis*	Hideaway Island	Vanuatu	7°41'49"S, 168°15'50"E	6/11/2014	+	+	+		+
W.48262	*Hydroides uniformis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	11/10/2015	+		+		+
W.48263	*Hydroides uniformis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	11/10/2015	+		+		+
W.48264	*Hydroides uniformis*	Mbabanga Island, Gizo	Solomon Islands	8°07'16.9"S, 156°53'41.5"E	11/10/2015	+		+		+
	Crucigera inconstans					DQ317113	EU184071	EU190464		
SAM E3587	*Crucigera tricornis*					EU184056	EU184067	EU190474		
	Crucigera zygophora					DQ242543		EU190470		
	Serpula columbiana					DQ317127		EU190469		
	Serpula concharum					DQ140408	EU184066	EU190468|		
SAM E3593	*Serpula uschakovi*					EU184065	EU184078			
SAM E3594	*Serpula vittata*					EU184064	EU184079	EU190471		
SAM E3595	*Serpula watsoni*					EU184057				
SAM E3696	*Galeolaria casepitosa*					AB106257	EU184080	FJ646538		



Appendix fig. 1 Sampling locations of specimens used in this thesis