Molecular Phylogenetics and Evolution xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Barcoding and multi-locus phylogeography of the globally distributed calcareous tubeworm genus *Hydroides* Gunnerus, 1768 (Annelida, Polychaeta, Serpulidae)

Yanan Sun[a,b,*], Eunice Wong[c], Shane T. Ahyong[a,d], Jane E. Williamson[b], Pat A. Hutchings[a,b], Elena K. Kupriyanova[a,b,*]

[a] Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia
[b] Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia
[c] School of Biological Sciences, University of Queensland, St Lucia, QLD 4072, Australia
[d] School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, NSW 2052, Australia

ARTICLE INFO

Keywords:
Marine
Calcareous tubeworm
Hydroides
Phylogeny
Barcoding
Biogeography

ABSTRACT

Hydroides is a large and diverse group of calcareous tubeworms (Serpulidae, Annelida) recognised by a distinctive but variable two-tiered operculum. Despite considerable research using several species of *Hydroides* as models in ecological and biofouling studies, phylogenetic and biogeographic relationships within the genus are still poorly understood. Using combined mitochondrial (COI, cytochrome *b*) and nuclear (18S, 28S and ITS) gene markers for 284 individuals of 45 morphospecies of *Hydroides*, we investigated the global phylogenetic and biogeographic relationships within the genus. Phylogenetic topologies were well supported and indicated high genetic diversity within *Hydroides*, revealing potential cryptic species. Present results also include the first COI barcoding data enabling rapid and effective species identification of *Hydroides* on a global scale. Phylogenetic relationships within *Hydroides* were more concordant with geographical distributions than morphological similarity of their opercula. Molecular divergence estimates suggested the origin and subsequent diversification in the western Tethys Sea followed by a shift of the historical centre of diversity 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 fossil *Hydroides* tubes would provide evidence to test this biogeographic hypothesis.

1. Introduction

1.1. Hydroides as a genus of Serpulidae

Serpulid polychaetes (Family Serpulidae Rafinesque, 1815), commonly known as “calcareous tubeworms”, are a unique and highly specialised group of marine segmented worms. These polychaetes inhabit self-secreted calcareous tubes usually attached to a wide range of hard substrates from the intertidal to abyssal zone (ten Hove and Kupriyanova, 2009). Serpulids can be recognised by their calcareous tube, in most cases, colourful radiolar crown, and a well-differentiated tube plug called the operculum, which has traditionally been one of the most important characters used in morphological taxonomy (reviewed by ten Hove and Kupriyanova, 2009).

With 105 nominal species, *Hydroides* Gunnerus, 1768, is the most speciose genus in Serpulidae (Read et al., 2017). Species of *Hydroides* are easily recognised by the complex two-tiered operculum consisting of a proximal funnel made of numerous radii, and a distal crown of chitinous spines called the verticil (Bastida-Zavala and ten Hove, 2002: Fig. 3). Species delimitation in *Hydroides* relies heavily on the distinctive and varied structure of the operculum, especially of the verticil spines. The verticils vary in terms of symmetrical or asymmetrical arrangement of the spines, presence of lateral, internal, and external spinules on spines, distal spine modifications, presence or absence of distinct dorsal spines, verticil spine bifurcation, and the presence of bulbous spines (Bastida-Zavala and ten Hove, 2002: Fig. 4). A standardised terminology for the *Hydroides* operculum was first proposed by ten Hove (1990) and then updated by Bastida-Zavala and ten Hove (2002).

Species of *Hydroides* are widely distributed in subtidal habitats of

* Corresponding authors at: Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia.
E-mail addresses: sunyanan8733@gmail.com (Y. Sun), Elena.Kupriyanova@austmus.gov.au (E.K. Kupriyanova).

https://doi.org/10.1016/j.ympev.2018.06.021
Received 28 July 2017; Received in revised form 22 December 2017; Accepted 11 June 2018

tropical and subtropical regions, and less commonly in temperate and cold waters. They encrust various natural hard substrata such as rocks, mollusc shells, coral skeletons, as well as artificial substrates such as aquaculture nets, seawater intake pipes, mooring buoys and ship hulls (Bastida-Zavala and ten Hove, 2003; Bastida-Zavala, 2008; ten Hove and Kupriyanova, 2009). Several species of *Hydroides*, such as *H. dianthus* (Verrill, 1873), *H. ezoensis* Okuda, 1934, *H. dirampha* Mörch, 1863, and *H. elegans* (Haswell, 1883), are well known as invasive biofoulers that can settle gregariously forming dense aggregations of calcareous tubes on underwater structures, and are easily dispersed *via* human related vectors, leading to cosmopolitan bioinvasions (Qiu and Qian, 1997, Okamoto et al., 1998; Toonen and Pawlik, 2001; Link et al., 2009). These biofoulers have been intensively studied in terms of 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.

1.2. Taxonomic status of the genus Hydroides

The genus *Hydroides* is morphologically closely related to *Serpula* Linnaeus, 1758 (the type genus of the family, characterised by a funnel-shaped operculum), and *Crucigera* Benedict, 1887 (characterised by a similar funnel-shaped operculum, but with basal knobs). Ever since *Hydroides* was erected by Gunnerus (1768) for *H. norvegica,* numerous generic and subgeneric taxa have been proposed to further distinguish the species with a two-tiered operculum based on the morphology of the verticil. Philippi (1844) erected *Eupomatus* for species without lateral spinules on their verticil spines, and this name was subsequently widely used to group up to 23 species (Hartman, 1959). Mörch (1863), who considered the presence or lack of lateral spinules of only subgeneric value, erected the subgenus *Eucarphus* for the species with spines having lateral spinules. Grube (1878) described two new species *S. minax* and *S. furcifera* with a two-tiered operculum under the genus *Serpula*. Bush (1905) erected the genus *Glossopsis* for *Serpula minax* Grube, 1878, to recognise 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. *Eucarphus*, *Glossopsis*, and *Schizocraspedon* were subsequently considered as redundant by some taxonomists who accepted only the generic names *Hydroides* and *Eupomatus* to subdivide the group (Pixell, 1913; Rioja, 1958; Hartman, 1959; Jones, 1962). Other taxonomists (Okuda, 1934; Dew, 1959; Pillai, 1960, 1961, 1971; Straughan, 1967a, b) recognised only the single *Hydroides,* without subgeneric divisions.

Uchida (1978) proposed *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. These two features, however, rendered the new genus indistinguishable from the genus *Serpula*; thus, the genus *Protohydroides* is currently considered invalid (ten Hove, 1984) and the consensus that *Hydroides* is a single genus prevails. Not surprisingly, with a long and complex list of synonyms, members of *Hydroides* show high intraspecific diversity in morphology that may confound reconstructing relationships among species based only on opercular morphology (Bastida-Zavala and ten Hove, 2002).

1.3. COI barcoding of the genus Hydroides

DNA barcoding using a fragment of the mitochondrial cytochrome oxidase subunit I (COI) has been demonstrated to be effective in distinguishing species and revealing cryptic species of polychaetes (Barroso et al., 2010; Carr et al., 2011; Sun et al., 2012). However, the barcoding fragments of COI in some groups of polychaetes, such as the families Cirratulidae, Nephtyidae, Spionidae, and Serpulidae (Pleijel et al., 2009; Nygren and Pleijel, 2011; Carr et al., 2011; Sun et al., 2012) are extremely difficult to amplify owing to the lack of appropriate primers. Worse still, new primer design is always limited by availability of reference sequences (Yuryev, 2007). In the case of *Hydroides*, COI barcoding has travelled a difficult road. As with all other serpulid genera, the earliest attempts to amplify COI fragments in *Hydroides* using the universal barcoding primers HCO2198-LCO1490 (hereafter “Folmer primers”, Folmer et al., 1994) completely failed (see Sun et al., 2012: 539–540). Sun et al. (2012) achieved the first success for three species of *Hydroides* using the polychaete-specific primers polyLCO/polyHCO designed by Carr et al. (2011). Using these three COI sequences as references, the first *Hydroides*-specific primers (Hydro-COIF/Hydro-COIR) were designed, and COI barcoding fragments from 11 out of 14 tested species were generated (Sun et al., 2012). Fortunately, next-generation sequencing technologies have made generation of mitochondrial genomes more feasible and less time intensive. Therefore, COI reference sequences of *Hydroides* for effective primer design could be readily obtained from mitochondrial genomic data (Sun, 2017), making the barcoding of the whole genus possible.

1.4. Phylogenetic relationships within Hydroides

Serpula, *Crucigera*, and *Hydroides* are currently accepted as forming a monophyletic group united by the presence of a funnel-shaped operculum, pseudo-perculum and bayonet-shaped special collar chaetae (ten Hove, 1984; ten Hove and Jansen-Jacobs, 1984). The monophyly of this group was further supported by the results of phylogenetic studies based on morphological (Kupriyanova, 2003), molecular (Lehrke et al., 2007) and combined datasets (Kupriyanova et al., 2006, 2008).

Jones (1962) proposed the first evolutionary scheme within *Hydroides,* dividing the 21 species of *Eupomatus* into four groups according to the morphology of the verticil spines: i.e., species with verticil spines of different sizes, with asymmetrically arranged verticil spines, straight verticil spines, as well as species with verticil spines with T-shaped tips. As *Eupomatus* was later synonymised with *Hydroides*, Pillai (1972) proposed an alternative scheme for 42 species of *Hydroides* world-wide, dividing them into two major categories according to the presence or absence of modified dorsal spines, and then further into 12 groups (Fig. 1A, Pillai, 1972: Figs. 2–4). Neither of the two proposed schemes, however, resulted from formal phylogenetic analyses. Moreover, with the synonymy of *Hydroides* and *Eupomatus*, exponentially increasing numbers of newly discovered species of *Hydroides* in subsequent years, as well as synonymisation of other species of *Hydroides* (see Read et al., 2017), the phylogenetic relationships based on morphological characters alone have remained contentious. The first formal cladistic analysis of the genus using traditional characters provided little phylogenetic resolution (Bastida-Zavala, pers. comm).

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 mitochondrial cytochrome *b* (cyt b) and two nuclear ribosomal RNA genes (18S and 28S) of eight species of *Hydroides*, Kupriyanova et al. (2008) were the first to confirm the monophyly of *Hydroides* and to recover a close relationship among 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 analysis of COI of ten species. Tovar-Hernández et al. (2015) described a new species, *H. dolabrus,* and corrected the identification of *H. recurvispina* by Sun et al. (2012) to *H. dolabrus.* They added *H. panamensis* to the *H. brachyacantha-H. dolabrus-H. sanctaecrucis* group and recovered the close relationship among *H. elegans* and *H. norvegica*, *H. ezoensis* and *H. fusicola*, as well as *H. pseudouncinata* and *H. operculata* (Tovar-Hernández et al., 2015) (Fig. 1C). Relationships among these groups, however, remained unclear owing to the low nodal support values in the resulting phylogenies. Moreover, because the taxon sampling of these studies was limited to only 18 out of 105 known species, the phylogenetic positions of most species within *Hydroides* remained unclear.

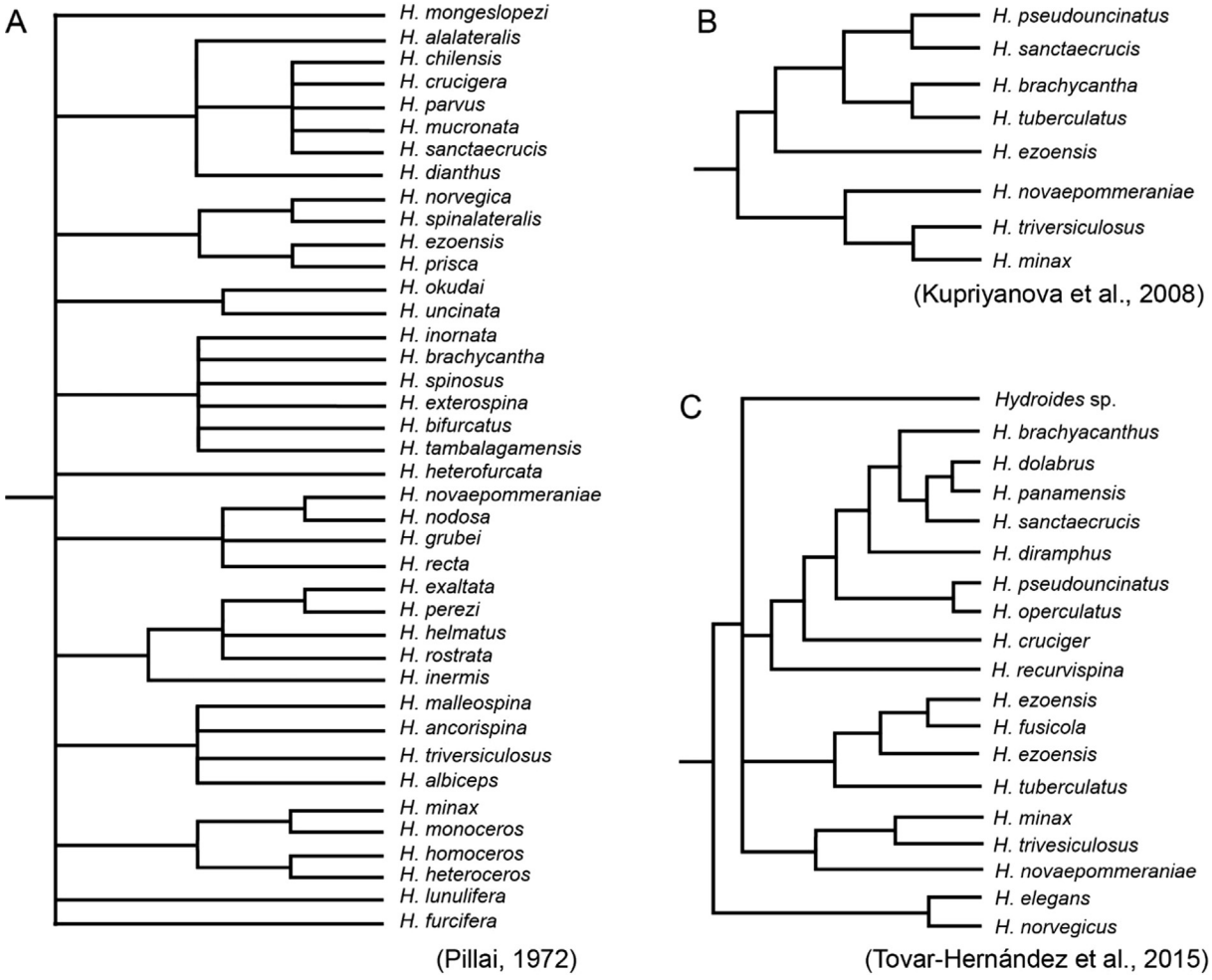

Fig. 1. Phylogenetic relationships within *Hydroides* indicated in previous studies.

Given that none of the molecular analyses above supported the morphology-based phylogenetic groups proposed by either Jones (1962) or Pillai (1972), the phylogenetic interpretation of the morphology of *Hydroides* remained unclear. Thus, an up-to-date phylogenetic study using broad taxon sampling to understand the phylogenetic relationships within *Hydroides* is undertaken here to create a framework within which the morphology and distributions can be interpreted.

1.5. Geographical distribution of Hydroides

Most species of *Hydroides* have been described from the Indo-West Pacific, followed by the tropical eastern Pacific and tropical western Atlantic (Caribbean region) (Bastida-Zavala and ten Hove, 2002). By linking morphological characteristics of species of *Hydroides* to their native distributions, Pillai (1972) recognised 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 centre of origin, consisting of species bearing an asymmetrical verticil. However, Pillai's hypothesis remained contentious because species with both symmetrical and asymmetrical verticils have been described from both of the main geographic regions since 1972.

As some species of *Hydroides* can easily disperse *via* anthropogenic vectors, the native ranges of reported "widespread" species such as *H. brachyacantha*, *H. dianthus*, and *H. elegans* also now seem uncertain (Sun et al., 2016). Recent phylogenetic studies (Sun et al., 2012; Tovar-Hernández et al., 2015) of *Hydroides* spp., however, demonstrated a geographic pattern where *Hydroides* spp. from the eastern Pacific and western Atlantic were more closely related to each other than to those collected from other regions, reflecting the Indo-West Pacific/Atlanto-East Pacific faunal separation widely observed in other warm-water coastal marine invertebrates (Ekman, 1953). This biogeographic pattern in *Hydroides*, however, is based on limited taxon sampling, and the geographic patterns of other species of the genus need to be clarified.

Although serpulids inhabiting calcareous tubes have the best fossil record among the mostly soft-bodied annelids, it is difficult to assign tube fossils to extant species, especially in *Hydroides,* due to the high similarity of the tube external macrostructure among species within the group (Ippolitov et al., 2015). The identities of most fossil tubes that were assigned to species of the *Serpula-Hydroides* group in earlier studies are taxonomically ambiguous (Ippolitov et al., 2015) and cannot serve as references for testing the historical geographic patterns of dispersal within the group. Recently, examination of tube fossils using scanning electron microscopy and X-ray diffraction demonstrated a high variety of ultrastructure and non-uniform mineralogical composition in serpulids. With these new tools, the most recent common ancestor of extant taxa of the *Serpula-Hydroides* group was dated to 66 Ma (Cenozoic) (Vinn, 2007; Vinn et al., 2008; Ippolitov et al., 2015), providing the most reliable calibration point with which to investigate the historical phylogeography of serpulids.

In this study, we performed phylogenetic analyses using the largest multi-locus dataset to date, based on five markers (three nuclear and two mitochondrial genes) with worldwide sampling of species of *Hydroides.* The aims of this study were to 1) generate a well-supported, comprehensive species-level phylogeny that can aid in species

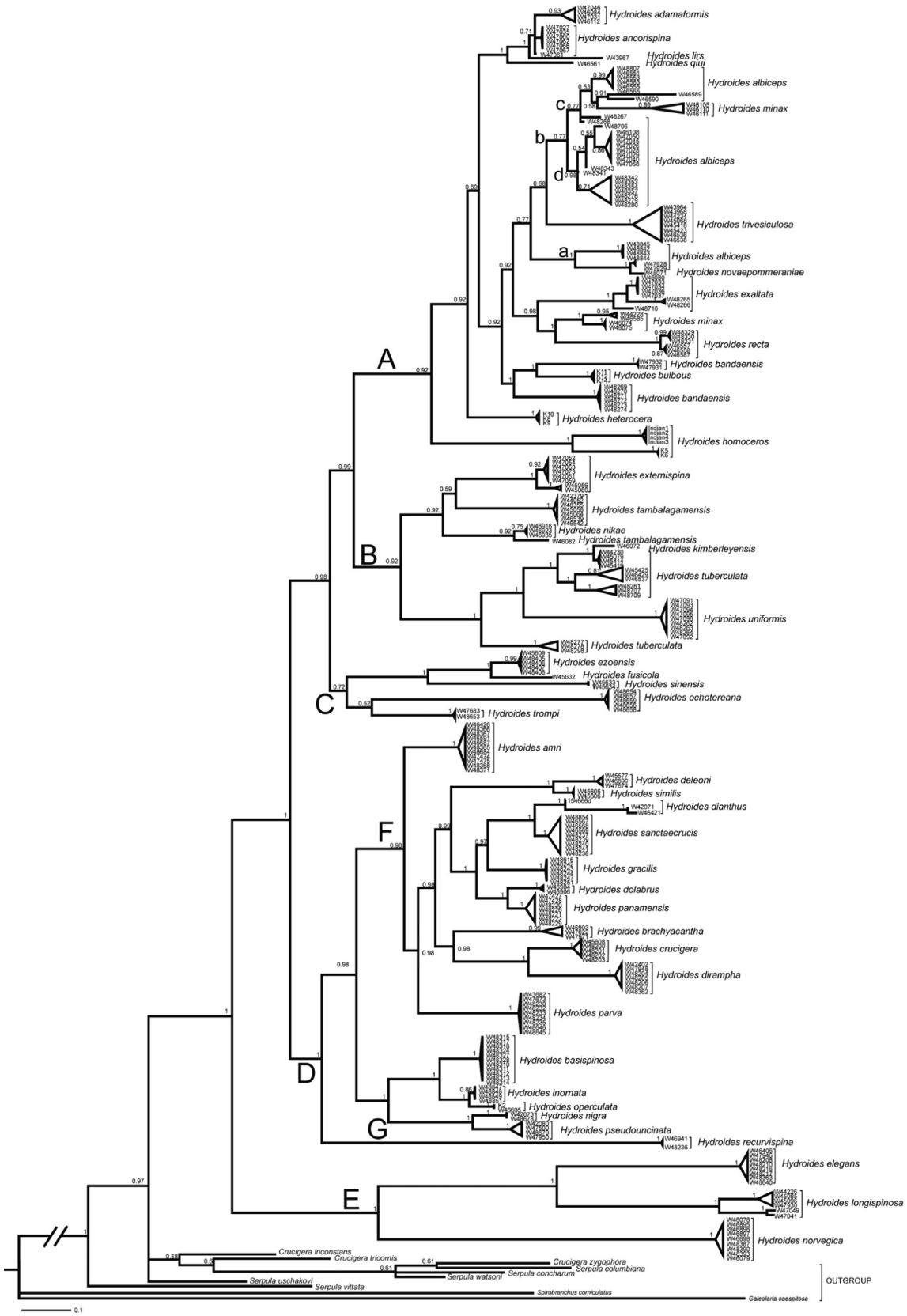

Fig. 2. Bayesian inference (BI) tree of 45 morphospecies of *Hydroides* based on combined dataset of five molecular markers yielded five main distinct clades and 70 distinct species-level lineages. Values at nodes represent posterior probabilities.

delimitation and cryptic species detection; 2) investigate the potential utility of COI barcoding for discriminating species of *Hydroides* from across a broad geographic range; 3) estimate the divergence times for the major clades of *Hydroides*; and 4) reconstruct the geographic patterns of dispersal within the group by linking the cladogenic history with the biogeographical framework.

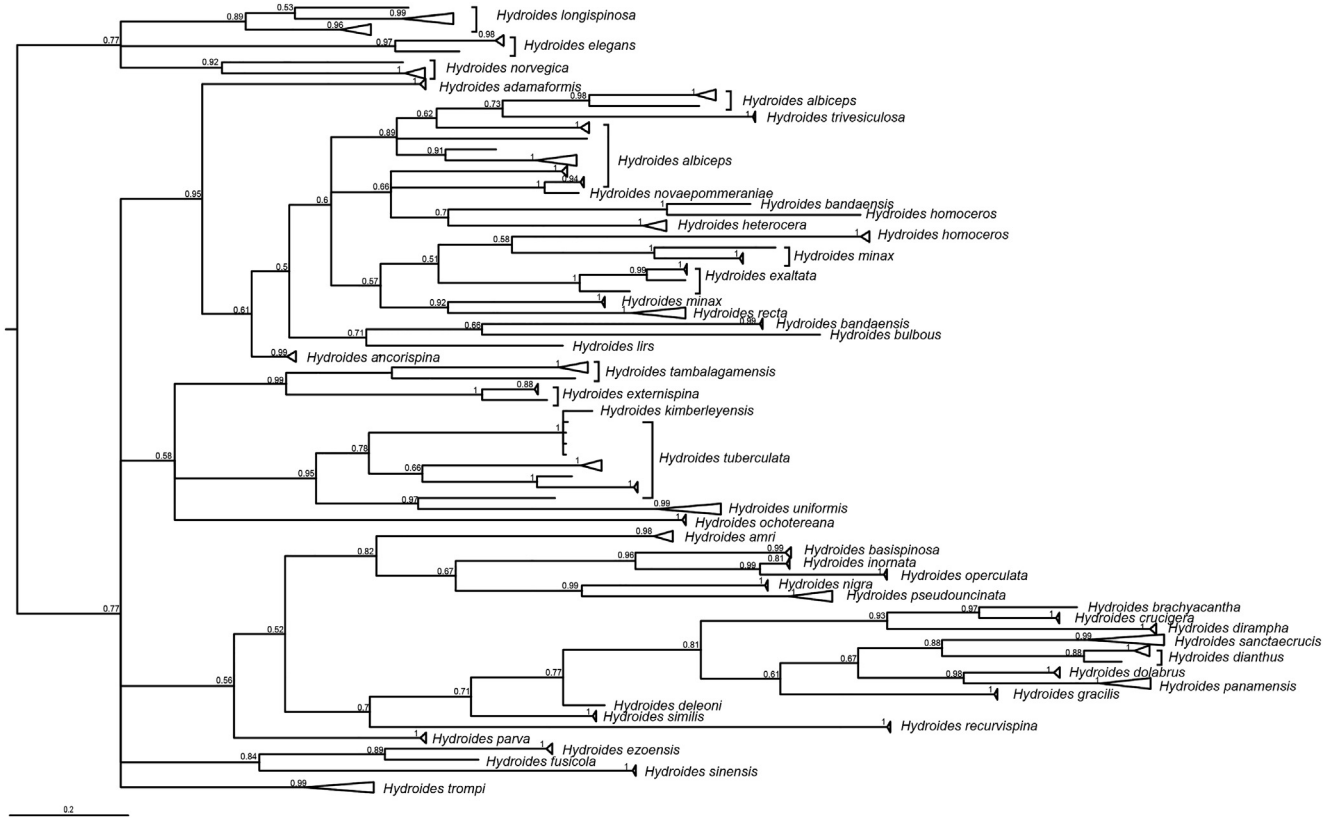

Fig. 3. Bayesian inference (BI) tree of 44 morphospecies of *Hydroides* based on COI barcoding sequences. Values at nodes represent posterior probabilities.

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 currently known species distributions globally, with the exception of Africa (Supplementary Fig. S1). Most specimens are deposited in the collection of Australian Museum (AM) in Sydney, Australia. Details of specimens, including collection localities and voucher numbers are given in Supplementary Table S1. Specimens were removed from their tubes under a dissecting microscope, identified to species according to their morphology (mainly structure of the operculum), and preserved in 95% ethanol. For each specimen, the posterior end of the abdomen was cut for DNA extraction. Representatives of 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 outgroups (Supplementary Table S1).

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's protocol. Owing to the lack of appropriate primers effective for *Hydroides*, new taxon-specific COI primer sets targeting the barcoding region of *Hydroides* were developed based on sequences obtained from mitochondrial genomes of eight *Hydroides* species (Sun, 2017). 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, and ~400 bp of cyt b. Amplifications 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. 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. GenBank accession numbers of sequences are shown in Supplementary Table S1.

2.2. Sequence alignments, phylogeny reconstruction and barcoding analyses

Two sequence datasets were analysed. The first (combined dataset), used in combined analysis (Olmstead and Sweere, 1994), concatenated sequences of the 18S, 28S, ITS2, COI, and cyt b gene fragments of 284 specimens representing 45 morphospecies of *Hydroides*, together with sequences of ten outgroup species downloaded from GenBank comprising species of *Serpula*, *Spirobranchus*, *Crucigera* and *Galeolaria*. To examine species delimitation in a "barcoding" context, the second dataset (COI dataset) included only sequences of the barcoding region of COI gene from the 248 specimens representing 44 morphospecies.

Both datasets 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 by eye for obvious misalignments and combined using BioEdit v7.0.5.3 (Hall, 1999). Each of the three nuclear genes, as well as each of the three codon positions of protein coding genes 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 shown in Table 1.

The final lengths of 18S, 28S, ITS2, COI, and cyt b alignments 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

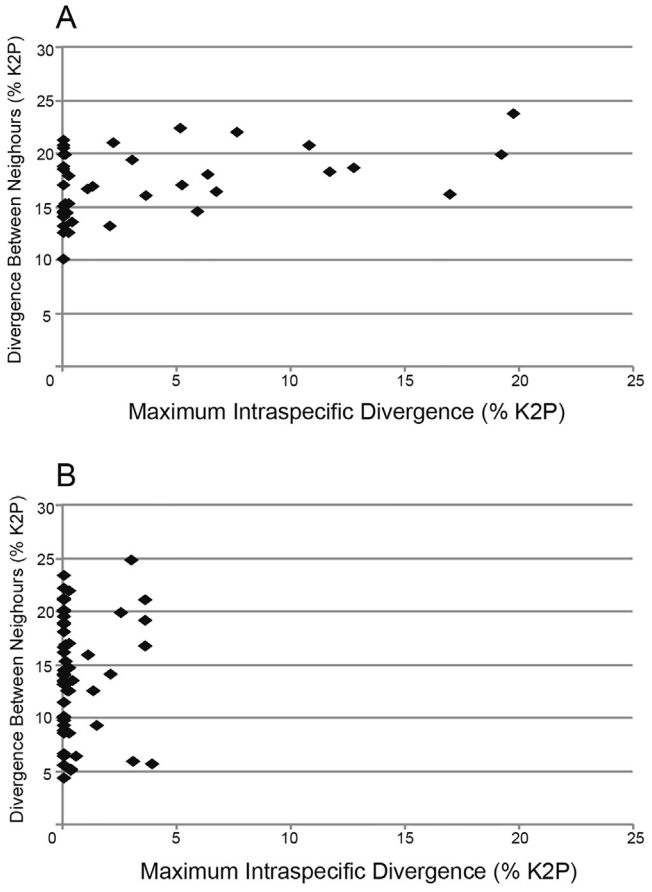

Fig. 4. Comparison of COI (K2P) distances between morphospecies (A) and genetic lineages recovered by phylogenetic analyses (B).

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 replicates (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.

Genetic distances for COI were calculated using the K2P model (Kimura, 1980) in MEGA 5.0 (Tamura et al., 2011). Initial molecular species delimitation used the "barcoding gap", defined by Hebert et al. (2004) as the existence of at least a 10 times greater average interspecific distance than average intraspecific genetic distance.

2.3. Divergence time estimates

Divergence times were estimated using BEAST v2.4.3 (Bouckaert et al., 2014). Taxa used for divergence time estimates 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 exemplar 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 chains, posterior distribution of all parameters and their associated statistics, including estimated sample sizes (ESS) and 95% highest posterior density (HPD) intervals. TreeAnnotator v. 1.5.4 was used to summarise the set of post burn-in trees and produce a maximum clade credibility chronogram.

From the recent review of tube fossils of the *Serpula-Hydroides* group (Ippolitov et al., 2015), two calibration points could be identified. Coiled tubes with slowly growing loops and a flattened upper side are considered nearest to *Hydroides* (Rovereto, 1904; Lommerzheim, 1981; Ippolitov et al., 2015), of which the most ancient fossil has been dated to the middle Paleogene (~61 Ma; Lommerzheim, 1981). Thus, one of the time constraints 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 the study of 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. (2015).

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 delineations of Spalding et al. (2007) and the reported distribution of *Hydroides*: (A) East Pacific, (B)

Table 1
Details of primers used for PCR of each gene and the best-fit models used in phylogenetic analyses.

Gene	Primer used for PCR (Sequence 5′ to 3′)	Annealing Temperature (°C) used in PCR	Fragment length used in phylogenetic analyses	Best-fit Model used in phylogenetic analyses
18S	TimA: AMCTGGTTGATCCTGCCAG (Nóren and Jordelius, 1999) 1100R2: CGGTATCTGATCGTCTTCGA (Nóren and Jordelius, 1999)	60	935	TIM2e + I + G
28S	LSUD1F: ACCCGCTGAATTTAAGCATA (Osborn et al., 2007) D2ar: ACGAACGATTTGCACGTCAG (Osborn et al., 2007)	61	983	TPM2u + I + G
COI	HyCOF190: TCNRTNTTKACDGTKACATGCTA (this study) HyCOR886: ACCCYATYATHCCRATAGARCACAT (this study)	51	581	1st coding position: GTR + I + G 2nd coding position: TIM + G 3rd coding position: GTR + G
cyt b	424-f: GGWTAYGTWYTWCCWTGRGGWCARAT (Boore and Brown, 2000) COBr825: AARTAYCAYTCIGGYTTRATRTG (Burnette et al., 2005)	51	301	1st coding position: TN + G 2nd coding position: TPM2u + G 3rd coding position: TPM2u + G
ITS	ITS3: GCATCGATGAAGAACGCAGC (White et al., 1990) ITS4: TCCTCCGCTTATTGATATGC (White et al., 1990)	55	602	TPM2 + G

West Atlantic, (C) East Atlantic and Mediterranean, (D) West Indo-Pacific, (E) Central Indo-Pacific, (F) Temperate Northern Pacific, (G) Temperate Australasia (Fig. 6). For widely reported invasive species, only the first cited distributions of these species (checked on WoRMS, 2017) were included in the analyses. *Hydroides elegans* was excluded from the historical biogeographic analyses because its native range is unclear. RASP allows constraints to be placed on each model to reflect past geographical events and configurations. Thus, the phylogeny was stratified into three time slices: T1, T2 and T3. T1 occurred between the root age of 62 and 30 Ma, for which we decreased 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 as 0 to reflect the connection of Australasia with Antarctica. T2 occurred from 30 Ma to 18 Ma, 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 Ma 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 as a result of the closure of the Isthmus of Panama around 3.1 Ma, were not included because no sister lineages spanning this boundary post-dating the closure could be identified according to the divergence time estimates.

3. Results

3.1. Phylogenetic analyses

Both BI (Fig. 2) and ML (Supplementary Fig. S2) 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 the group including representatives of the genera *Serpula* and *Crucigera*. Five distinct clades of *Hydroides* could be recognised (A–E in Fig. 2) in the BI phylogram. Of the five clades, three (clades A, B, D) were well-supported under both BI and ML (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), but also 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 the 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. COI barcoding

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 design. Altogether, the following four primers, including one coding-strand and three anti-coding-strand primers, were designed and tested: HyCOF190 (TCNRTNTTKACDGTKACATGCTA), HyCOR688 (AAYCTMGTHK- WAAARTGHCGATC), HyCOR673 (TGHCGATCHRYYAAAAGCATAGT), and HyCOR886 (ACCCYATYAT-HCCRATAGARCACAT). In the code names of primers, 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) (Supplementary Table S2).

BI analyses based on available COI barcoding data recovered 56 distinct, well-supported lineages of 44 *Hydroides* morphospecies (Fig. 3), which is consistent with the lineages generated from the combined dataset. The genetic distances among morphologically distinct species ranged from 12.6% to 37.1% (mean 26.2%), while those among totally molecular-based lineages ranged from 5.5% to 37.1% (mean 25.9%) (Fig. 4), compared to the genetic divergence within lineages that ranged from 0 to 3.6% (mean 1.5%). Intermediate distances ranging from 5% to 12% were only found among lineages of single morphospecies, such as *H. albiceps* (10.2%), *H. exaltata* (5.5%), *H. trivesiculosa* (9.3%), and *H. tuberculata* (8.6%, 8.8%).

3.3. Divergence time estimates

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. 5) diverged from other clades around 62.4 Ma (61–65.1 Ma, 95% HPD), during the Mid-Paleocene. The main eastern Pacific and Atlantic clade (Clade D) appeared approximately 42.7 Ma (32.6–51.7 Ma, 95% HPD). Divergence between species from the temperate northern Pacific and Indo-Pacific occurred approximately 43.5 Ma (34–53 Ma, 95% HPD), with the subsequent divergence of the two main Indo-Pacific lineages (splits of clades A and B) occurring around 39 Ma (29.7–47.9 Ma, 95% HPD), during the Mid-Eocene (Fig. 5). The most significant diversification within *Hydroides* occurred during the Miocene (~5–23 Ma).

3.4. Inference of biogeographic history

Biogeographical analyses estimated that the ancestor of *Hydroides* had a wide distribution in the eastern Atlantic and Indian Ocean during the Palaeocene (clade K; Fig. 6). Further dispersal events occurred during the early Eocene (clade J; Fig. 6), including the eastward dispersal to the tropical western and northern Pacific (clade I; Fig. 6), as well as the dispersal westwards to eastern Pacific *via* the Atlantic (clade D; Fig. 6). Subsequent diversification of clade I was the cladogenic event that separated the ancestor of *H. ezoensis* + *H. fusicola* + *H. sinensis* (Fig. 6, clade C) in the temperate Northern Pacific from the tropical and subtropical species in this clade. Within the Indo-Pacific (clade H), the reconstruction shows a within-region origin of clade A and B. 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 temperate Australasia by the late Neogene. In the east Pacific and Atlantic clade (Fig. 6, clade D), the split between the western Atlantic and the Mediterranean groups (Fig. 6, clade F) occurred in the late Oligocene.

4. Discussion

4.1. Phylogenetic analyses, COI barcoding, and taxonomic implications

The analyses in this study were based on more extensive sampling of

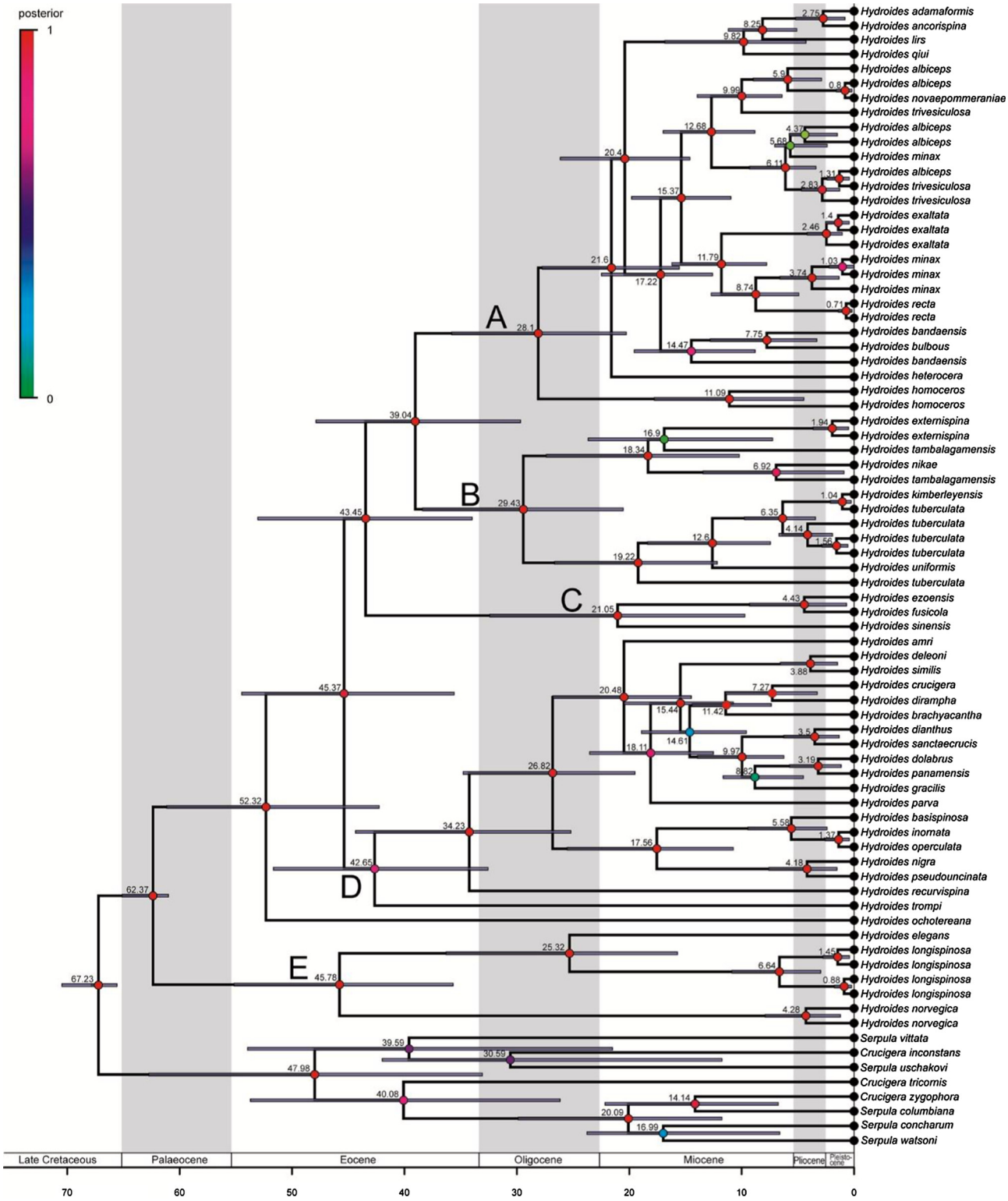

Fig. 5. Chronogram of *Hydroides* produced from BEAST analysis. Maximum clade credibility tree with mean nodal ages and 95% highest posterior density (HPD) intervals are indicated by bars. Time-scale in millions of years and geological time periods shown at the bottom. The colour bar indicates the posterior probability on the node.

taxa and loci than any previous study of *Hydroides*, resulting in the most comprehensive and best-resolved phylogeny of the genus to date. The present results corroborate those of previous studies (Kupriyanova et al., 2008; Tovar-Hernández et al., 2015) finding *Hydroides* to be monophyletic. Within *Hydroides*, analyses based on the 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 45 initial morphospecies names used in phylogenetic analyses, the new molecular phylogeny suggests 70 distinct species-level 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*.

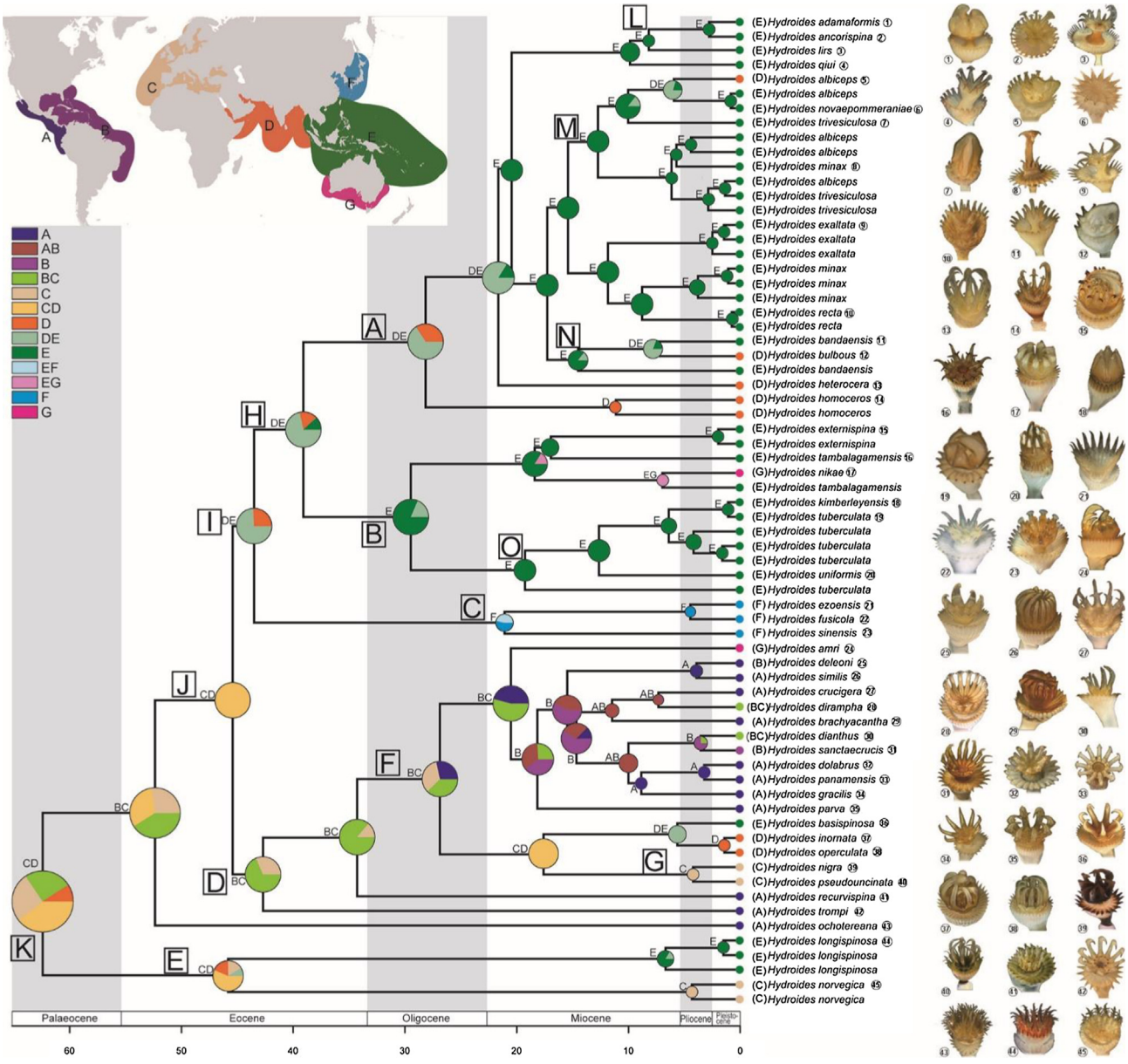

Fig. 6. Biogeographical reconstruction of ancestral ranges in *Hydroides* using DEC with time-slicing. Map on top-left corner illustrates the biogeographic regions used in this study. A: East Pacific, B: West Atlantic, C: East Atlantic and Mediterranean, D: West Indo-Pacific, E: Central Indo-Pacific, 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. Photos on left show operculum of each species. Numbers in circle correspond to species names in the tree.

This indicates a high genetic diversity in the Indo-Pacific region.

Although the first *Hydroides* specific primers (Hydro-COIF/Hydro-COIR) generated COI barcoding fragments from 11 out of 14 tested species (Sun et al., 2012), attempts to use the primer set on a wider range of species resulted in successful amplification of only 17 of the 45 species of *Hydroides* in this study. However, with the new primer set HyCOF190/HyCOR886, 218 COI barcoding sequences were obtained from 44 morphospecies representing 47% of currently recognised species in the genus *Hydroides*. The new primer set of HyCOF190/HyCO673 covered the same barcoding region with that of Hydro-COIF/Hydro-COIR (Sun et al., 2012). The main difference of the two primer sets was that the "A" at 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. The new primer set of HyCOF190/HyCO886 also covered barcoding region of approximately 200 bp longer than that of Hydro-COIF/Hydro-COIR. The high success rate (Supplementary Table S2) represents a significant improvement over the previous primer set (Sun et al., 2012).

With the new primer set developed herein, the present results show the possibility for the broad application of DNA barcoding in the genus *Hydroides*. In most cases, delineation of clade boundaries from COI barcode data was straightforward. The 17-fold higher mean inter- as compared to intra- lineages genetic divergence in COI, and the rarity of intermediate divergences indicated that the COI barcodes had high discrimination power for *Hydroides* species delineation. 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.

Of the multi-lineage morphospecies we detected three genetic lineages within the *H. exaltata*, *H. longispinosa*, and *H. operculata*-complex (Fig. 7A–C), as well as two genetic lineages within *H. homoceros*. Lineages of each species were recovered as a single monophyletic group, with genetic distance 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

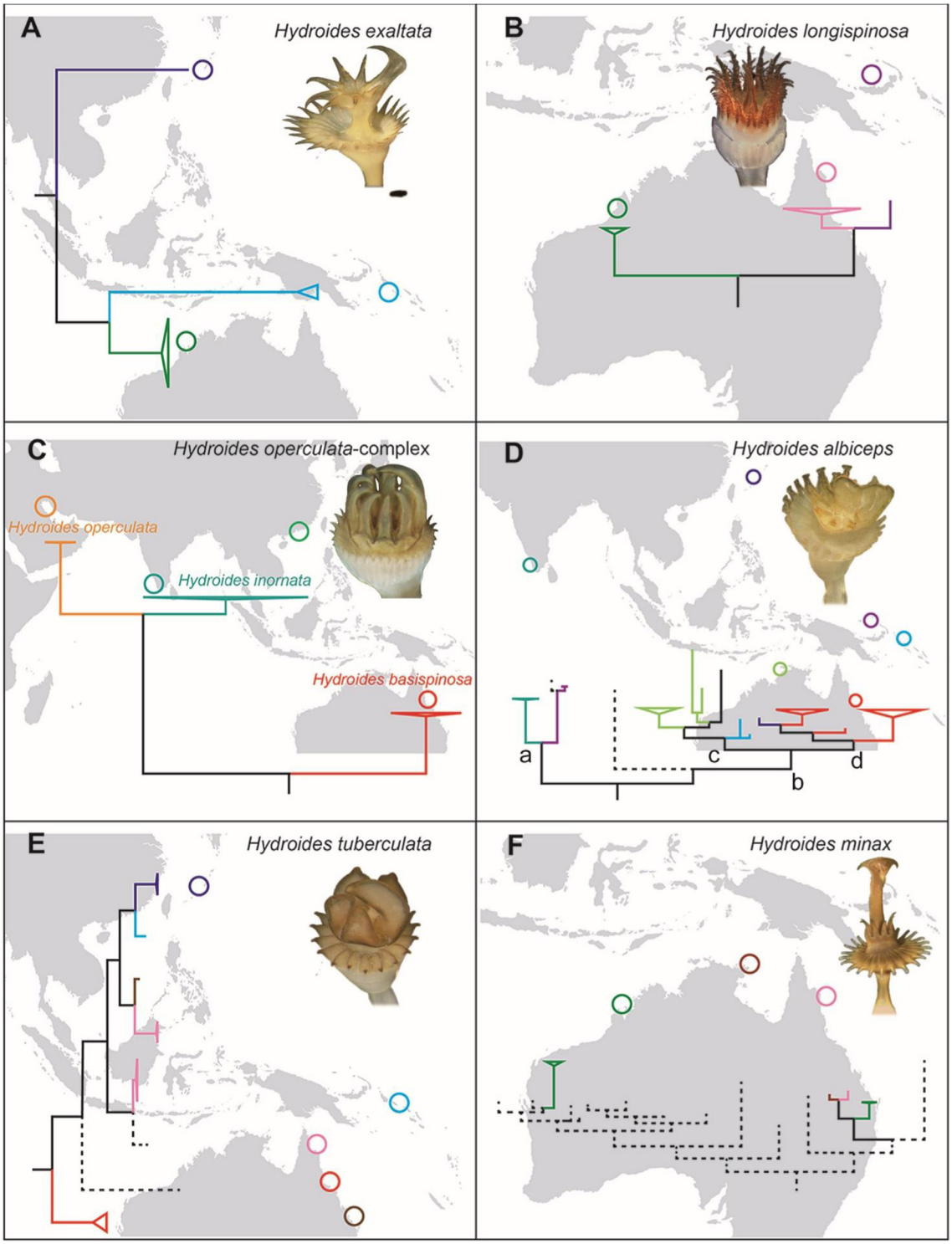

Fig. 7. Widespread *Hydroides* species with multiple lineages reconstructed from phylogenetic analysis, with photos of the operculum of each species. Topologies from the phylogenetic tree in Fig. 2. Coloured circles indicate sampling locations. The colours of the circles correspond to those of the lineages.

19.4% in the *H. operculata*-complex. *Hydroides operculata*, which has a long and convoluted taxonomic history and has been reported nearly world-wide (Ben-Eliahu, 1991; Ben-Eliahu and ten Hove, 1992; Çinar, 2006; Kubal et al., 2012; Sun et al., 2012, 2015), has been recently revised as a species complex consisting of at least three morphologically similar species: *H. inornata*, *H. basispinosa* and *H. operculata*, with different distribution ranges. (Fig. 7C; details see Sun et al., 2017).

The multiple lineages of *H. albiceps* and *H. tuberculata* were recovered as paraphyletic. *Hydroides albiceps* distinguished from other species of *Hydroides* by the operculum having a large vesicular spine and several bottle-shaped smaller spines (Fig. 7D), has been accorded a wide Indo-Pacific distribution, from the Red Sea to Polynesia (Sun et al., 2015). Our results indicate a strikingly high genetic diversity within this species by recovering seven distinct genetic lineages, based on specimens from Augustus Island (Western Australia), Mission Beach (Queensland, Australia), Darwin (Northern Territory, Australia), Maharashtra (India), Okinawa (Japan), Papua New Guinea, and the Solomon Islands, respectively (Fig. 7D). Pair-wise genetic distances for

COI among the eight groups ranged from 10.2% 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. 7D, clade a, b). The lineages from India and Papua New Guinea were grouped together in both BI and ML analyses (Figs. 2, 5D, clade a; PP: 1.00; BP: 100), and recovered as the sister group to a clade of six other genetic lineages. The other clade (Figs. 2, 5D, clade b) consisted of a subclade of lineages from Okinawa, Augustus Island and Mission Beach (Figs. 2, 5D, clade c; PP: 0.99; BP: 98), and specimens from the Solomon Islands and Darwin with good support (Figs. 2, 5D, clade d; PP: 0.99; BP: 98). *Hydroides trivesiculosa*, which is morphologically similar to *H. albiceps*, was nested within the two main clades of the latter (Fig. 2, clade a, b), though with low nodal support. 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 features made it difficult to distinguish specimens from different locations. Our phylogenetic results, however, indicate that there are at least four highly divergent lineages of *H. albiceps* morphotypes that potentially constitute separate species. Apart from *H. albiceps*, *H. tuberculata* was recovered as paraphyletic with the incursion of *H. kimberleyensis* and *H. uniformis*. Within *H. tuberculata*, the main clade grouped specimens from Okinawa (Japan), the Solomon Islands, as well as Broomfield Reef and Lizard Island (Queensland, Australia). Specimens collected from Lizard Island were split into two paraphyletic lineages, except for the specimens grouped in the main clade, the other specimens from Lizard Island were grouped with *H. kimberleyensis*, which indicates possible presence of sympatric cryptic diversity in the *H. tuberculata* morphotype. Specimens from Mission Beach were grouped outside of the clade consisting of other specimens of *H. tuberculata*, *H. kimberleyensis* and *H. uniformis* (from Vanuatu).

Two genetic lineages of *H. bandaensis* from specimens from Papua New Guinea and the Solomon Islands did not form a clade, and had *H. bulbosa* nested within. Three genetic lineages were detected in *H. minax*. Two of the lineages included one specimen was from Lizard Island and one from Bremer Island (Northern Territory, Australia), forming a clade together with two of the five specimens from Augustus Island (WA), with the 11.5–29.4% genetic divergences in COI. The other three specimens of *H. minax* from Augustus Island also formed a monophyletic group, but were nested within 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 are presently 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, differences 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 descriptions that support the genetic identification.

4.2. Operculum evolution

The hypothesis of the opercular transformation series in serpulids was proposed on the basis of the ontogenetic change in opercular structure from a simple 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 indicate that the opercular structure within *Hydroides* does not show a transformation series from simple to complex and as such does not reprise the ontogenetic trajectory. The ancestor of species such as *H. norvegica*, *H. elegans* and *H. longispinosa* (Fig. 6, clade E), 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 localised. Species with thick, swollen, or bulbous verticil spines (Fig. 6, clades L–O) originated in the Indo-Pacific, while species that originated in the east Pacific commonly displayed elongated verticil spines (Fig. 6, clade D). *Hydroides ezoensis* and *H. sinensis* contained only internal spinules on the verticil spines and occurred in the temperate northern Pacific (Fig. 6, clade C). The divergence estimates indicated that most opercular diversification occurred during the Miocene (5–23 Ma). The lineages formed from the Pliocene onwards showed subtle morphological differences (e.g., multiple genetic lineages of morphospecies discussed above) or overlapped in the extent of variation with respect to morphology (e.g., *H. dolabrus* and *H. panamensis*, Fig. 6). In some marine organisms, the high genetic diversity as found in the central Indo-Pacific that 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), or simply, recency of cladogenesis. The diversity and distributions of the present species in the central Indo-Pacific could correspond to sea-level changes during the 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 distribution of the most recent common ancestor of *Hydroides* was in the western Atlantic and the Indian Ocean, which was a tropical Indo-Mediterranean region during the Mid-Palaeocene (Briggs, 2006)—the so-called 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., 2015). Our results are consistent with the record of the oldest fossil of *Hydroides* from the Mediterranean (Lommerzheim, 1981). The results indicate isolation between the Indo-Pacific clade (Fig. 6, clade I) and the eastern tropical Pacific and Atlantic clade (Fig. 6, 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., 2015).

During the closure of the Tethys in the early Miocene, the central Indo-Pacific region was a centre of palaeodiversity of molecular lineages (Fig. 6, clade H), evidenced by the highly localised cladogenesis. Also, the central Indo-Pacific region appeared to act as a source for the nearby Indian Ocean and temperate Australia since the Miocene *via* dispersal and subsequent vicariant separation of *H. albiceps* and *H. nikae*. The pattern of palaeodiversity in the Indo-Mediterranean region and the central Indo-Pacific reflects an eastward shift in historical biodiversity hotspots in marine organisms over the past 50 million years (Renema et al., 2008). The western Tethys Sea, which was a biodiversity hotspot during the Eocene 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, 2011), which led to large extinctions in the western Tethys and an eastward shift of the western 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 emergence of the central Indo-Pacific as a centre for diversity.

5. Conclusions

This is the first study to include a near comprehensive representation of *Hydroides* lineages on a global scale. Although not all described species of the genus could be sampled, this study demonstrates that phylogenetic relationships within *Hydroides* are more concordant with geographical distribution than expectations based on morphology. Species of *Hydroides* are not naturally widely distributed, so any such reported wide distributions should be treated as suspicious unless evidence of human-mediated translocations exists. Species from the central Indo-Pacific region show high levels of homoplasy in the most taxonomically useful morphological features, such as the opercular morphology, compared to their high intra-specific genetic diversity, which calls for further investigation of life history traits in addition to morphological characteristics or defining ecological differences in the delimiting of new species. This study provides the first barcode reference library for species identification of *Hydroides* on a global scale to help with rapid detection of potential cryptic species or new invasive species. 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 centres 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.

Acknowledgements

The study was supported by the Australian Biological Resource Survey (ABRS) [grant number RF213-19] to EKK and PH, and the Australian Biological Resource Survey (ABRS) National Taxonomy Research Grant Programme-2015/2016 Student Travel Grant [STG15-01] to YS. 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, PAH, and EKK for sampling at Lizard Island, Great Barrier Reef. We are grateful to Chris Glasby from the Museum and 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, Mathan Magesh, 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 advice on analyses using BEAST, Michael Gillings for enlightening discussions, and Alexei Ippolitov for his comments on fossil records of *Hydroides*.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.021.

References

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

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., 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., ten Hove, H.A., 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, pp. 515–528.

Ben-Eliahu, M.N., ten Hove, H.A., 1992. Serpulids, Annelida: Polychaeta, along the Mediterranean coast of Israel—New population build-ups of Lessepsian migrants. Israel. J. Zool. 38 (1), 35–53.

Boore, J.L., 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. Mol. Biol. Evol. 17 (1), 87–106.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., 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.

Burnette, A.B., Struck, T.H., Halanych, K.M., 2005. Holopelagic *Poeobius meseres* ('Poeobiidae', Annelida) is derived from benthic flabelligerid worms. Biol. Bull. 208, 213–220.

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. Nat. 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.

Ekman, S., 1953. Zoogeography of the Sea. Sidgwick & Jackson Limited, xiv + 417 pp.

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.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

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.

Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., Francis, C.M., 2004. Identification of birds through DNA barcodes. Plos. Biol. 2 (10), e312.

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.

ten Hove, H.A., 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.

ten Hove, H.A., 1984. Towards a phylogeny in serpulids (Annelida; Polychaeta). In: Hutchings, P.A. (Ed.), Proceeding of the First International Polychaete Conference, Sydney. Linnean Society of New South Wales, Sydney, pp. 181–196.

ten Hove, H.A., Jansen-Jacobs, M.J., 1984. A revision of the genus *Crucigera* (Polychaeta; Serpulidae); a proposed methodical approach to serpulids, with special reference to variation in *Serpula* and *Hydroides*. In: Hutchings, P.A. (Ed.), Proceeding of the First International Polychaete Conference, Sydney. Linnean Society of New South Wales, Sydney, pp. 143–180.

ten Hove, H.A., 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*. Biofouling 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.
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.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16 (2), 111–120.
Kupriyanova, E.K., 2003. Evolution of life history in Serpulimorph polychaetes: a phylogenetic analysis. Hydrobiologia 496, 105–114.
Kupriyanova, E.K., Bastida-Zavala, R., Halt, M.N., Lee, M., 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., Macdonald, T.A., Rouse, G.W., 2006. Phylogenetic relationships within Serpulidae (Sabellida, Annelida) inferred from molecular and morphological data. Zool. Scr. 35, 421–439.
Kupriyanova, E.K., Nishi, E., ten Hove, H.A., 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.
Lehrke, J., ten Hove, H.A., Macdonald, T.A., Bartolomaeus, T., Bleidorn, C., 2007. Phylogenetic relationships of Serpulidae (Annelida, Polychaeta) based on 18S rDNA sequence data and implications for opercular evolution. Org. Divers. Evol. 7, 195–206.
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.
Mörch, O.A.L., 1863. Revisio critica Serpulidarum. Et Bidrag til Rørormenes Naturhistorie. Naturh. Tidsskr. 3, 347–470.
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.
Nóren, M., Jordelius, U., 1999. Phylogeny of Prolecithophora (Platyhelminthes) inferred from 18S rDNA sequences. Cladistics 15, 103–112.
Nygren, A., Pleijel, F., 2011. From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. Mol. Phylogenet. Evol. 58, 132–141.
Okamoto, K., Watanabe, A., Sakata, K., 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. J. Mar. Biotechnol. 6, 7–10.
Okuda, S., 1934. Some tubicolous annelids from Hokkaido. Journal of the Faculty of Science, Hokkaido University, Series 6. Zoology 3 (4), 233–246.
Olmstead, R.G., Sweere, J.A., 1994. Combining data in phylogenetic systematics – an empirical approach using 3 molecular data sets in the Solanaceae. Syst. Biol. 43, 467–481.
Osborn, K.J., Rouse, G.W., Goffredi, S.K., Robison, B.H., 2007. Description and relationships of *Chaetopterus pugaporcinus,* an unusual pelagic polychaete (Annelida, Chaetopteridae). Biol. Bull. 212, 40–54.
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.
Pleijel, F., Rouse, G., Nygren, A., 2009. Five colour morphs and three new species of *Gyptis* (Hesionidae, Annelida) under a jetty in Edithburgh, South Australia. Zool. Scr. 38, 89–99.
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.
Qiu, J.W., Qian, P.Y., 1997. Combined effects of salinity, temperature and food on early development of the polychaete *Hydroides elegans*. Mar. Ecol. Prog. Ser. 152, 79–88.
Read, G., ten Hove, H.A., Sun, Y., Kupriyanova, E.K., 2017. *Hydroides* Gunnerus, 1768 (Annelida: Serpulidae) is feminine: a checklist of updated names. Zookeys 642, 1–52.
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.
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., 1967. 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, Y., Kupriyanova, E.K., Qiu, J.W., 2012. COI barcoding of *Hydroides*: a road from impossible to difficult. Invertebr. Syst. 26, 539–547.
Sun, Y., Wong, E., ten Hove, H.A., 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.
Sun, Y., Al-Kadari, M., Kubal, P., Walmiki, N., Kupriyanova, E.K., 2017. Cutting a Gordian knot of tubeworms with DNA data: the story of *Hydroides operculata* (Annelida, Serpulidae). Zootaxa 4323 (1), 39–48.
Sun, Y., 2017. Taxonomy, barcoding and phylogeny of *Hydroides* (Serpulidae, Annelida), the largest genus of notorious fouling and invading calcareous tubeworms. PhD thesis, Macquarie University, Sydney, Australia.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28 (10), 2731–2739.
Toonen, R.J., Pawlik, J.R., 2001. Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). I. Gregarious and nongregarious settlement. Mar. Ecol. Prog. Ser. 244, 103–114.

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.

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.

Verrill A.E., XVIII, 1873. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. Report on the condition of the sea fisheries of the south coast of New England [later becomes Reports of the United States Commissioner of Fisheries]. 1, 295–778.

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., ten Hove, H.A., Mutvei, H., Kirsimäe, K., 2008. Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida). Zool. J. Linnean Soc. 154, 633–650.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, NY, pp. 315–322.

WoRMS, 2017. *Hydroides* Gunnerus, 1768. In: Read, G., Fauchald, K., (Eds.) World Polychaeta database. Accessed through: < http://www.mariWoRcies.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.

Yuryev, A., 2007. PCR Primer Design (Methods in Molecular Biology). Humana Press, Totowa, New Jersey, pp. 431.