UNIVERSITY OF CALIFORNIA, SAN DIEGO

Phylogeny, biogeography, and systematics of *Branchipolynoe* (Polynoidae, Annelida), with descriptions of four new species from Costa Rican methane seeps

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Johanna I. Lindgren

Committee in charge:

Professor Greg Rouse, Chair
Professor Eric Allen, Co-Chair
Professor Andrew Barton

2017

The Thesis of Johanna I. Lindgren is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California, San Diego

2017

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ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Greg Rouse for his support as the chair of my committee. His guidance and encouragement has made this an enjoyable and fulfilling experience.

I would like to thank Dr. Eric Allen and Dr. Andrew Barton for their membership on my committee and interest in my project.

Finally, I am immensely grateful to my family for their endless love and support.

The Thesis is currently being prepared for submission for publication. Lindgren, Johanna; Hourdez, Stéphane; Rouse, Greg. The thesis author was the primary investigator and author of this material.

ABSTRACT OF THE THESIS

Phylogeny, biogeography, and systematics of *Branchipolynoe* (Polynoidae, Annelida), with descriptions of four new species from Costa Rican methane seeps

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Johanna I. Lindgren

Master of Science in Biology

University of California, San Diego, 2017

Professor Greg Rouse, Chair

Professor Eric Allen, Co-Chair

There are currently four named species of *Branchipolynoe*, all found living symbiotically in mytilid mussels in the genus *Bathymodiolus*, which all occur at hydrothermal vents or methane seeps. Here, phylogenetic analyses using mitochondrial (CO1 and 16S) and nuclear (ITS) genes, as well as morphological analysis were

conducted on a collection of *Branchipolynoe* from Pacific Costa Rican methane seeps. This revealed four new species of *Branchipolynoe* and these are formally described here. These newly discovered species live in three different species of *Bathymodiolus* mussels (also new) at depths ranging from 1000 m to 1800 m. *Branchipolynoe kajsae* sp. nov. and *Branchipolynoe halliseyae* sp. nov. are found in hosts *Bathymodiolus billschneideri*, *B. earlougheri,* and *B. nancyschneiderae. Branchipolynoe meridae* sp. nov. is found in hosts *Bathymodiolus earlougheri* and *B. billschneideri. Branchipolynoe eliseae* sp. nov. is found in hosts *Bathymodiolus billschneideri* and *B. nancyschneiderae.* The phylogenetic and biogeographical implications of this high sympatric diversity of *Branchipolynoe* are discussed.

Introduction

Scaleworms refers to a variety of family-ranked taxa (e.g. Polynoidae, Sigalionidae) placed in Aphroditiformia, which is a clade of annelids with over 1000 described species (Fauchald 1977; Norlinder et al. 2012). While most scaleworms are free living, many within Polynoidae are commensals with other animals. One polynoid genus, *Branchipolynoe*, currently contains four named species that are only found living symbiotically in mytilid mussels of the genus *Bathymodiolus,* which in turn are only known from hydrothermal vents and methane seeps.

Currently there are 14 named polynoid species with branchiae (gills). These belong to five genera: *Peinaleopolynoe*, *Thermopolynoe*, *Branchinotogluma*, *Branchiplicatus*, and *Branchipolynoe* (Hourdez & Jouin-Toulmond 1998) and all are found in the deep sea at hydrothermal vents, methane seeps, or whale falls. These habitats provide abundant hydrogen sulphide needed for certain chemosynthetic bacteria to thrive (Lutz & Kennish 1993). The bacteria are the food source to a variety of filter-feeders and grazers (such as scaleworms), and are symbionts with many animal species, including *Bathymodiolus* mussels (Nelson et al. 1995).

The scaleworm genus *Branchipolynoe* contains species that are only found living inside of oxygen-limited mussel mantle cavities (Pettibone 1984). Notably, they have very well-developed branchiae, much larger than the other branchiate polynoid species. Their gills do not contain blood vessels but are instead filled with coelomic fluid that circulates throughout the body cavity and contains extracellular hemoglobins, producing their red color (Hourdez & Jouin-Toulmond 1998). *Branchipolynoe* are also unusual for

their small scales, since most scaleworms have scales which completely cover the dorsum. However, the scales of *B. pettiboneae*, *B. seepensis,* and *B. symmytilida* only cover the parapodia at most. This may be an adaptation to their lives inside of mussels, not requiring the scales for defense (Pettibone 1984).

Branchipolynoe have been historically regarded as commensal symbionts because they did not appear to cause any noticeable harm to the host mussels (Pettibone 1984). However, recent studies have suggested that they may be parasitic (Desbruyères et al. 1985; Colaço et al. 2002; Britayev et al. 2003; Britayev et al. 2007). One study found pseudofaeces and small amounts of mussel tissue in the gut contents of *B. symmytilida* (Desbruyères et al. 1985). Another study found that the guts of *B. seepensis* contained no mussel tissue and instead contained detritus, a food source to *Bathymodiolus* mussels (Britayev et al. 2003). From this it was inferred that *Branchipolynoe* may be kleptoparasites, stealing food from their hosts (Britayev et al. 2003). However, a study on the carbon and nitrogen signatures of *Branchipolynoe seepensis* and *Bathymodiolus*, found that while they share the same carbon signature, *B. seepensis* had a significantly higher nitrogen signature reflecting a higher trophic level (Colaço et al. 2002). This suggests that *Branchipolynoe* are consuming the mussels themselves rather than the mussels' food.

As well as consuming their hosts, *Branchipolynoe* also cause tissue damage and stunt growth. Infected mussels have been found to have damaged gill filaments (Ward et al. 2004), as well as deformed labial palps and feet (Britayev et al. 2003). Because no mussel tissue was found in the guts of the worms, these traumas were attributed not to parasitism but to collateral damage caused by the feeding of the worms (Britayev et al.

2003). *Branchipolynoe* are often found inside tunnels within the gills of their hosts (Britayev et al. 2003). Infected mussels also have a higher width to length ratio, indicating stunted growth (Britayev et al. 2003). This further supports a parasitic relationship rather than a commensal one.

Branchipolynoe are sexually dimorphic, with larger females, and have a sex ratio skewed from 1:1 (ranging from 0.5:1 to 0.7:1), in favor of females (Van Dover et al. 1999). This corresponds with their behavior, as females will remain in their host mussel their whole life and males must leave their host to find a female to breed with (Britayev et al. 2007). *Branchipolynoe* have internal fertilization and the females can store sperm and are therefore able to produce many offspring from one pairing (Van Dover et al. 1999; Britayev et al. 2007). Their fecundity is limited by their very large (up to 400 μm), likely lecithotrophic eggs; however, this may be an adaptation to development and dispersal in the extreme conditions of vents and seeps (Jollivet et al. 2000).

Branchipolynoe currently has four named species (Pettibone 1984; Pettibone 1986; Miura & Hashimoto 1991; Zhou et al. 2017). *Branchipolynoe symmytilida* Pettibone 1984 was described from Galapagos Rift hydrothermal vents and lives in *Bathymodiolus thermophilus* (Pettibone 1984; Fisher et al. 1988). It has also been found in the East Pacific Rise (EPR) (Chevaldonné et al. 1998) in hosts *Bathymodiolus thermophilus and B. antarcticus* (Johnson et al. 2013; Johnson pers. comm.). Though the EPR *Branchipolynoe symmytilida* and Galapagos *B. symmytilida* show some genetic differentiation in terms of haplotype frequency distribution (Hurtado et al. 2004), there is no doubt that it is a widely-distributed species.

Branchipolynoe pettiboneae Miura & Hashimoto 1991 was described from hydrothermal vents at the Kaikata Seamount (Japan) in the hosts *Bathymodiolus brevior*, *B. platifrons*, and *B. japonicus* (Miura & Hashimoto 1991; Miura 1997). It was also recorded from Izena Hole, Okinawa Trough (Miura & Hashimoto 1991). It has been reported from other vent areas in the western Pacific, including Fiji (Chevaldonné et al. 1998), though this would appear to be a yet undescribed species (Qiu pers. comm.). *Branchipolynoe longqiensis* Zhou, Zhang & Wang 2017 was described from the Longqi vent field (Southwest Indian Ridge) in host *Bathymodiolus marisindicus* (Zhou et al. 2017). Two sequences available from Indian Ocean hydrothermal vents for unnamed species of *Branchipolynoe* (Van Dover et al. 2001; Copley et al. 2016) can be attributed to *Branchipolynoe longqiensis* (Zhou pers. comm.).

Branchipolynoe seepensis Pettibone 1986 was described from Florida Escarpment (Gulf of Mexico) methane seeps in an unidentified *Bathymodiolus* mussel (Pettibone 1986). The host mussel is most likely *Bathymodiolus heckerae* from Florida Escarpment (Faure 2015). *Branchipolynoe seepensis* has also been recorded several times, based on morphology, from the Mid-Atlantic Ridge (MAR) (Segonzac 1992; Desbruyères 1994). There, it is found in hosts *Bathymodiolus azoricus* and *B. puteoserpentis* (Chevaldonné et al. 1998; Daguin & Jollivet 2005). However, the MAR *Branchipolynoe seepensis* and the Gulf of Mexico *B. seepensis* are most likely separate species based on three factors. One is that there is marked genetic distance of more than 5% for Cytochrome Oxidase 1 (COI) between specimens from the two localities (Chevaldonné et al. 1998), though unfortunately the COI sequences are not available. Also, they are found thousands of

kilometers apart, with the true *Branchipolynoe seepensis* found at methane seeps, while the MAR records are from hydrothermal vents (Chevaldonné et al. 1998).

In this paper, we present morphological and molecular sequence data from samples of *Branchipolynoe* from Pacific Costa Rican methane seeps that have been explored in recent years (Levin et al. 2012; Levin et al. 2015). We sequenced DNA for the mitochondrial COI and 16S rDNA, as well as the nuclear ITS1 and ITS2 genes. From this, the phylogeny and biogeography of *Branchipolynoe* were assessed.

Materials and Methods

Material studied

Specimens of *Branchipolynoe* and *Bathymodiolus* were collected from several cruises in Costa Rica from 2009-2017. *Branchipolynoe* specimens were either placed in 95% ethanol or in 10% formaldehyde, then transferred to 70% ethanol. A piece of the foot of *Bathymodiolus* specimen was placed in 95% ethanol. Table 1 outlines the collection details and host *Bathymodiolus* species of *Branchipolynoe* used in this study for DNA sequencing.

Morphology

A Canon EOS Rebel T3i digital camera mounted on a Leica S8APO stereomicroscope or a Leica DMR HC compound microscope was used for micrography. Helicon Focus v. 4.2.8 (Helicon Soft Ltd.) was used to merge images to create stacked pictures.

DNA extraction, amplification, and sequencing

Fragments of the mitochondrial CO1 gene were obtained for 103 *Branchipolynoe* individuals. Mitochondrial 16S and nuclear ITS genes were also sequenced for a subset of *Branchipolynoe* specimens. Fragments of the mitochondrial CO1 gene were obtained for 74 *Bathymodiolus* individuals. DNA was extracted using the Zymo Research Genomic DNA™ -Tissue MiniPrep Kit or the Zymo Research *Quick*-DNA™ 96 Plus Kit (Zymo Research, CA, USA) following the manufacturer's protocol. Genes were

amplified with a PCR mixture of 12.5 uL of either Apex™ Taq RED Master Mix (Genesee Scientific, USA) or Conquest™ PCR Master Mix 1 (Lamba Biotech, USA), 8.5 uL H2O, 1 uL of each primer, and between 50-100 ng DNA.

For *Branchipolynoe*, a 700bp fragment of CO1 was amplified with the primers LCO1490 and HCO2198 (Folmer et al. 1994) and the following protocol: 3 min. 94°C; 5 x (30 sec 94°C, 45 sec 47°C, 1 min. 72°C); 30 x (30 sec 94°C, 45 sec 52°C, 1 min. 72°C); 5 min. 72°C. A 500bp region of 16S was amplified with the protocol: 3 min. 95°C; 40 x (40 sec 95°C, 40 sec 50°C, 50 sec 72°C); 5 min. 72°C and the primers 16Sarl and 16Sbrh (Palumbi et al. 2002). Either both the ITS1 and ITS2 genes were amplified together with primers ITS18SFPOLY and POLY_28R (Nygren et al. 2009; Glover et al. 2005) and the protocol: 4 min. 96°C; 45 x (30 sec 94°C, 30 sec 48°C, 1 min. 72°C); 8 min. 72°C, or the ITS2 gene was amplified with the primers POLY_ITS3F and POLY_28R (Glover et al. 2005) and the same protocol. For *Bathymodiolus*, a 500-600bp fragment of CO1 was amplified with the primers BathCO1-F and BathCO1-R (Olu-Le Roy et al. 2007) and the following protocol: 2 min. 94°C; 5 x (35 sec 94°C, 35 sec 48°C, 70 sec 72°C); 35 x (35 sec 94°C, 35 sec 52°C, 70 sec 72°C); 10 min. 72°C.

The PCR products were purified using 2uL ExoSAP-IT™ (USB Corporation, Ohio, USA) and the following protocol: 20 min. 37°C; 15 min. 80°C. The cleaned PCR products were sent to Eurofins Genomics (Louisville, KY, USA) or Retrogen, Inc. (San Diego, CA, USA) for sequencing. The sequences were edited by eye on Geneious v. 7.1.9 (Kearse et al. 2012) and aligned using MAFFT v. 7 server (Katoh et al. 2002) under default settings.

Phylogenetic analysis

Phylogenetic trees were obtained for the individual *Branchipolynoe* CO1 gene and for the concatenated CO1, 16S, and ITS genes using maximum likelihood (ML) and maximum parsimony (MP). Aligned sequences for CO1, 16S, and ITS were concatenated using SequenceMatrix v 1.8 (Vaidya et al. 2011) and exported as non-interleaved nexus or phylip formatted files. Maximum likelihood (ML) analyses were done using RAxML v. 1.5 (Stamatakis 2014) with the data partitioned by gene, support assessed via a minimum of 100 thorough bootstrap replicates. *Branchinotogluma sandersi* was set as the outgroup based on the phylogenetic results of Norlinder et al (2012). Alternative rooting analysis was also explored using another branchiate polynoid, *Peinaleopolynoe sillardi,* as an outgroup.

Maximum parsimony (MP) analyses were conducted using PAUP* v. 4.0 (Swofford 2002), using heuristic searches with the tree-bisection-reconnection branch-swapping algorithm and 1000 random addition replicates. Support values were determined using 1000 jackknife replicates each with 100 random addition searches, heuristic search with tree-bisection-reconnection, and 37% character deletion.

To find the most suitable model for assessing genetic distances, an AIC calculation using jModelTest v. 2.1.4 (Darriba et al. 2012; Guindon & Gascuel 2003) was implemented on the separate CO1 and ITS data sets with default settings. The models selected were TIM1+G for CO1 and HKY+G for ITS. The uncorrected CO1 and ITS pairwise distances and the corrected pairwise distances with the corresponding models were found in PAUP* v. 4.0 (Swofford 2002).

ML analysis to assess species delineation was also performed on the *Bathymodiolus* CO1 data set using RAxML v. 1.5 (Stamatakis 2014) with support assessed via a minimum of 100 thorough bootstrap replicates, and *Benthomodiolus lignicola* as the outgroup based on the phylogenetic results of Miyazaki et al. (2010).

Character transformations

Character transformations for locality and habitat were individually performed using Mesquite v. 3.1 (Maddison & Maddison 2017) on the ML and MP trees obtained from CO1, 16S, and ITS data. The states for the locality character are East Pacific, West Pacific, West Atlantic, and Indian Ocean. The states for habitat are hydrothermal vents and methane seeps. The Mk1 model was used for the transformations (Lewis 2001) as this incorporates branch length information into the transformation.

Haplotype networks

Haplotype networks for each species were obtained from CO1 data with PopART (Bandelt et al. 1999), using a median joining network and epsilon set to 0. Two color-coded haplotype networks were made for each species, one coded for host mussels and one for depth.

Results

Species delimitation

Table 2 shows the uncorrected and TIM1+G corrected CO1 pairwise distances for the *Branchipolynoe* species. Table 3 shows the uncorrected and HKY+G corrected ITS pairwise distances. The combined CO1 and ITS data suggest the recognition of four new species: *B. kajsae* sp. nov., *B. halliseyae* sp. nov., *B. meridae* sp. nov., and *B. eliseae* sp. nov., which are formally described below. Both the CO1 and ITS pairwise distances show much higher interspecific distances than intraspecific distances for each species. Justification for the species delineation can be found in the Discussion.

The minimum corrected CO1 distance between *B. meridae* and *B. eliseae* was 8.37%. Their respective minimum CO1 distances to *B. symmytilida* were 10.99% and 11.90%. The minimum corrected CO1 distances between *B. seepensis* and *B. kajsae* and *B. halliseyae* was 6.65% and 7.11%, respectively. The minimum CO1 distance between *B. kajsae* and *B. halliseyae* was relatively close, at 4.75%. However, the minimum corrected ITS distance between them was found to be 1.91%. This is larger than between *B. symmytilida* and both *B. meridae* and *B. eliseae*, which were 1.30% and 1.08% respectively.

Phylogeny

Figure 1 shows the ML tree (likelihood score of -6369.8) obtained from CO1, 16S, and ITS concatenated data from one individual from each species, with *Branchinotgluma sandersi* as the outgroup. Each relevant node shows the ML bootstrap

and MP jackknife support values. The same tree topology was obtained from ML analysis run with an alternative branchiate scaleworm, *Peinaleopolynoe sillardi,* as the outgroup, and is therefore not shown. Two most parsimonious trees with lengths of 723 steps were obtained from the CO1, 16S, and ITS concatenated data. One of the two trees had the same topology as the ML result (Figure 1). Figure 2 shows the second MP tree, with MP jackknife values on the nodes. This second tree differs from the ML result and the other MP tree only with respect to the positions of *B. eliseae* and *B. meridae* and *B. symmytilida*. The ML and one MP trees have *B. eliseae* and *B. symmytilida* as sisters while the MP tree in Figure 2 has *B. eliseae* and *B. meridae* as sister taxa, though with low support (jackknife value= 41%). Other nodes across the tree were reasonably well supported, though the ML bootstrap support value for *B. eliseae* and *B. symmytilida* as sisters was 65 (Figures 1 & 2).

Host mussels

All the new *Branchipolynoe* species were found in the host mussel *Bathymodiolus billschneideri*. All except *B. meridae* were also found in host *B. nancyschneiderae*, and all except *B. eliseae* were found in host *B. earlougheri* (Table 1). *Bathymodiolus nancyschneiderae* was only found at 1000 m, *B. billschneideri* was found at 1400 m and 1800 m but not 1000 m, and *B. earlougheri* was found at 1000 m and 1800 m.

Character transformations

Figure 3 shows the ML tree topology (with ML branch lengths incorporated, though not shown) obtained from CO1, 16S, and ITS data with the transformation for the

character based locality. This also matched the tree topology for one of the MP trees. Figure 4 shows the same ML tree with transformation for habitat. Figure 5 shows the alternative MP tree obtained from CO1, 16S, and ITS data with the transformation for locality and Figure 6 also shows this MP tree with transformation for habitat. The ancestral locality for *Branchipolynoe* is most likely the East Pacific though the likelihood for this was only 47% based on the ML tree topology and 52% based on the MP tree topology (Figures 3 & 5). The ancestral habitat for *Branchipolynoe* is somewhat ambiguous, as it is most likely seeps, based on Figure 4, though the likelihood for this was only 61%. The alternative MP tree topology slightly favors vents as the most likely ancestral habitat, though with a likelihood of 54% (Figure 6).

Haplotype networks

Figures 7–9 show that most individuals of each species sequenced for this study have their own haplotype, with few sharing the same haplotype. *Branchipolynoe kajsae* has no individuals with the same haplotype (Figure 7), while *B. halliseyae* also has a few shared haplotypes, though one is relatively well-represented (Figure 8). *Branchipolynoe meridae* and *B. eliseae* both have several individuals with unique haplotypes, and only one shared haplotype (Figure 9). The haplotype networks do not show any obvious correlation between similar haplotypes and depth or host mussel. Some of the same haplotypes are found in all three hosts and across depths.

Discussion

This study revealed much more diversity than would be expected based on previous studies of *Branchipolynoe*. There have only been four previously described *Branchipolynoe* species from hydrothermal vents and methane seeps across the world (Pettibone 1984; Pettibone 1986; Miura & Hashimoto 1991; Zhou et al. 2017), though there is evidence of two other yet undescribed species (Chevaldonne et al. 1998, Qui pers. com.). The discovery and naming of four new species from Pacific Costa Rican seeps doubles the number of named *Branchipolynoe* species. These species are genetically distinct, but have few morphological differences, with *B. halliseyae* and *B. kajsae* showing no clear morphological differences. They represent cryptic species, which are morphologically similar yet genetically distinct species (Bickford et al. 2007). Cryptic animal species have been found repeatedly from hydrothermal vents and methane seeps (Vrijenhoek et al. 1994; Peek et al. 1997; Johnson et al. 2008; Borda et al. 2013; Stiller et al. 2013). Cryptic speciation in vents and seeps may be due in part to stabilizing selection imposed by the extreme conditions, or to differences in development or habitat and host preferences (Bickford et al. 2007).

Describing animal species with no obvious morphological differences is becoming more routine based on molecular data alone (Halt et al. 2009). Doing so may separate previously believed cosmopolitan species (Stiller et al. 2013; Borda et al. 2013), which is important for understanding biogeographical patterns, species distributions, and depth and habitat preferences (Nygren 2013).

There is no consensus on a minimum CO1 distance required to delimitate species, however substantially larger minimum interspecific distances over maximum intraspecific distances suggest the recognition of new species (Meyer & Paulay 2005; Meier et al. 2008). The minimum-corrected CO1 distance of 8.37% between *B. meridae* and *B. eliseae* is much higher than their maximum intraspecific variation of 2.42% and 0.75%, respectively (Table 2). This supports their recognition as two new species.

Nuclear ITS distances were found as well as the mitochondrial CO1 distances, as the ITS region has been shown to be useful as a marker for the separation of cryptic species (Nygren et al. 2011; Nygren & Pleijel 2011; Nygren 2013). ITS distances can be used in addition to CO1 data if the CO1 results are unclear, and are typically smaller than CO1 distances (Nygren et al. 2011; Nygren & Pleijel 2011; Borda et al. 2013). *Branchipolynoe kajsae* and *B. halliseyae* have a relatively close minimum-corrected CO1 distance of only 4.75%, however the minimum-corrected ITS distance between *B. kajsae* and *B. halliseyae* is 1.91%, which is greater than the distance between *B. symmytilida* and *B. meridae* (1.30%) and *B. eliseae* (1.08%) (Table 3). Based on previous studies of cryptic species in the deep sea (Nygren & Pleijel 2011; Borda et al. 2013), a 4.75% CO1 distance and a 1.91% ITS distance has been considered enough to delimitate two species. Therefore, the minimum corrected CO1 and ITS distances suggests the recognition of *B. kajsae* and *B. halliseyae* as two distinct species, though further sampling and sequencing to validate this would seem wise.

There was no correlation between similar haplotypes and host mussel or depth (Figures 7–9). There were also no marked genetic distances between individuals of the same species living in different depths or hosts. Some of the same haplotypes were found

at different depths and different hosts. This suggests that *Branchipolynoe* species at Costa Rican methane seeps have no preference for host mussel species and disperse between 1000 m and 1800 m with no difficulty. However, two species were found mostly at 1000 m (*B. halliseyae* and *B. meridae*) and the other species were mostly found at 1800 m (*B. kajsae* and *B. eliseae*) (Table 1).

The four new species are the only known sympatric *Branchipolynoe* species to date. However, despite sharing the same locality, they appear to share different evolutionary histories. As *Branchipolynoe halliseyae* and *B. kajsae* are most closely related to *B. seepensis* which is found in the Gulf of Mexico, they likely diverged from *B. seepensis* by a vicariant event resulting from the formation of the Panama Isthmus, which began initial forming 23 to 25 million years ago and finally became a land barrier around 3.5 million years ago (Farris et al. 2011). The dispersal of deep-sea taxa, such as *Branchipolynoe* would likely have been impacted some tens of millions of years ago (Bacon et al. 2015). Molecular clock assessment of the sequence data for *Branchipolynoe* may be able to pinpoint this vicariant event, but independent calibration could not be determined for this study.

The phylogeny of *B. symmytilida*, *B. meridae*, and *B. eliseae* showed different scenarios under maximum likelihood and maximum parsimony (Figures 1 & 2). The ML tree, as well as one of the two MP trees, places *B. symmytilida* as sister to *B. eliseae* (Figure 1). The other MP tree places *B. meridae* as sister to *B. eliseae* (Figure 2). Based on Figure 4, the most recent common ancestor for the clade likely lived at methane seeps, with the ancestor of *B. symmytilida* having had a habitat shift to hydrothermal vents.

Figure 6 however shows that the ancestral habitat for the ancestor of *B. symmytilida*, *B. meridae*, and *B. eliseae* is equally likely to have been at a vent or a seep.

The circumstances under which *B. halliseyae* and *B. kajsae* diverged from each other is unclear, as they share the same habitat, host mussels, and depth. However, most *B. halliseyae* individuals were found at 1000 m and most *B. kajsae* individuals were found at 1800 m, so depth may have been a contributing factor to their speciation.

Other studies on polychaete diversity have found differing biogeographical patterns. A study on *Amphisamytha* found a similar scenario to *Branchipolynoe,* where sympatric species were not the most closely related species (Stiller et al. 2013). Conversely, a study on *Archinome,* found that the geographically closest species were also the most closely related species, with an Atlantic species sister to a clade formed by three East Pacific species, that diverged due to vicariant events (Borda et al. 2013). This scenario is somewhat like that recovered here for *B. symmytilida*, *B. meridae*, and *B. eliseae.*

The ancestor to *Branchipolynoe* likely lived in the East Pacific (Figures 3 & 5) in methane seeps (Figure 4) and may have moved west to colonize vents in the West Pacific and Indian Ocean as well as east to colonize seeps in the Gulf of Mexico.

The most closely related *Branchipolynoe* species are not found in the most closely related *Bathymodiolus* species. The host mussels of the four new *Branchipolynoe* species (*Bathymodiolus earlougheri*, *B. nancyschneiderae*, and *B. billschneideri*) are closely related to each other and to the hosts of *B. symmytilida* (*B. thermophilus* and *B. antarcticus*) (Rouse pers. comm.), forming a single clade. This clade is sister to a clade containing the hosts of *Branchipolynoe seepensis* and *B. longqiensis*, and one of the hosts

of *B. pettiboneae* (Miyazaki et al. 2010). Together, both clades are sister to a clade containing the remaining two hosts of *B. pettiboneae* (Miyazaki et al. 2010).

Branchipolynoe meridae and *B. eliseae* are most closely related to *B. symmytilida* while *B. halliseyae* and *B. kajsae* form a clade sister to *B. seepensis*, which in turn form a clade sister to *B.* cf. *pettiboneae* and *B. longqiensis*. This shows a different phylogenetic relationship to the hosts, and suggests that speciation of *Bathymodiolus* and *Branchipolynoe* did not follow the same pattern, though further sampling of *Bathymodiolus* for potential undiscovered species of *Branchipolynoe* would be worth pursuing.

Branchipolynoe forms part of a clade of scaleworms associated with vent and seep habitats (Norlinder et al. 2012, Gonzalez et al. 2017). The other known species though (e.g. *Branchinotogluma*) are free-living forms and not symbiotic. Our results suggest that the ancestral *Branchipolynoe* likely colonized *Bathymodiolus* from a pre-existing scaleworm living at a seep habitat. This may have been in East Pacific seeps (Figures 3–5) and further colonization of different *Bathymodiolus* likely moved west and east. This would account for the different phylogenetic relationships between *Branchipolynoe* and *Bathymodiolus* (Miyazaki et al. 2010). Divergence time estimates for *Branchipolynoe* should be made and compared to *Bathymodiolus* to see if the *Bathymodiolus* hosts diverged before the *Branchipolynoe* species.

There are undoubtedly more *Branchipolynoe* species to be discovered at hydrothermal vents and methane seeps across the world. Further research into these vent and seep communities may provide valuable insight into the dispersal and adaptation of these fascinating worms.

New Species Descriptions

Subfamily Branchipolynoinae Pettibone, 1984

Genus *Branchipolynoe* Pettibone, 1984

***Branchipolynoe kajsae*, new species**

(Figures 10 & 11)

Material- Costa Rica, Eastern Pacific, from dives of *Alvin* in 2010 and 2017, associated with mussels near hypersaline seeps. Dive 4909, 24 May 2017, 8.93048° N, 84.31250° W, 1000 m, collectors E. Cordes & R. Smith, holotype (A6611). Dive 4590, 11 Jan 2010, 9.1175667° N, 84.839534° W, 1800 m, collector G. Rouse, 9 paratypes. Dive 4907, 22 May 2017, 8.93042° N, 84.31278° W, 999 m, collectors L. Levin & C. Seid, 2 paratypes. Dive 4910, 25 May 2017, 8.93042° N, 84.31263° W, 1004 m, collectors G. Rouse & T. Litke, 1 paratype. Dive 4914, 29 May 2017, 9.11753° N, 84.83953° W, 1886 m, collectors C. Roman & A. Durkin, 2 paratypes. Dive 4917, 1 June 2017, 8.92930° N, 84.31500° W, 1000 m, collectors G. Rouse & B. Moran, 1 paratype. Dive 4922, 5 June 2017, 8.92955° N, 84.30780° W, 1009 m, collectors J. Le & C. Roman, 1 paratype. Dive 4924, 7 June 2017, 9.03048° N, 84.62020° W, 1409 m, collectors L. Levin & K. Krasnosky, 1 paratype. (See table 1 for catalog numbers).

Etymology- Named in honor of Carin “Kajsa” Lindgren, mother of the first author.

Host- *Bathymodiolus earlougheri, Bathymodiolus nancyschneiderae,* and *Bathymodiolus billschneideri*

Diagnosis- Body long with 21 segments, including first achaetous tentacular segment. Ten pairs of elytra attached on elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, round, and smooth, leaving middle third of body uncovered. Dorsal cirri on non-elytra bearing segments. Short ventral cirri. Enlarged ventral papillae on segments 11 and 12. Parapodia subbiramous. Notopodia small with few, short notosetae. Two branches of branchiae above dorsal cirri. Bilobed prostomium with short median antenna, one pair of palps, and rounded anterior lobes, lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous and fused to the prostomium with two pairs of tentacular cirri. Branchiae are dense and start on segment 2 or 3 with larger upper and smaller lower groups. Pygidium with pair of anal cirri.

Measurements- The holotype is 35 mm long, 15 mm wide, including parapodia, with 21 segments.

Description- (Figures 10 & 11) Body long, slightly tapered posteriorly. Adults have 21 segments, including the first achaetous tentacular segment.

Ten pairs of elytra attached anteriorly to elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, round, and smooth. Elytra leave most of the dorsum uncovered, ranging from partially covering to completely covering the parapodia. Non-

elytra bearing segments have cylindrical cirrophores and long, slender dorsal cirri extending past the setae.

Dense and aborescent branchiae emerge from the body in two groups, a larger dorsal group and a smaller ventral group. Branchiae begin on either segment 2 or segment 3, completely covering the parapodia.

The parapodia subbiramous. Notopodia much smaller than neuropodia with fewer notosetae. Notosetae smooth and shorter and thicker than neurosetae. Neurosetae long and slender with tapered tip. No difference in shape between dorsal and ventral neurosetae.

Prostomium bilobed with rounded anterior lobes with two bumps on either side, short conical median antenna, and pair of long, slender palps. Prostomium lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous, fused to the prostomium, with two pairs of long tentacular cirri. Buccal cirri similar in size to tentacular cirri. Ventral cirri smaller, attached to middle of neuropodia. Enlarged ventral papillae on segments 11 and 12 project posteriorly. Pygidium small with pair of thick, tapered anal cirri.

Remarks- *Branchipolynoe kajsae* has large elytra and short, dense branchiae, while *B. pettiboneae* has long filamentous branchiae, and *B. symmytilida*, *B. meridae*, and *B. eliseae* have long filamentous branchiae and small elytra. *Branchipolynoe kajsae* has subbiramous parapodia while *B. seepensis* and *B. longqiensis* have biramous parapodia. *Branchipolynoe kajsae* has long dorsal cirri, while *B. seepensis*, *B. symmytilida*, *B. pettiboneae*, *B. longqiensis*, *B. meridae*, and *B. eliseae* all have short dorsal cirri.

Branchipolynoe halliseyae has branchiae starting on segment 2 and *B. kajsae* has branchiae starting on segment 2 or 3. There are no clear morphological differences between *B. halliseyae* and *B. kajsae.*

***Branchipolynoe halliseyae*, new species**

(Figures 12 & 13)

Material- Costa Rica, Eastern Pacific, from dives of *Alvin* in 2009, 2010, and 2017, associated with mussels near hypersaline seeps. Dive 4501, 22 Feb 2009, 8.93° N, 84.3133° W, 1008 m, collectors G. Rouse & D. Huang, holotype (A1322), 10 paratypes. Dive 4511, 5 Mar 2009, 8.9305° N, 84.3123° W, 1001 m, collectors G. Rouse & D. Huang, 1 paratype. Dive 4586, 7 Jan 2010, 8.9308° N, 84.313° W, 1000 m, collector G. Rouse, 7 paratypes. Dive 4588, 9 Jan 2010, 8.9307833° N, 84.312533° W, 997 m, collector G. Rouse, 3 paratypes. Dive 4907, 22 May 2017, 8.93042° N, 84.31278° W, 999 m, collectors L. Levin & C. Seid, 9 paratypes. Dive 4909, 24 May 2017, 8.93048° N, 84.31250° W, 1000 m, collectors E. Cordes & R. Smith, 4 paratypes. Dive 4910, 25 May 2017, 8.93042° N, 84.31263° W, 1004 m, collectors G. Rouse & T. Litke, 7 paratypes. Dive 4911, 26 May 2017, 9.11507° N, 84.84683° W, 1891 m, collectors L. Levin & J. Le, 3 paratypes. Dive 4912, 27 May 2017, 9.11538° N, 84.83618° W, 1859 m, collectors V. Orphan & K. Dawson, 1 paratype. Dive 4914, 29 May 2017, 9.11753° N, 84.83953° W, 1886 m, collectors C. Roman & A. Durkin, 5 paratypes. Dive 4915, 30 May 2017, 9.11800° N, 84.84043° W, 1885 m, collectors S. Goffredi & D. Forsman, 1 paratype. Dive 4917, 1 June 2017, 8.92930° N, 84.31500° W, 1000 m, collectors G. Rouse & B. Moran, 9 paratypes. Dive 4922, 5 June 2017, 8.92955° N, 84.30780° W, 1009 m,

collectors J. Le & C. Roman, 2 paratypes. Dive 4924, 7 June 2017, 9.03048° N, 84.62020° W, 1409 m, collectors L. Levin & K. Krasnosky, 4 paratypes. (See table 1 for catalog numbers).

Etymology- Named in honor of Jessica Hallisey, in appreciation for her efforts at Seacamp who have supported the Scripps Oceanographic Collections Endowment in her honor.

Host- *Bathymodiolus earlougheri, Bathymodiolus nancyschneiderae,* and *Bathymodiolus billschneideri*

Diagnosis- Body long with 21 segments, including first achaetous tentacular segment. Ten pairs of elytra attached on elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, round, and smooth, leaving middle third of body uncovered. Dorsal cirri on non-elytra bearing segments. Short ventral cirri. Enlarged ventral papillae on segments 11 and 12. Parapodia subbiramous. Notopodia small with few, short notosetae. Two branches of branchiae above dorsal cirri. Bilobed prostomium with short median antenna, one pair of palps, and rounded anterior lobes, lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous and fused to the prostomium with two pairs of tentacular cirri. Branchiae are dense and start on segment 2 with larger upper and smaller lower groups. Pygidium with pair of anal cirri.

Measurements- The holotype is 43 mm long, 17 mm wide, including parapodia, with 21 segments.

Description- (Figures 12 & 13) Body long, slightly tapered anteriorly and posteriorly, and arched dorsally. Adults have 21 segments, including the first achaetous tentacular segment.

Ten pairs of elytra attached anteriorly to elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, round, and smooth. Elytra partially or completely cover the parapodia, leaving most of the dorsum uncovered. Non-elytra bearing segments have cylindrical cirrophores and long tapered dorsal cirri that extend slightly past the setae.

Branchiae dense and aborescent, emerging from the body in two groups, a larger dorsal group and a smaller ventral group. Branchiae begin on segment 2, increasing in size to completely cover parapodia.

The parapodia subbiramous. Notopodia much smaller than neuropodia with fewer notosetae. Notosetae smooth and shorter and thicker than neurosetae. Neurosetae long and slender with a tapered tip. No clear separation or difference in shape between dorsal and ventral neurosetae.

Prostomium bilobed with rounded anterior lobes, short conical median antenna, and pair of long palps. Prostomium lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous, fused to the prostomium, with two pairs of short tentacular cirri. Buccal cirri similar in size to tentacular cirri. Ventral cirri smaller,

attached to middle of neuropodia. Enlarged ventral papillae on segments 11 and 12 project posteriorly. Pygidium small with pair of short anal cirri.

Remarks- *Branchipolynoe halliseyae* has large elytra and short, dense branchiae, while *B. pettiboneae* has long filamentous branchiae, and *B. symmytilida*, *B. meridae*, and *B. eliseae* have long filamentous branchiae and small elytra. *Branchipolynoe halliseyae* has subbiramous parapodia while *B. seepensis* and *B. longqiensis* have biramous parapodia. *Branchipolynoe halliseyae* has long dorsal cirri, while *B. seepensis*, *B. symmytilida*, *B. pettiboneae*, *B. longqiensis*, *B. meridae*, and *B. eliseae* all have short dorsal cirri. *Branchipolynoe halliseyae* has branchiae starting on segment 2 and *B. kajsae* has branchiae starting on segment 2 or 3. There are no clear morphological differences between *B. halliseyae* and *B. kajsae.*

***Branchipolynoe meridae*, new species**

(Figures 14 & 15)

Material- Costa Rica, Eastern Pacific, from dives of *Alvin* in 2009, 2010, and 2017, associated with mussels near hypersaline seeps. Dive 4911, Dive 4911, 26 May 2017, 9.11507° N, 84.84683° W, 1891 m, collectors L. Levin & J. Le, holotype (A6616). Dive 4511, 5 Mar 2009, 8.9305° N, 84.3123° W, 1001 m, collectors G. Rouse & D. Huang, 2 paratypes. Dive 4501, 22 Feb 2009, 8.93° N, 84.3133° W, 1008 m, collectors G. Rouse & D. Huang, 1 paratype. Dive 4586, 7 Jan 2010, 8.9308° N, 84.313° W, 1000 m, collector G. Rouse, 6 paratypes. Dive 4591, 12 Jan 2010, 9.11821667° N, 84.839116° W, 1802 m, collector G. Rouse, 1 paratype. (See table 1 for catalog numbers).

Etymology- Named after Merida from Disney/Pixar movie "Brave", due to the resemblance of its branchiae to her hair.

Host- *Bathymodiolus earlougheri* and *Bathymodiolus billschneideri*

Diagnosis- Body long with 21 segments, including first achaetous tentacular segment. Ten pairs of elytra attached on elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, oval, and smooth, leaving middle third of body uncovered. Dorsal cirri on non-elytra bearing segments. Short ventral cirri. Enlarged ventral papillae on segments 11 and 12. Parapodia subbiramous. Notopodia small with few, short notosetae on segments with elytra. Two branches of branchiae above dorsal cirri. Bilobed prostomium with short median antenna, one pair of palps, and rounded anterior lobes, lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous and fused to the prostomium with two pairs of tentacular cirri. Branchiae are long and start on segment 2 with larger upper and smaller lower groups. Pygidium with pair of anal cirri.

Measurements- The holotype is 50 mm long, 20 mm wide including parapodia, with 21 segments.

Description- (Figures 14 & 15) Body short and wide, tapered anteriorly and posteriorly. There are 21 segments, including first achaetous tentacular segment.

Ten pairs of elytra attached anteriorly to elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra oval, smooth, very small, only covering a third to half of parapodia. Non-elytra bearing segments have cylindrical cirrophores and short tapered dorsal cirri not visible dorsally.

Branchiae aborescent with long filaments, attached in two groups, one larger dorsal group and one smaller ventral group, beginning on segment 2.

The parapodia subbiramous with extremely reduced notopodia bearing short, tapered notosetae on segments with elytra. Neuropodia larger with very short, slender, and hooked neurosetae. No clear separation or difference in shape between dorsal and ventral neurosetae.

Prostomium bilobed with rounded anterior lobes, without frontal filaments, lateral antennae, or eyes. Prostomium has a very short conical median antenna and pair of tapered palps. First tentacular segment achaetous, fused to prostomium with two pairs of short tentacular cirri. Buccal cirri similar in size to tentacular cirri. Ventral cirri much smaller, attached to middle of neuropodia. Enlarged ventral papillae on segments 11 and 12 project posteriorly. Pygidium is small with pair of long anal cirri.

Remarks- *Branchipolynoe meridae* has small elytra and long filamentous branchiae, while *B. seepensis*, *B. longqiensis*, *B. halliseyae*, and *B. kajsae* have large elytra and short, dense branchiae. *Branchipolynoe meridae* has branchiae starting on segment 2, while *B. seepensis*, *B. pettiboneae,* and *B. longqiensis* have branchiae starting on segment 3. *Branchipolynoe symmytilida*, *B. seepensis*, *B. pettiboneae*, and *B. longqiensis* have short neurosetae with stout dorsal neurosetae and hooked ventral neurosetae, while *B.*

meridae has no difference between dorsal and ventral neurosetae, hooked neurosetae, and notosetae only on segments with elytra and *B. eliseae* has hooked ventral neurosetae and straight dorsal neurosetae.

***Branchipolynoe eliseae*, new species**

(Figures 16 & 17)

Material- Costa Rica, Eastern Pacific, from dives of *Alvin* in 2010 and 2017, associated with mussels near hypersaline seeps. Dive 4922, 5 June 2017, 8.92955° N, 84.30780° W, 1009 m, collectors J. Le & C. Roman, holotype (A6660). Dive 4591, 12 Jan 2010, 9.11821667° N, 84.839116° W, 1802 m, collector G. Rouse, 1 paratype. Dive 4590, 11 Jan 2010, 9.1175667° N, 84.839534° W, 1800 m, collector G. Rouse, 5 juveniles. (See table 1 for catalog numbers).

Etymology- Named in honor of Elise Lindgren, sister of the first author.

Host- *Bathymodiolus billschneideri* and *Bathymodiolus nancyschneiderae*

Diagnosis- Body long with 21 segments, including first achaetous tentacular segment. Ten pairs of elytra attached on elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra small, oval, and smooth, leaving middle third of body uncovered. Dorsal cirri on non-elytra bearing segments. Short ventral cirri. Enlarged ventral papillae on segments 11 and 12. Parapodia subbiramous. Notopodia small with few, short notosetae. Two branches of branchiae above dorsal cirri. Bilobed prostomium with short median antenna,

one pair of palps, and rounded anterior lobes, lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous and fused to the prostomium with two pairs of tentacular cirri. Branchiae are long and start on segment 2 with larger upper and smaller lower groups. Pygidium with pair of anal cirri.

Measurements- The holotype is 45 mm long, 6 mm wide, including parapodia. It has 21 segments.

Description- (Figures 16 & 17) Body long, tapered anteriorly and posteriorly. Adults have 21 segments, including first achaetous tentacular segment.

Ten pairs of elytra attached to elytrophores on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19. Elytra oval and very reduced, only covering one fifth of parapodia. Non-elytra bearing segments have short, stout dorsal cirri on cylindrical cirrophores.

Branchiae aborescent with long filaments, beginning on segment 2, and emerging in one large dorsal group and one smaller ventral group.

Parapodia subbiramous with extremely reduced notopodia with short, tapered notosetae. Neuropodia short with slender, tapered neurosetae. Ventral neurosetae slightly hooked and dorsal neurosetae straight. No clear separation between dorsal and ventral neurosetae. Notosetae thicker, smoother, and shorter than neurosetae.

Prostomium bilobed with rounded anterior lobes, very short conical median antenna, and pair of very short conical palps. Prostomium lacking frontal filaments, lateral antennae, and eyes. First tentacular segment achaetous, fused to prostomium with two pairs of short tentacular cirri. Buccal cirri similar in size to tentacular cirri. Ventral

cirri much smaller, attached to middle of neuropodia. Enlarged ventral papillae on segments 11 and 12 project posteriorly. Pygidium round with pair of short, conical anal cirri.

Remarks- *Branchipolynoe eliseae* has small elytra and long filamentous branchiae, while *B. seepensis*, *B. longqiensis*, *B. halliseyae*, and *B. kajsae* have large elytra and short, dense branchiae. *Branchipolynoe eliseae* has branchiae starting on segment 2, while *B. seepensis*, *B. pettiboneae,* and *B. longqiensis* have branchiae starting on segment 3. *Branchipolynoe symmytilida*, *B. seepensis*, *B. pettiboneae*, and *B. longqiensis* have short neurosetae with stout dorsal neurosetae and hooked ventral neurosetae, while *B. meridae* has no difference between dorsal and ventral neurosetae, hooked neurosetae, and notosetae only on segments with elytra and *B. eliseae* has hooked ventral neurosetae and straight dorsal neurosetae.

The Thesis is currently being prepared for submission for publication. Lindgren, Johanna; Hourdez, Stéphane; Rouse, Greg. The thesis author was the primary investigator and author of this material.

Table 1. Collection data and hosts of *Branchipolynoe* samples used in this study for DNA sequencing. E.P.R.= East Pacific Rise.

Species	Voucher SIO-BIC	Location	Site	Latitude; Longitude	Depth (m)	Date	Host *Bathymodiolus* Species
Branchipolynoe symmytilida (Pettibone 1984)	A6554	E.P.R.	38°S German Flats	37.67252° S; 110.87695° W	2236	3/25/05	*B. antarcticus*
	A6555	E.P.R.	38°S German Flats	37.67252° S; 110.87695° W	2236	3/25/05	*B. antarcticus*
	A6556	E.P.R.	9°N Choo Choo	9.82367° N; 104.29550° W	2518	4/14/00	*B. thermophilus*
Branchipolynoe seepensis (Pettibone 1986)	A6553	Gulf of Mexico	Florida Escarpment	26.03083° N; 84.91817° W	3296	10/11/03	-
Branchipolynoe c.f. *pettiboneae* (Miura et al. 1991)	A5460	E.P.R.	Lau Basin	22.0° S; 176.0° W	1991	5/27/05	-
Branchipolynoe kajsae sp. nov.	A2159	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A2160	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A2161	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A2162	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A6516	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A6517	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A6518	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. earlougheri*
	A6519	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A6520	Costa Rica	Jaco Scarp	9.11757° N; 84.83953° W	1800	1/11/10	*B. billschneideri*
	A6574	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
	A6597	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. nancyschneiderae*
	A6611	Costa Rica	Mound 12	8.93048° N; 84.31250° W	1000	5/24/17	-
	A6626	Costa Rica	Mound 12	8.93042° N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6662	Costa Rica	Mound 12	8.92955° N; 84.30780° W	1009	6/5/17	*B. nancyschneiderae*
	A6668	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. billschneideri*
	A6669	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. billschneideri*
	A6703	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6713	Costa Rica	Quepos Seep	9.03048° N; 84.62020° W	1409	6/7/17	*B. billschneideri*
Branchipolynoe halliseyae sp. nov.	A1322	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
	A2127	Costa Rica	Yettisburgh	8.93078° N; 84.31253° W	997	1/9/10	-
	A2128	Costa Rica	Yettisburgh	8.93078° N; 84.31253° W	997	1/9/10	-

Table 1. continued

A2144	Costa Rica	Yettisburgh	8.93078° N; 84.31253° W	997	1/9/10	-
A6530	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6531	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6532	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6533	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6534	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6535	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6536	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6537	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6538	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6539	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
A6540	Costa Rica	Mound 12	8.9305° N; 84.3123° W	1001	3/5/09	-
A6541	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
A6542	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
A6543	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. nancyschneiderae*
A6544	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. nancyschneiderae*
A6545	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. nancyschneiderae*
A6546	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	-
A6547	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
A6565	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6568	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6570	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6575	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6578	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6582	Costa Rica	Mound 12	8.93048° N; 84.31250° W	1000	5/24/17	*B. nancyschneiderae*
A6589	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
A6592	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6593	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. earlougheri*
A6595	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. nancyschneiderae*
A6596	Costa Rica	Mound 12	8.93075° N; 84.31280° W	1001	5/21/17	*B. nancyschneiderae*
A6605	Costa Rica	Mound 12	8.93048° N; 84.31250° W	1000	5/24/17	*B. nancyschneiderae*
A6609	Costa Rica	Mound 12	8.93048° N; 84.31250° W	1000	5/24/17	*B. nancyschneiderae*
A6612	Costa Rica	Mound 12	8.93048° N; 84.31250° W	1000	5/24/17	-
A6613	Costa Rica	Jaco Scarp	9.11507° N; 84.84683° W	1891	5/26/17	-

Table 1. continued

	A6617	Costa Rica	Jaco Scarp	9.11507° N; 84.84683° W	1891	5/26/17	*B. billschneideri*
	A6619	Costa Rica	Jaco Scarp	9.11507° N; 84.84683° W	1891	5/26/17	*B. billschneideri*
	A6623	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6625	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6628	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6629	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6633	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6637	Costa Rica	Mound 12	8.93042°N; 84.31263° W	1004	5/25/17	*B. nancyschneiderae*
	A6650	Costa Rica	Jaco Scarp	9.11538° N; 84.83618° W	1859	5/27/17	*B. billschneideri*
	A6651	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. earlougheri*
	A6653	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. billschneideri*
	A6654	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. billschneideri*
	A6656	Costa Rica	Mound 12	8.92955° N; 84.30780° W	1009	6/5/17	*B. nancyschneiderae*
	A6657	Costa Rica	Mound 12	8.92955° N; 84.30780° W	1009	6/5/17	*B. nancyschneiderae*
	A6667	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. earlougheri*
	A6670	Costa Rica	Jaco Scarp	9.11753° N; 84.83953° W	1886	5/29/17	*B. billschneideri*
	A6672	Costa Rica	Jaco Scarp	9.11800° N; 84.84043° W	1885	5/30/17	*B. earlougheri*
	A6675	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6676	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6677	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6685	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6690	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6696	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6699	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6701	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6704	Costa Rica	Mound 12	8.92930° N; 84.31500° W	1000	6/1/17	*B. nancyschneiderae*
	A6706	Costa Rica	Quepos Seep	9.03048° N; 84.62020° W	1409	6/7/17	*B. billschneideri*
	A6708	Costa Rica	Quepos Seep	9.03048° N; 84.62020° W	1409	6/7/17	*B. billschneideri*
	A6709	Costa Rica	Quepos Seep	9.03048° N; 84.62020° W	1409	6/7/17	*B. billschneideri*
	A6711	Costa Rica	Quepos Seep	9.03048° N; 84.62020° W	1409	6/7/17	*B. billschneideri*
Branchipolynoe meridae sp. nov.	A2131	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/12/10	-
	A6521	Costa Rica	Mound 12	8.93° N; 84.3133° W	1008	2/22/09	-
	A6522	Costa Rica	Mound 12	8.9305° N; 84.3123° W	1001	3/5/09	-
	A6523	Costa Rica	Mound 12	8.9305° N; 84.3123° W	1001	3/5/09	-

Table 1. continued

	A6524	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
	A6525	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
	A6526	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	-
	A6527	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
	A6528	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
	A6529	Costa Rica	Yettisburgh	8.9308° N; 84.313° W	1000	1/7/10	*B. earlougheri*
	A6616	Costa Rica	Jaco Scarp	9.11507° N; 84.84683° W	1891	5/26/17	*B. billschneideri*
Branchipolynoe eliseae sp. nov.	A2132	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/12/10	*B. billschneideri*
	A6548	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/11/10	-
	A6549	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/11/10	-
	A6550	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/11/10	-
	A6551	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/11/10	-
	A6552	Costa Rica	Jaco Scarp	9.11822° N; 84.83912° W	1800	1/11/10	-
	A6660	Costa Rica	Mound 12	8.92955° N; 84.30780° W	1009	6/5/17	*B. nancyschneiderae*

Table 2. Minimum uncorrected (lower diagonal), minimum TIM1+G corrected distances (upper diagonal, italics), and maximum TIM1+G corrected intraspecific distance (diagonal, bold) among species of *Branchipolynoe* for CO1 data.

	1	**2**	**3**	**4**	**5**	**6**
1. *B. symmytilida*	**0.007710**	*0.277006*	*0.273014*	*0.296518*	*0.109852*	*0.118956*
2. *B. seepensis*	0.135785	**0.009308**	*0.066497*	*0.071138*	*0.270865*	*0.255810*
3. *B. kajsae* sp. n.	0.132480	0.054015	**0.033998**	*0.047535*	*0.254550*	*0.283976*
4. *B. halliseyae* sp. n.	0.138829	0.056976	0.040519	**0.020805**	*0.293910*	*0.276707*
5. *B. meridae* sp. n.	0.076227	0.137210	0.128725	0.142778	**0.024247**	*0.083718*
6. *B. eliseae* sp. n.	0.082087	0.130831	0.139059	0.137608	0.062228	**0.007508**

Table 3. Minimum uncorrected (lower diagonal), minimum HKY+G corrected distances (upper diagonal, italics), and maximum HKY+G corrected intraspecific distance (diagonal, bold) among species of *Branchipolynoe* for ITS data.

	1	2	3	4	5
B. symmytilida	**0.001788**	*0.073863*	*0.074597*	*0.012994*	*0.010825*
B. kajsae sp. nov.	0.104569	**0.017893**	*0.019142*	*0.139787*	*0.117557*
B. halliseyae sp. nov.	0.107143	0.021366	**0.032901**	*0.181512*	*0.172290*
B. meridae sp. nov.	0.013646	0.223873	0.341990	**0.033737**	*0.035136*
B. eliseae sp. nov.	0.011952	0.182995	0.330000	0.043843	**0.001788**

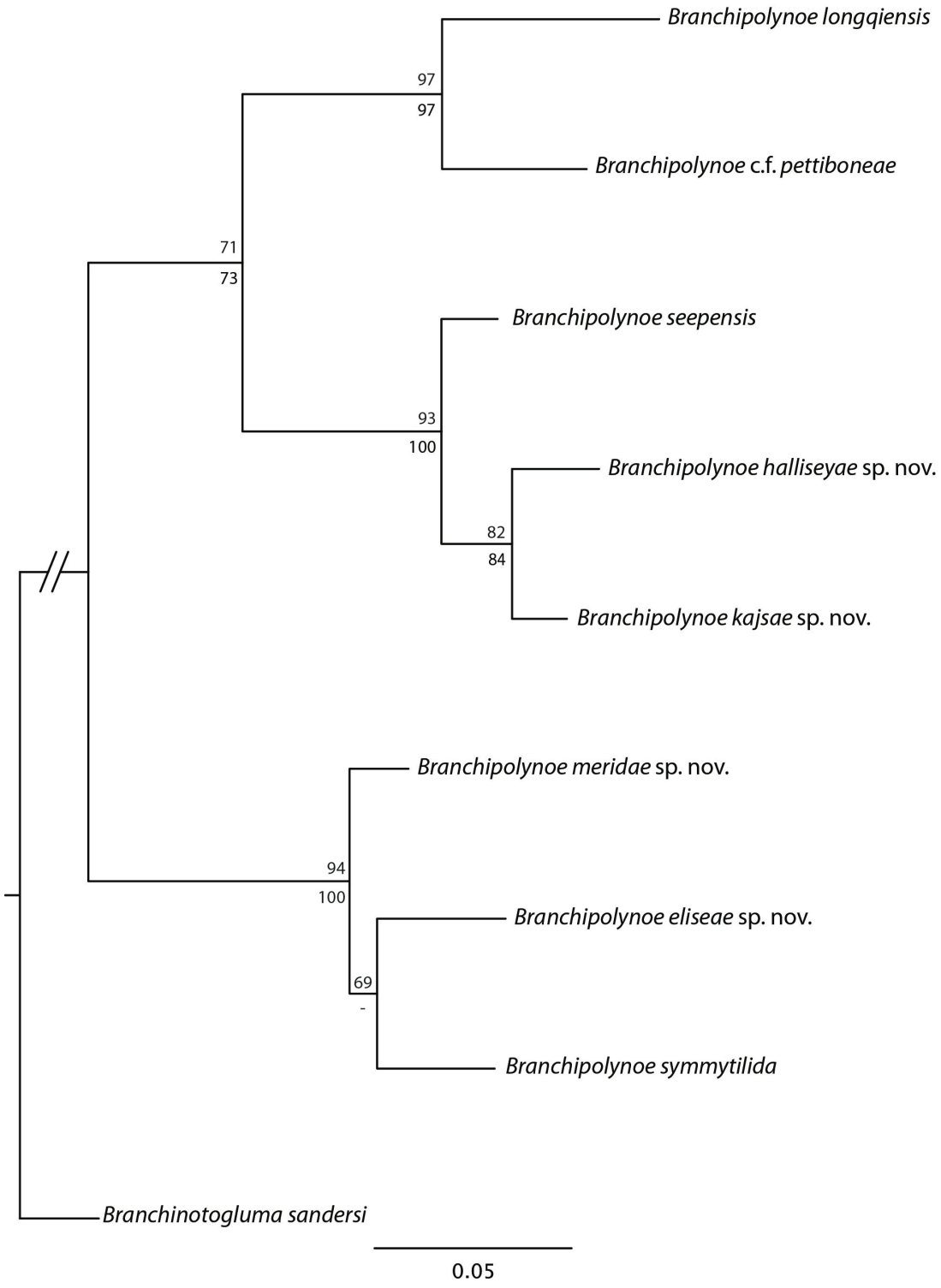

Figure 1. Maximum likelihood tree from the analysis of the combined sequences from CO1, 16S, and ITS. This is also the topology for one the MP trees. Numbers of support on relevant nodes are ML bootstrap followed by maximum parsimony jackknife. Tree not shown to scale. Dash indicates no jackknife support value.

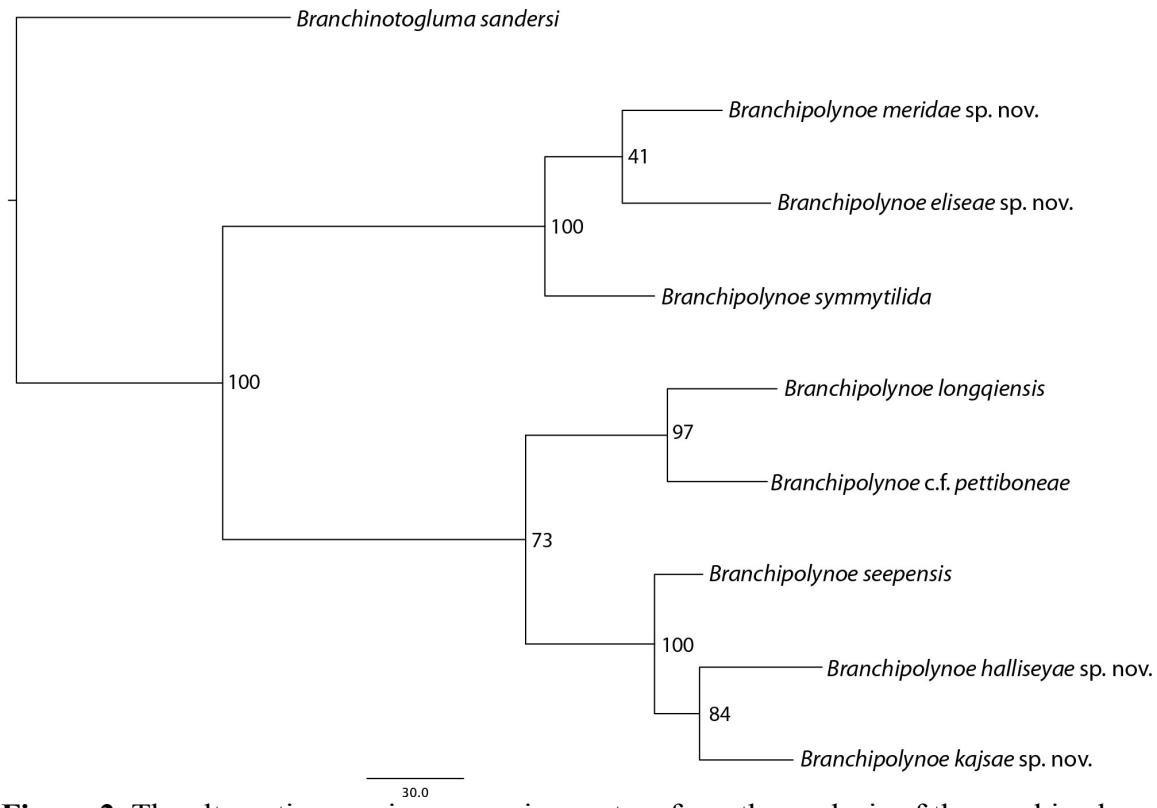

Figure 2. The alternative maximum parsimony tree from the analysis of the combined sequences from CO1, 16S, and ITS. Numbers of support on nodes are MP jackknife.

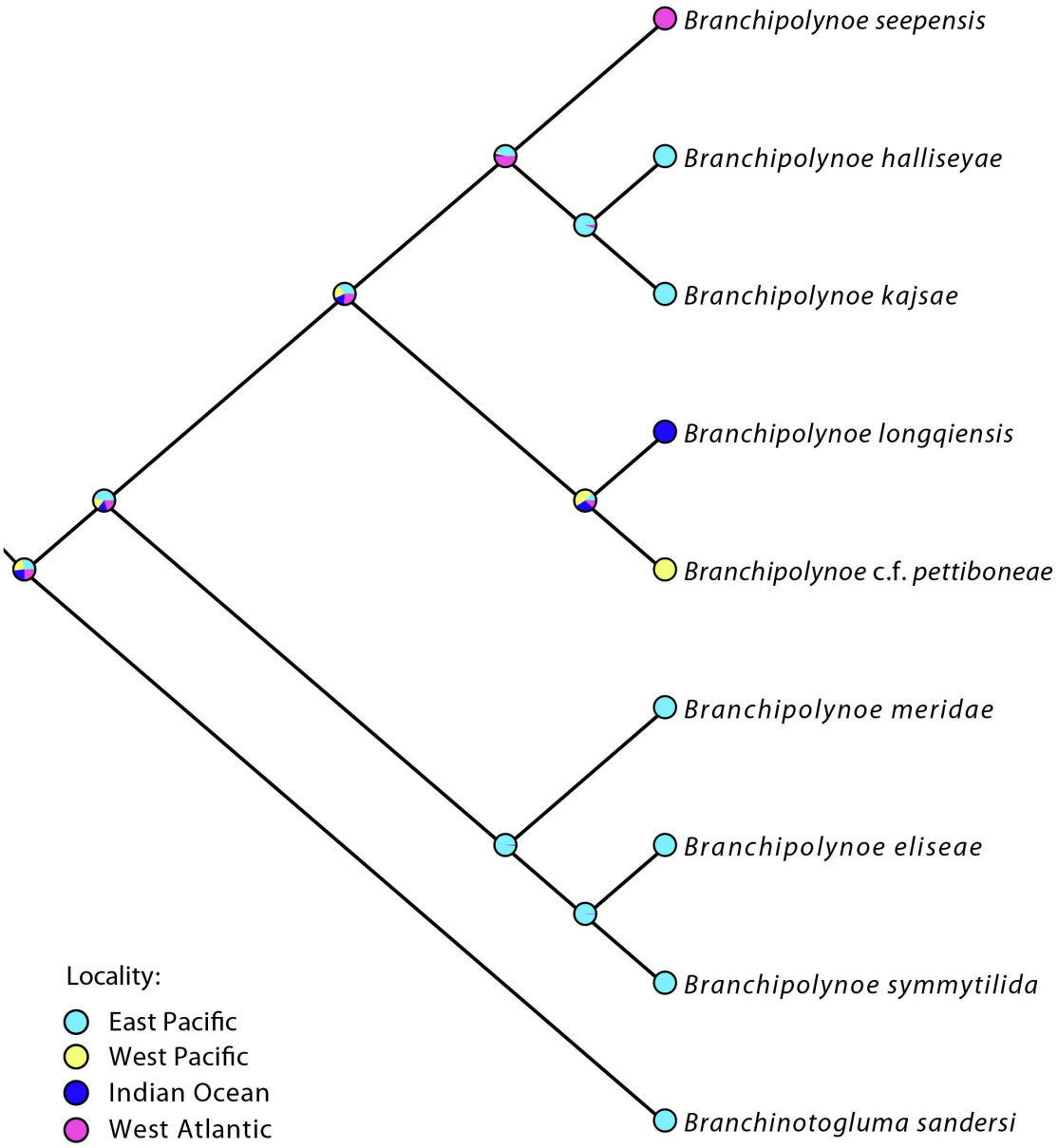

Figure 3. Maximum likelihood tree topology (and that of one of the most parsimonious trees) from the analysis of the combined sequences from CO1, 16S, and ITS, with the transformation for locality under the MK1 model. The 'pie charts' at the nodes represent the probabilities for the relevant states.

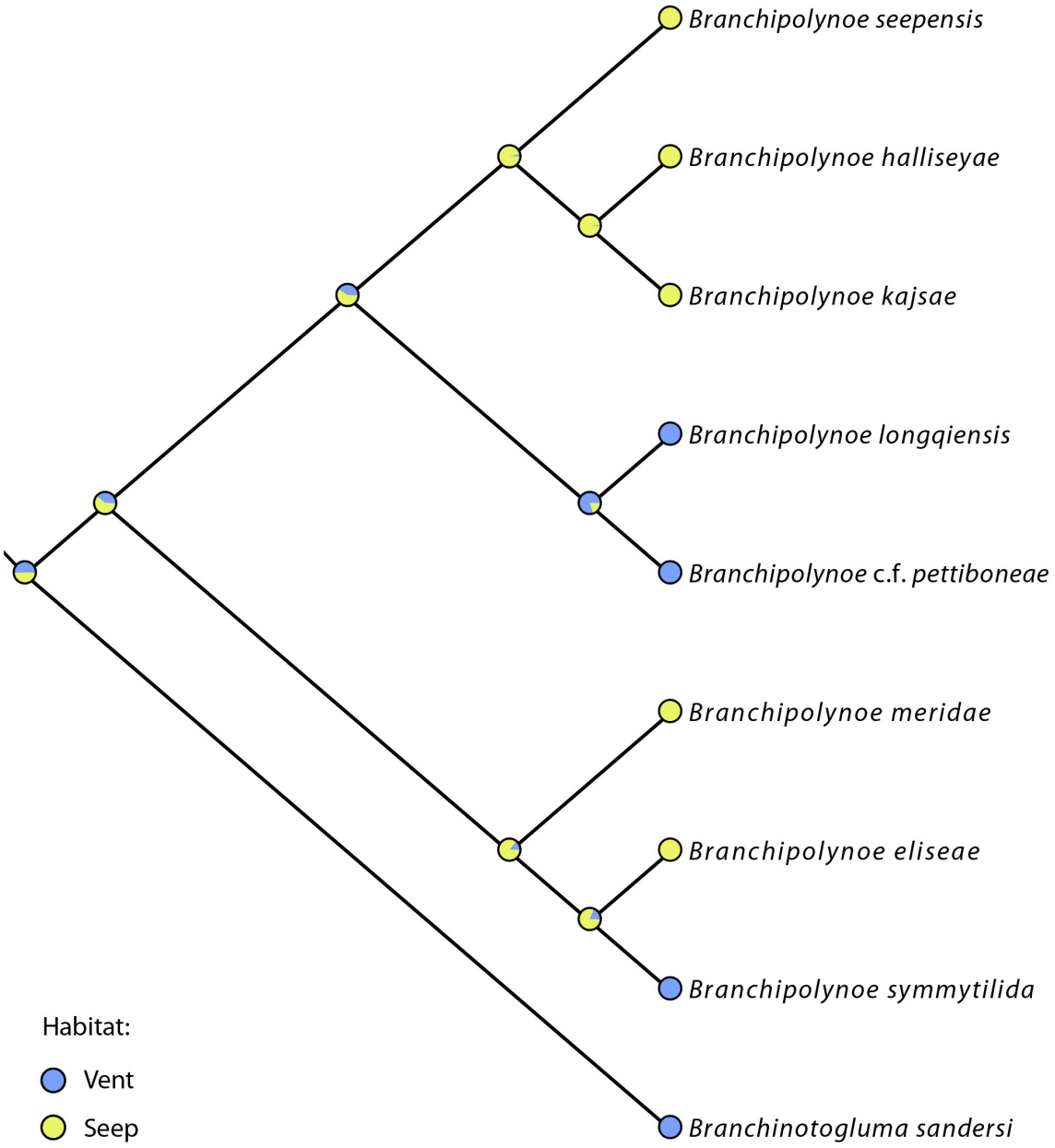

Figure 4. Maximum likelihood tree topology (and that of one of the most parsimonious trees) from the analysis of the combined sequences from CO1, 16S, and ITS, with transformation for habitat. The 'pie charts' at the nodes represent the probabilities for the relevant states.

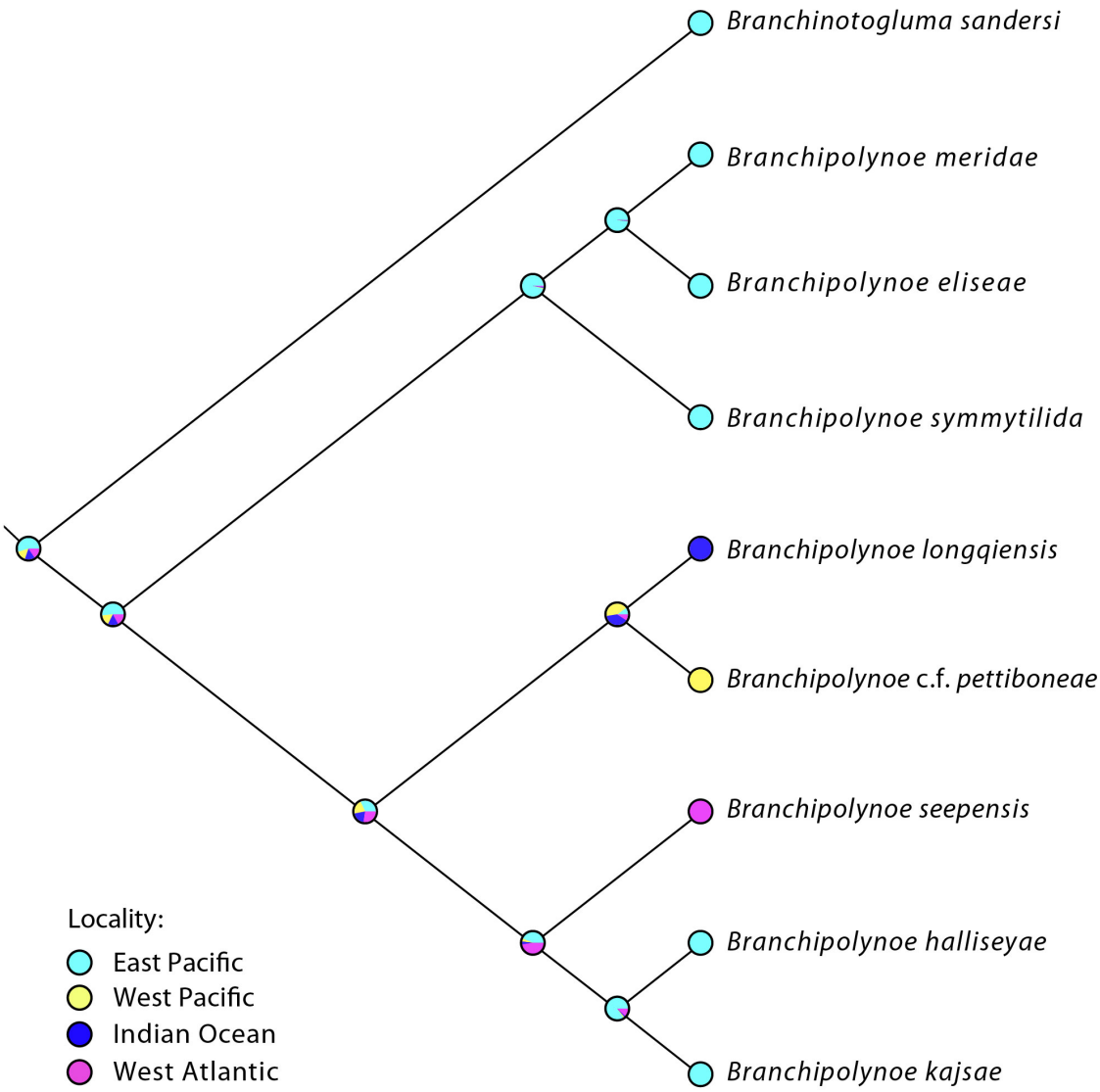

Figure 5. The 2nd maximum parsimony tree from the analysis of the combined sequences from CO1, 16S, and ITS, with transformation for locality. The 'pie charts' at the nodes represent the probabilities for the relevant states.

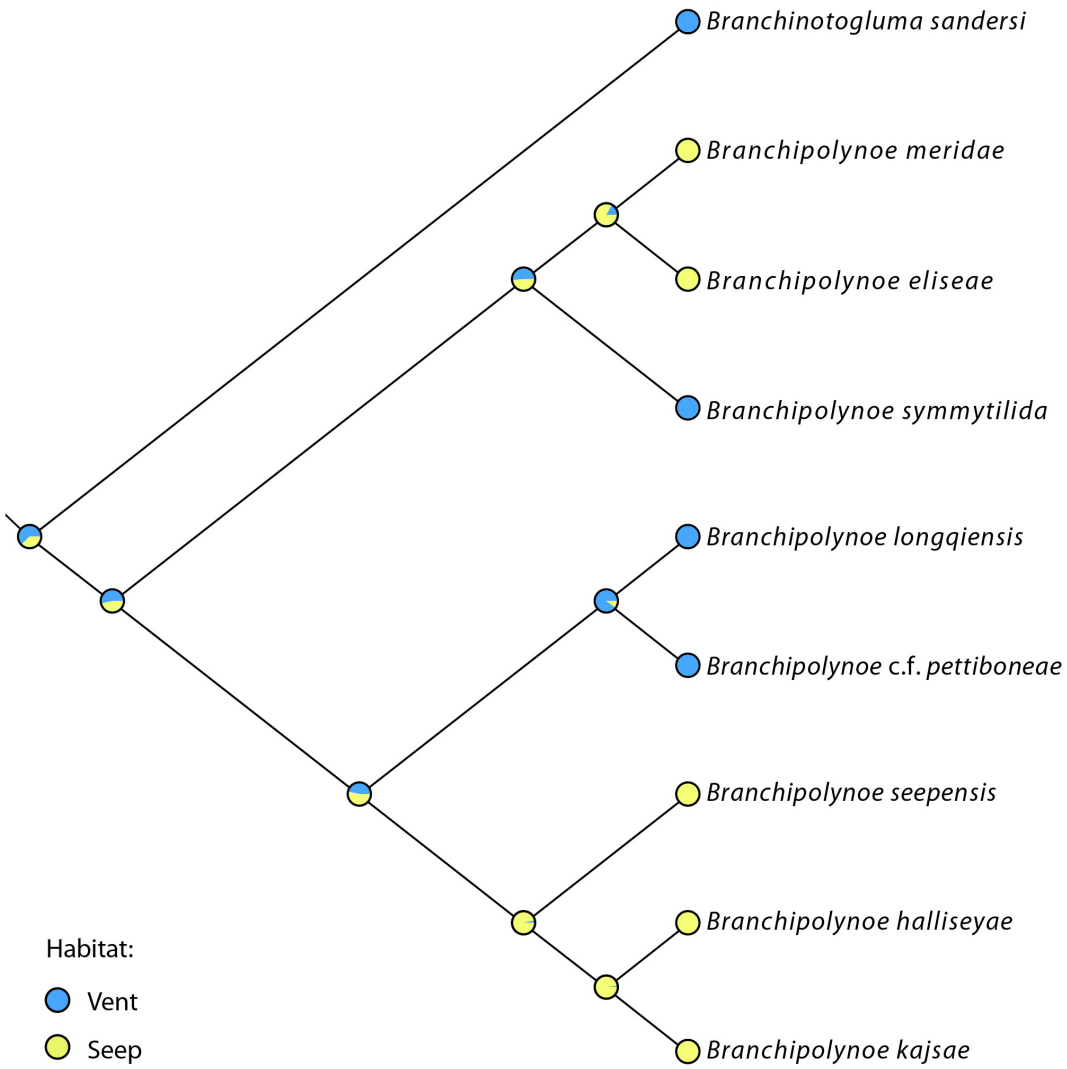

Figure 6. The 2nd maximum parsimony tree from the analysis of the combined sequences from CO1, 16S, and ITS, with transformation for habitat. The 'pie charts' at the nodes represent the probabilities for the relevant states.

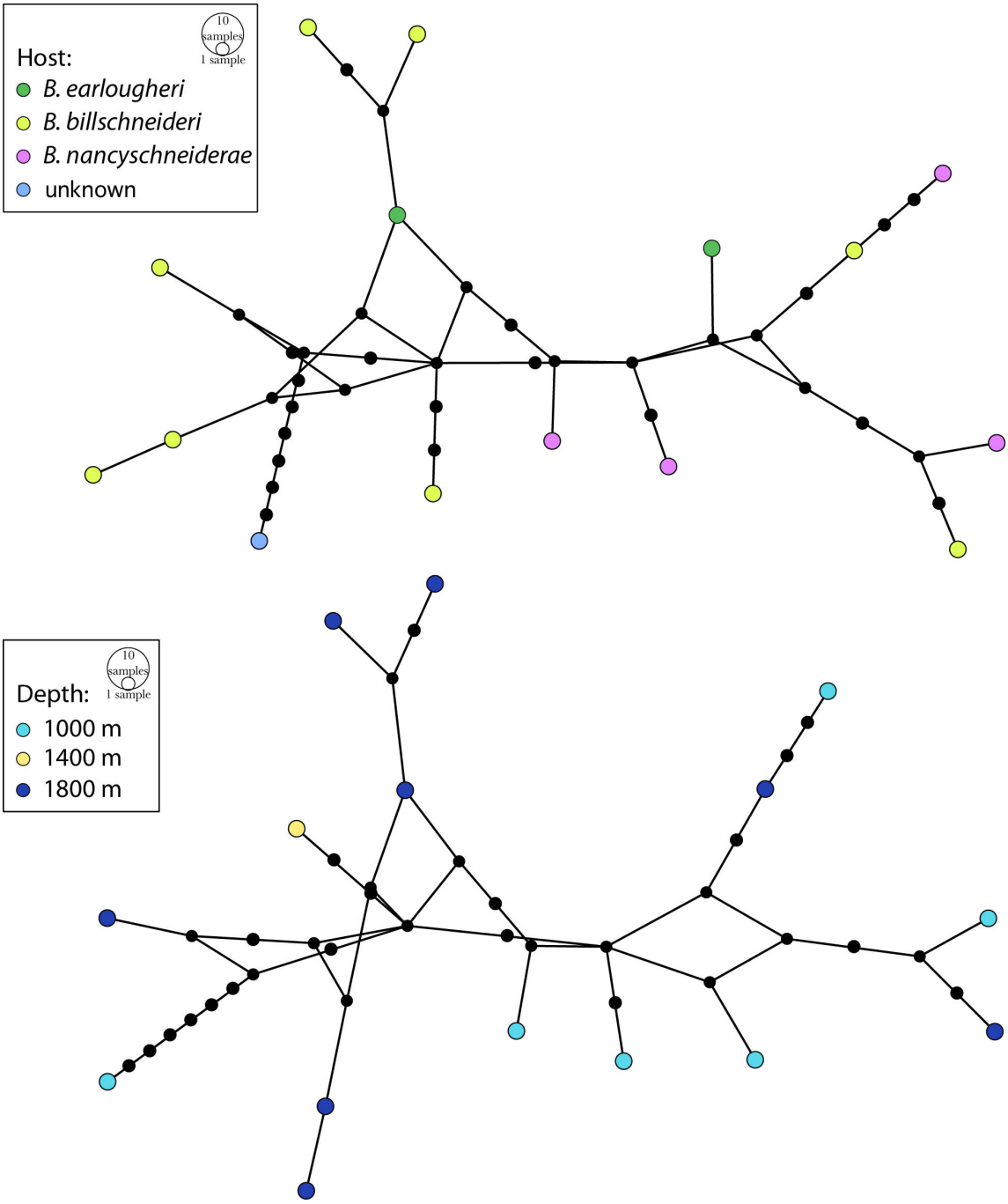

Figure 7. Haplotype networks for *B. kajsae* with colors indicating host mussel (top) and depth (bottom).

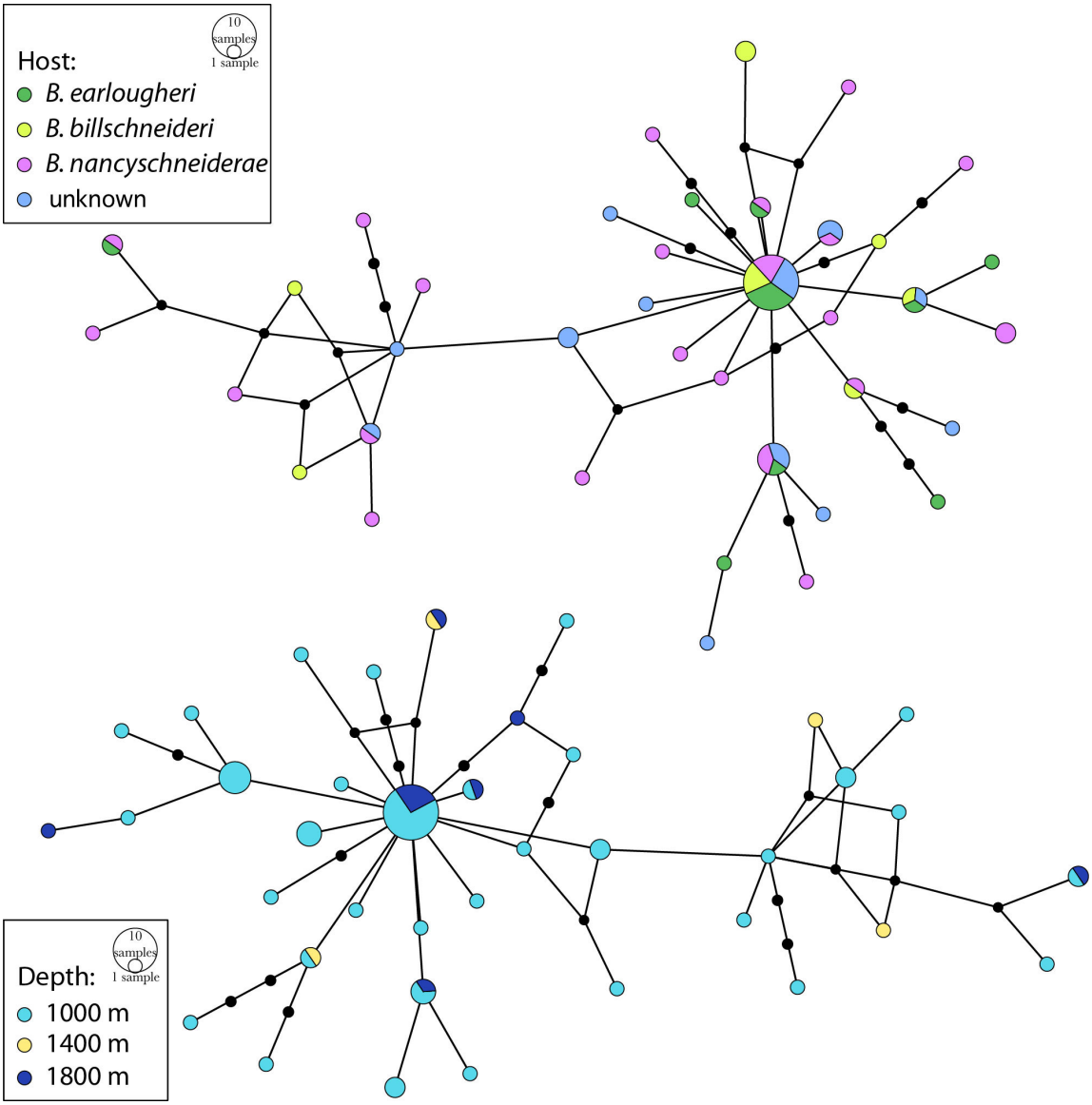

Figure 8. Haplotype networks for *B. halliseyae* with colors indicating host mussel (top) and depth (bottom).

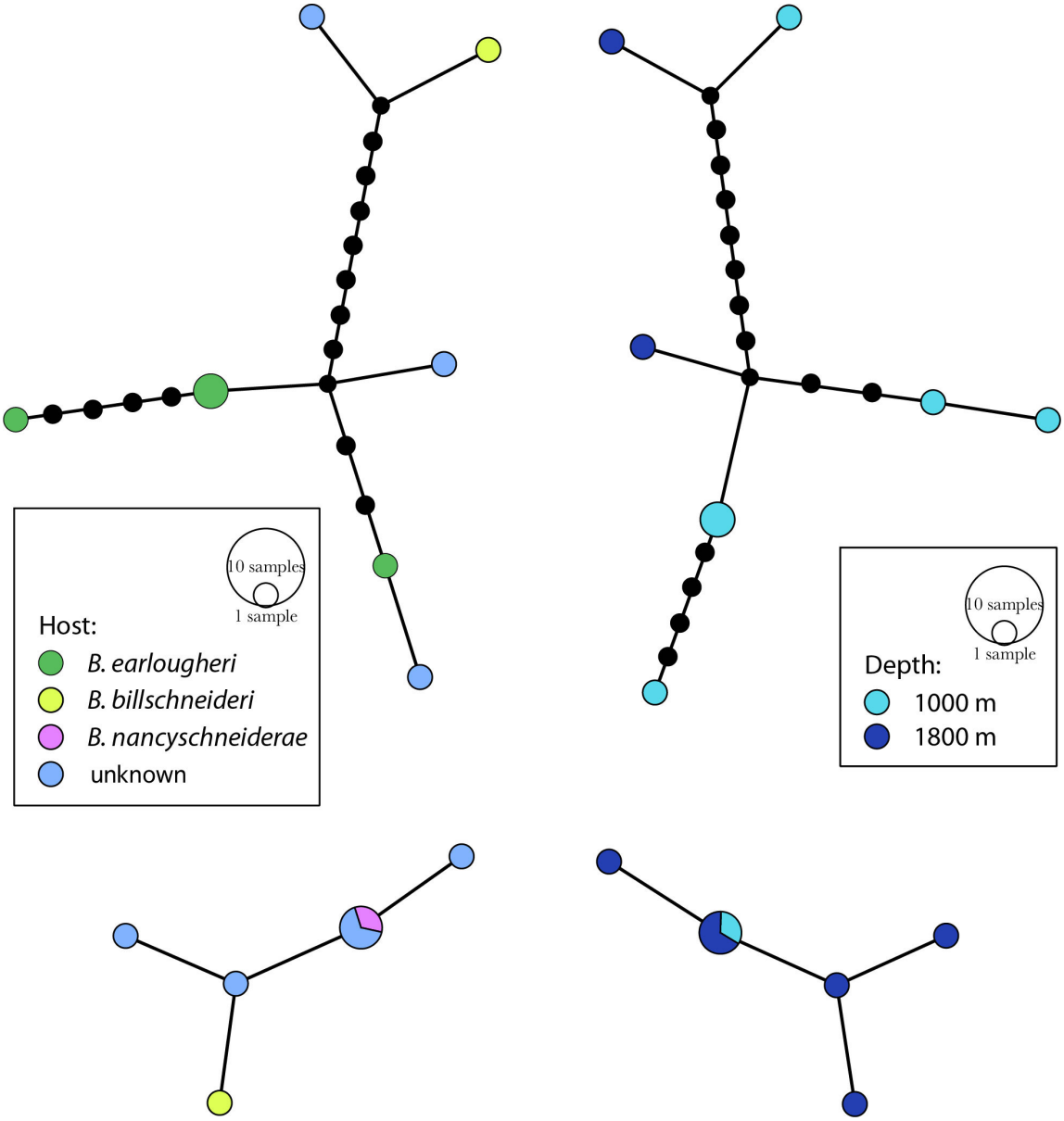

Figure 9. Haplotype networks for *B. meridae* (top) and *B. eliseae* (bottom) with colors indicating host mussel (left) and depth (right).

Figure 10. *B. kajsae* dorsal (left) and ventral (right) live images. Scale bars are 4 mm.

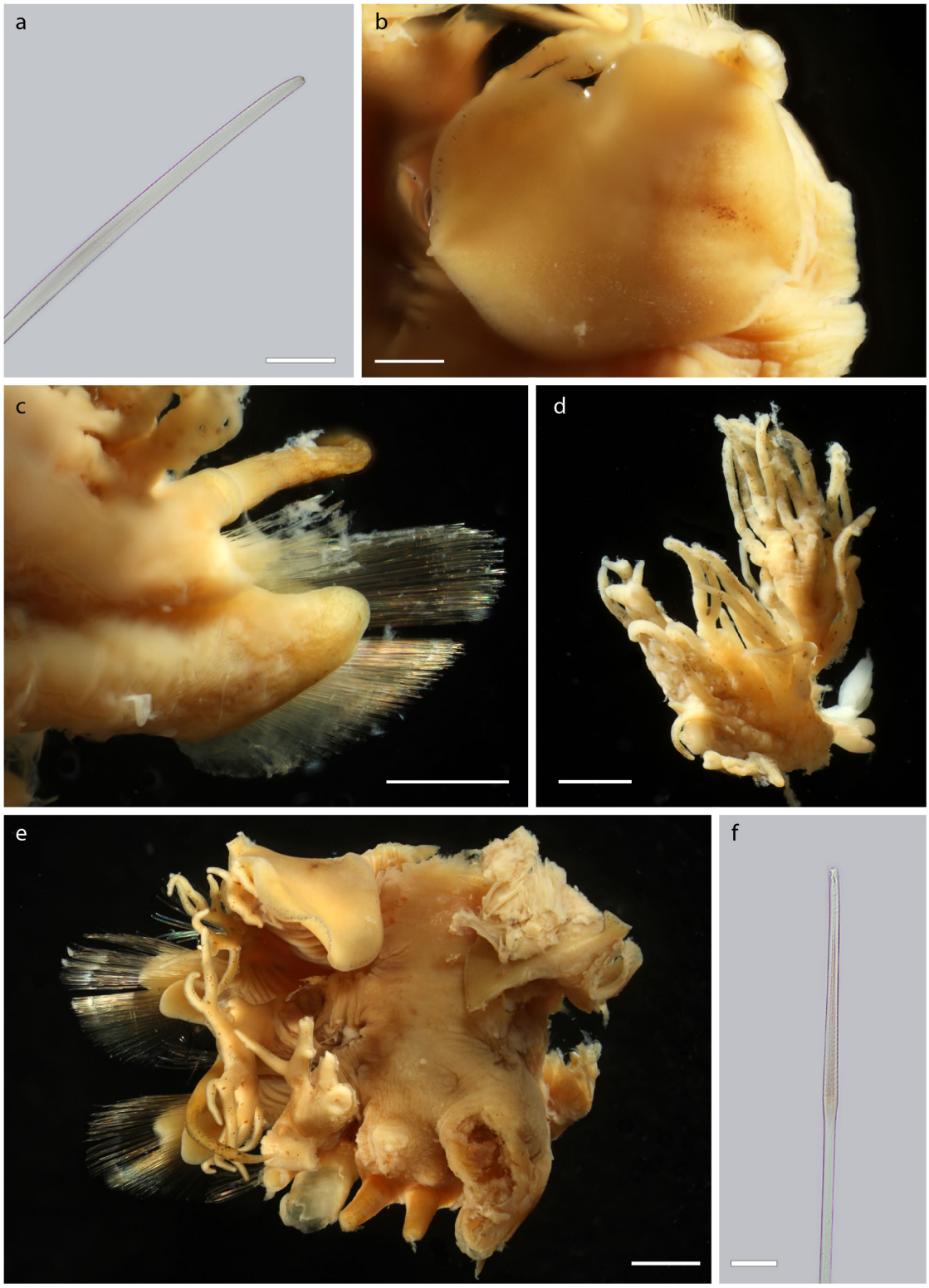

Figure 11. Images of *B. kajsae*: a. notosetae. b. elytra. c. parapodia with dorsal cirri. d. branchiae. e. 20th and 21st segments and pygidium with anal cirri. f. dorsal neurosetae. Scale bar sizes are: a. 10 µm. b. 0.5 mm. c. 1 mm. d. 1 mm. e. 1 mm. f. 5 µm.

Figure 12. *B. halliseyae* dorsal (left) and ventral (right) live images. Scale bars are 5 mm.

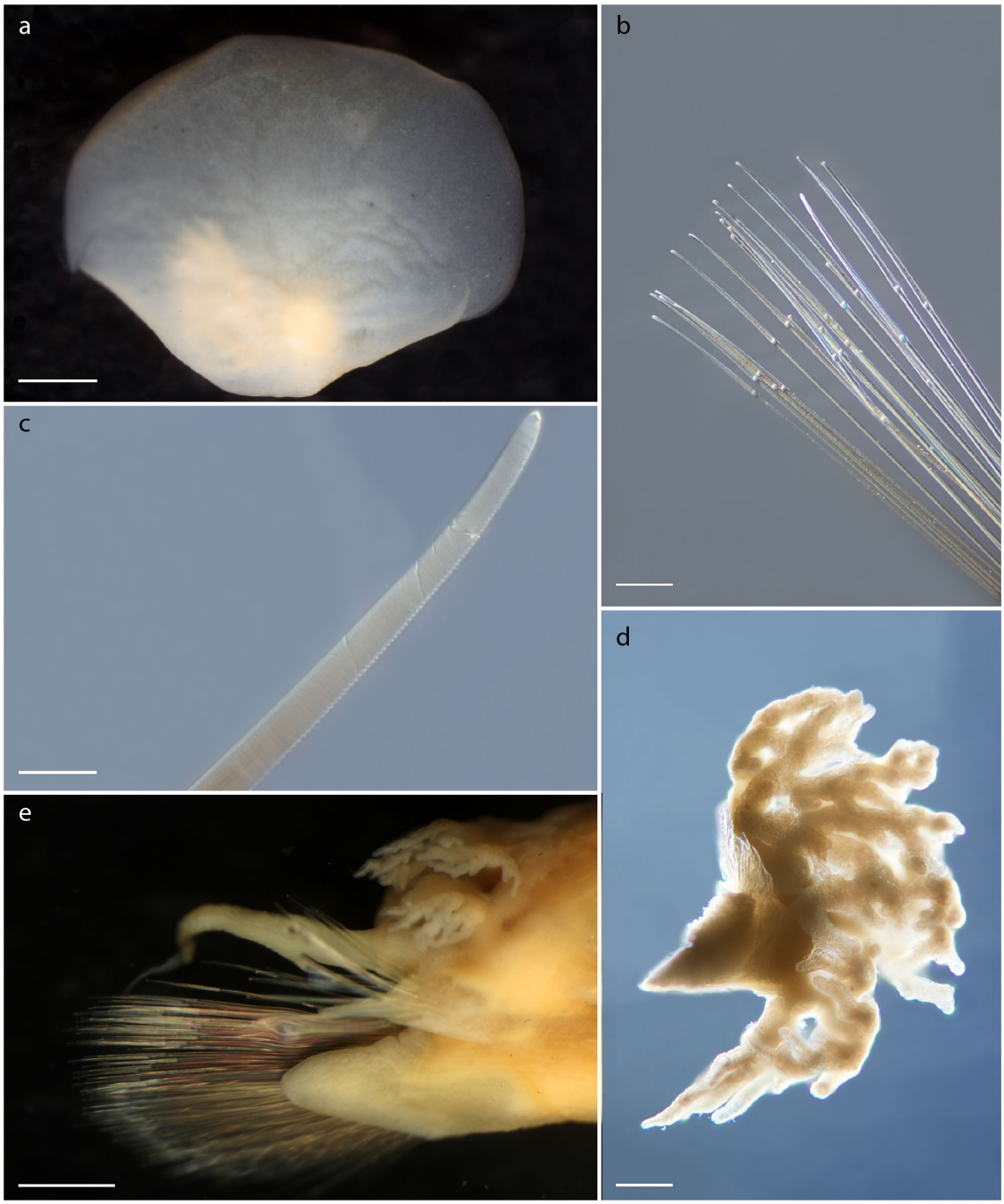

Figure 13. Images of *B. halliseyae*: a. elytra. b. neurosetae. c. notosetae. d. branchiae. e. parapodia with dorsal cirri. Scale bar sizes are: a. 0.5 mm. b. 15 µm. c. 5 µm. d. 15 µm. e. 0.5 mm.

Figure 14. *B. meridae* dorsal (top) and ventral (bottom) live images. Scale bars are 6 mm.

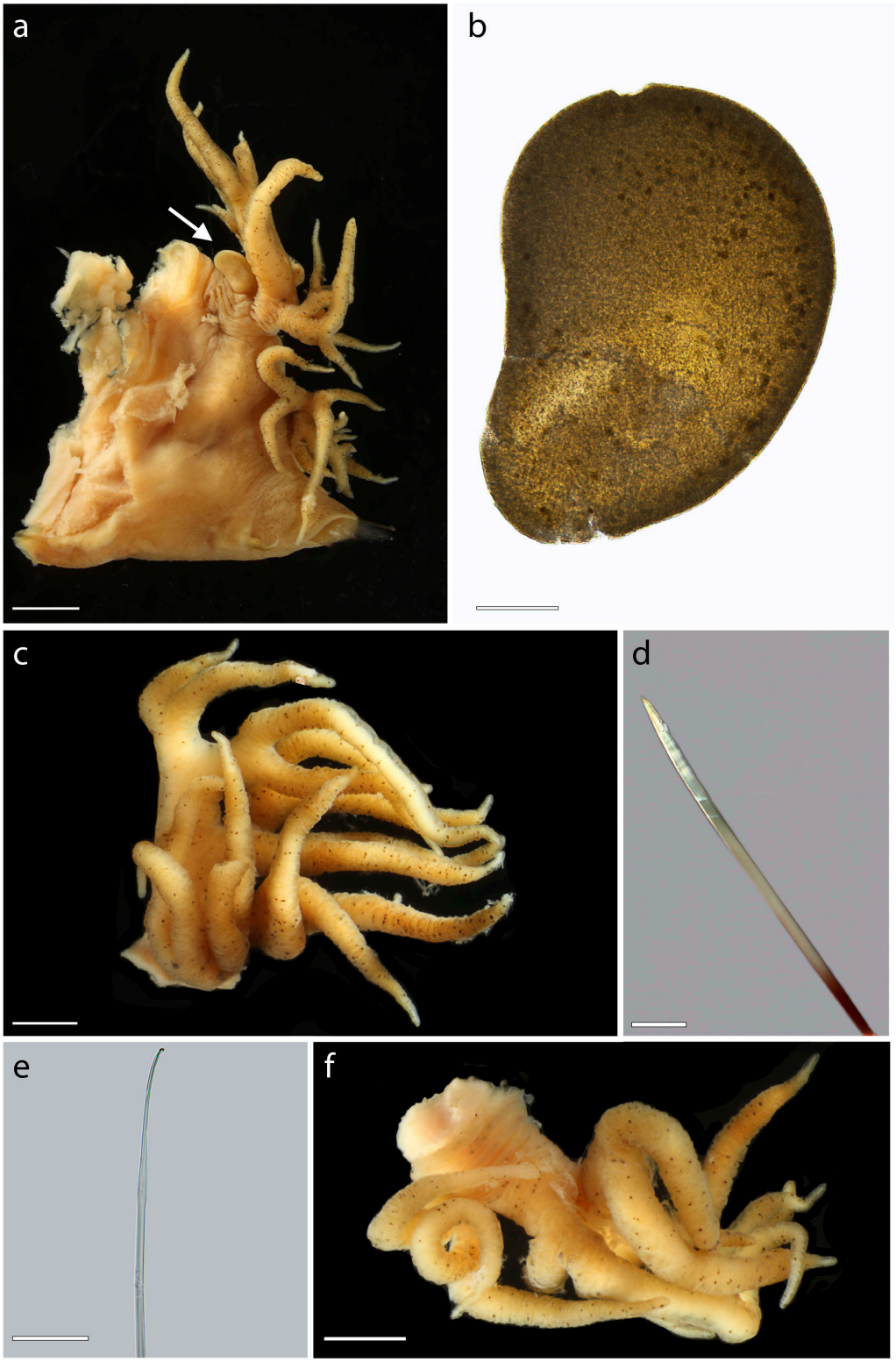

Figure 15. Images of *B. meridae*: a. parapodia with elytra (arrow). b. elytra. c. dorsal branchiae. d. notosetae. e. ventral neurosetae. f. ventral branchiae. Scale bar sizes are: a. 1 mm. b. 20 µm. c. 0.5 mm. d. 0.25 mm. e. 10 µm. f. 0.5 mm.

Figure 16. *B. eliseae* dorsal (left) and ventral (right) images. Scale bars are 5 mm.

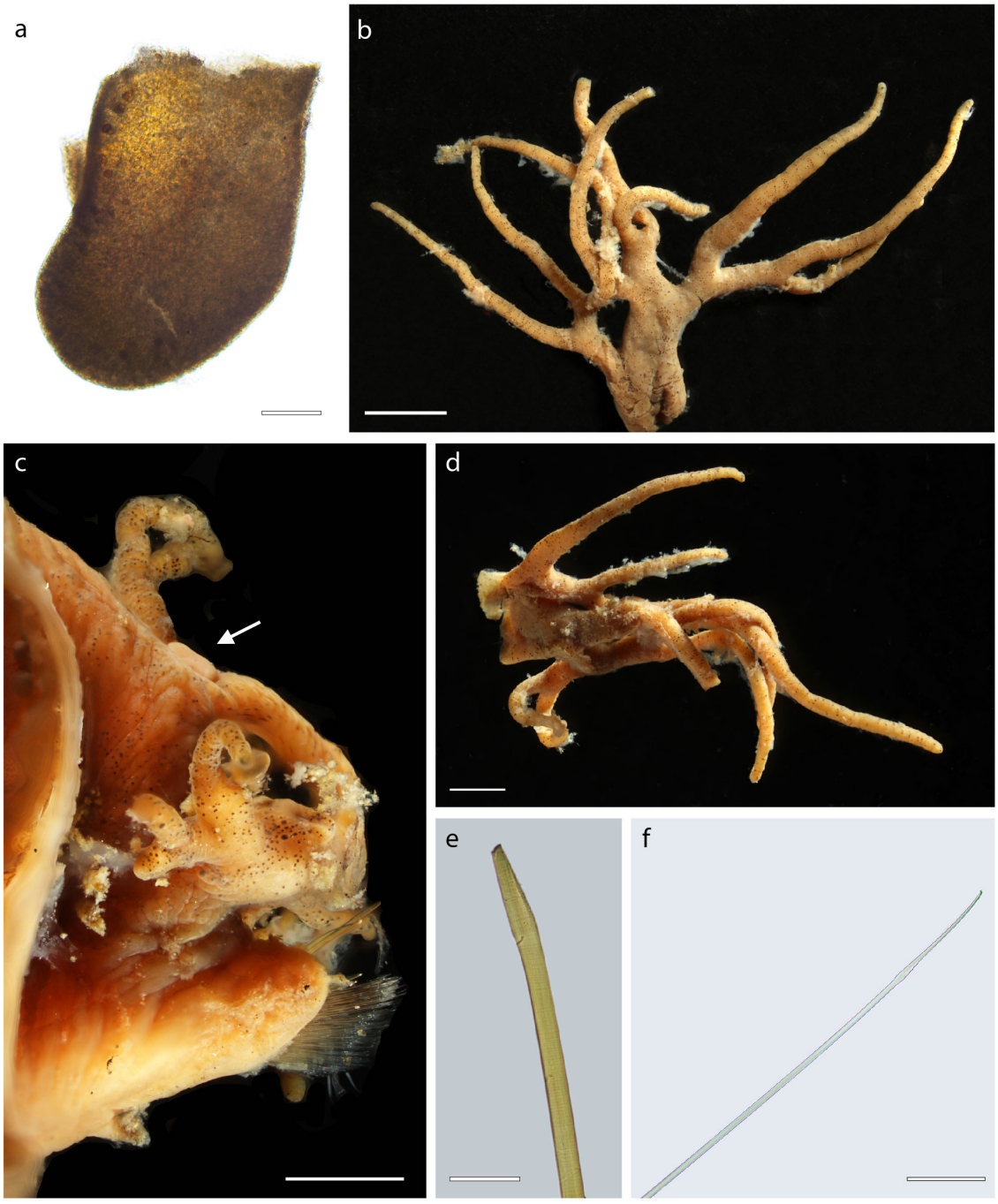

Figure 17. Images of *B. eliseae*: a. elytra. b. dorsal branchiae. c. parapodia with elytra (arrow). d. ventral branchiae. e. notosetae. f. ventral neurosetae. Scale bar sizes are: a. 15 µm. b. 2 mm. c. 1 mm. d. 1 mm. e. 20 µm. f. 10 µm.

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