# Phylogeny of Cladobranchia (Gastropoda: Nudibranchia): a total evidence analysis using DNA sequence data from public databases 

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#### Abstract

Cladobranchia is a clade of charismatic and exclusively marine slugs (Gastropoda: Nudibranchia). Though Cladobranchia and its sister taxon, Anthobranchia, have been supported by molecular data, little resolution among the higher-level groups within these two clades has emerged from previous analyses. Cladobranchia is traditionally divided into three taxa (Dendronotida, Euarminida, and Aeolidida), none of which have been supported by molecular phylogenetic studies. Reconstructions of the evolutionary relationships within Cladobranchia have resulted in poorly supported phylogenies, rife with polytomies and non-monophyletic groups contradicting previous taxonomic hypotheses. In this study, we present a working hypothesis for the evolutionary history of Cladobranchia, utilizing publicly available data that have been generated since the last attempt at a detailed phylogeny for this group (we include approximately 200 more taxa and a total of five genes). Our results resolve Cladobranchia as monophyletic and provide support for a small proportion of genera and families, but it is clear that the presently available data are insufficient to provide a robust and well-resolved phylogeny of these taxa as a whole.


Keywords: Cladobranchia, Nudibranchia, marine slugs, sea slugs, phylogenetics, maximum likelihood, concatenation, 16S, 18S, 28S, COI, H3, Mollusca

## Introduction

Cladobranchia is a diverse and charismatic clade of exclusively marine slugs. These organisms live in globally distributed habitats from the intertidal to the deep ocean, and are characterized by having branched digestive glands [1]. Though not as speciose as some other gastropod clades, cladobranchs have developed remarkable biological features that are rare among animals, many of which are related to defensive strategies. As this is a clade within Nudibranchia, which is characterized by the loss of the shell in adult animals [2], selection likely favored the evolution of defense mechanisms to compensate for the loss of a protective shell. The development of many different chemical and physical defense mechanisms has been hypothesized to have led to the large-scale diversification of Nudibranchia, and within it, Cladobranchia [3]. In order to understand this diversity, as well as the evolution of the ecological roles of taxa within Cladobranchia, an accurate phylogenetic framework is needed. However, given the depth of the evolutionary divergences and the diversity within this clade, reconstruction of the phylogenetic relationships among taxa in this group has proven difficult.

Both Cladobranchia ( $\sim 1000$ species) and its sister taxon, Anthobranchia ( $\sim 2000$ species) [4], have been supported as monophyletic by molecular data $[1,2,5]$, but thus far there has been little resolution among the higher-level groups within these two clades. Within Cladobranchia, there are three traditional taxa characterized on the basis of morphology: Dendronotida, Euarminida and Aeolidida [5]. Though a number of studies on the evolutionary history of Cladobranchia have been undertaken, the majority have been limited to specific clades, often at the family or genus level (e.g., Scyllaeidae [6], Aeolidiidae [7],

Tritoniidae [8], and Babakina [9]). Due to this focus on more recent divergences within Cladobranchia, there is little data that either support or reject the traditional classification of the three major taxa, making it difficult to understand the deeper evolutionary history within these groups.

To date, there has been only one large-scale phylogeny attempted for Cladobranchia [1], which was based on the three most commonly used genes in nudibranch systematics: mitochondrial 16S rRNA and Cytochrome Oxidase I, and nuclear Histone 3. In this phylogeny, the majority of relationships between higher-level taxa remained unresolved, both between and within the three traditional taxonomic divisions of Cladobranchia. Consequently, the evolution of traits within Cladobranchia remains poorly understood.

A robust phylogeny of Cladobranchia is necessary to provide a framework for our understanding of adaptations within this clade. Here we present the "current state of knowledge": a phylogeny for Cladobranchia as inferred from all publicly available DNA sequence data.

## Materials and Methods

## Taxon and data selection

The Cladobranchia sequence data used in our analyses (Dendronotina [=Dendronotida], Arminina [=Euarminida], and Aeolidina [=Aeolidida]; taxa in brackets reflect equivalent taxonomic designations in the literature) were downloaded from GenBank [10] in February 2014. These data comprise 297 species and five genes, including the mitochondrial genes coding for cytochrome oxidase I (COI) and 16S rRNA, and nuclear genes coding for Histone 3 (H3), 18S rRNA and 28S rRNA (Appendix A). The two outgroups for this analysis, Discodoris atromaculata and Cadlina laevis, were selected to maximize the number of genes for each outgroup as well as provide some taxonomic breadth from within Anthobranchia, the sister taxon to Cladobranchia. D. atromaculata was the only species in GenBank from Anthobranchia for which sequences were available for all five genes, and C. laevis was the only remaining species for which four of the genes were available.

## Multiple sequence alignment and data matrix construction

Alignments were generated for each gene using the auto function in MAFFT 7.130 [11]. In each gene alignment, multiple sequences from the same taxon (identified by GenBank taxon ID) were reduced to a single consensus sequence, using nucleotide ambiguity codes [12] as necessary. The GenBank taxon ID number is the most accurate identifier of species in GenBank because it reflects taxonomic rearrangements (e.g., a genus change), and as such was used to identify taxa. Consensus sequences were generated by providing the nucleotide coding sequence alignment as input to the consensus_iupac BioPerl subroutine [13].

There are a few principal motivations for using consensus sequences. The first is a desire to incorporate all information about the variability of specific nucleotide states for positions in each gene, both within species and within individuals. A second motivation is to mitigate the effects of mistaken taxon identification within GenBank and prevent errors resulting from the incorrect choice of a single representative sequence. A major challenge of working with previously published sequences is the lack of access to morphology and other means of confirming the identification of samples; the use of consensus sequences can mitigate the effects of possible taxonomic misidentification. Finally, by utilizing more available sequence data, the consensus procedure yields somewhat longer final sequences for each taxon.

The individual gene alignments were concatenated into a single matrix, and sites containing data for fewer than four taxa were removed. This matrix (ALL_TAXA) contained 297 species. Three additional data matrices were generated using subsets of this data: one that contained only taxa for which two or more genes were present (MIN_TWO_GENES; 271 species), a second that contained only taxa for which three or more genes were present (MIN_THREE_GENES; 196 species), and a third that includes all species for which either COI, H3 or 16S rRNA genes are present, thereby eliminating taxa for which only 18 S or 28 S were present (THREE_GENES; 290 species). An additional matrix was generated (MIN_149_TAXA; 297 species) to minimize missing data. For this matrix, the five genes were
concatenated and sites containing data for fewer than 149 taxa ( $\sim 50 \%$ ) were removed. All five alignments, plus each separate gene consensus alignment (for a total of ten) are available as supplementary files.

## Phylogenetic analyses

To complete the phylogenetic analyses we used GARLI 2.0 (Genetic Algorithm for Rapid Likelihood Inference; [14]) through the GARLI web service hosted at molecularevolution.org [15]. We used a general time reversible nucleotide model [16] with a proportion of invariant sites and an among site rate heterogeneity model with a discrete gamma distribution (GTR $+\mathrm{I}+\mathrm{G}$ ) together with GARLI default settings, including stepwise-addition starting trees. Three analyses were run for all matrices except MIN_149_TAXA: one without data partitioning; another with data partitioned into four possible subsets by type of gene: 1) COI mitochondrial, 2) H3 nuclear, 3) 16 S mitochondrial rRNA, and 4) 18 S and 28 S nuclear rRNA, for a total of at most three partitions; and a third, unpartitioned, with all sequences from the genus Melibe removed (due to an extremely long Melibe branch in our analyses). For MIN_149_TAXA, only a full, unpartitioned analysis was run. Two analyses were also run for each gene, one including and one excluding Melibe. For all analyses, non-parametric bootstrap values were determined using 2000 bootstrap replicates with five search replicates per bootstrap replicate. Postprocessing of the phylogenetic inference results was done by the GARLI web service at molecularevolution.org using DendroPy [17] and the R system for statistical computing [18], which includes the construction of a bootstrap consensus tree for each analysis. The estimation of the number of replicates required to recover the "best" topology follows Regier et al. [19].

## Results

## Data matrix properties

The matrix of five genes containing 297 species (ALL_TAXA) contained 6,475 nucleotide positions and was $26.9 \%$ complete, while the MIN_TWO_GENES (271 taxa) and MIN_THREE_GENES (196 taxa) data matrices each contained 6,484 nucleotide positions and were $28.0 \%$ and $29.7 \%$ complete, respectively. The THREE_GENES data matrix (290 taxa) contained 2,920 sites and was $41.0 \%$ complete. Finally, the MIN_149_TAXA data matrix ( 297 taxa) contained 1,419 sites and was $78.0 \%$ complete (Table 1). The full data matrix represented at least 65 genera ( $62.5 \%$ ) and 20 families ( $66.7 \%$ ) of all known families and genera within Cladobranchia.

Table 1. Size and completeness of aligned data matrices from GenBank sequences.

|  | Five Genes |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Matrix name | ALL_TAXA | MIN_2_GENES | MIN_3_GENES | MIN_149_TAXA | THREE_GENES |
| Number of taxa | 297 | 271 | 196 | 297 | 290 |
| Number of <br> nucleotide <br> positions | 6,475 | 6,484 | 6,484 | 1419 | 2,920 |
| Number of <br> nucleotides <br> (non-gap <br> characters) in <br> alignment | $1,923,075$ | $1,757,164$ | $1,270,864$ | 328,771 | 846,800 |
| Matrix <br> completeness <br> (number nt $\div$ <br> number possible <br> nt | $26.9 \%$ | $28.0 \%$ | $29.7 \%$ | $78 \%$ |  |
| Percentage of <br> ambiguous <br> nucleotides (non- | $0.10 \%$ | $0.11 \%$ | $0.13 \%$ | $0.51 \%$ | $0.21 \%$ |


| gap, nonA/C/G/T chars) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Percentage of all possible internal nodes with bootstrap $\geq 80$ (non-partitioned) | 36.4\% | 40.4\% | 41.3\% | 32.4\% | 38.4\% |
| Percentage of all possible internal nodes with bootstrap $\geq 80$ (partitioned) | 36.4\% | 37.4\% | 39.8\% | Not applicable | 23.9\% |

## Phylogenetic analyses

We performed two phylogenetic analyses for four of our five data matrices (ALL_TAXA, MIN_TWO_GENES, MIN_THREE_GENES, THREE_GENES). For the MIN_149_TAXA matrix, only an unpartitioned analysis was run (Appendix Figure B20). The MIN_TWO_GENES tree represented the best combination of comprehensive taxon sampling and proportion of well-supported nodes (those with a bootstrap value $\geq 80$ ), in which Cladobranchia had high bootstrap support. The ALL_TAXA tree contained a smaller percentage of resolved nodes, and the MIN_THREE_GENES tree contained 75 fewer taxa and only a slightly higher percentage of resolved internal nodes ( $+0.9 \%$ ) (Table 1). Therefore, we consider the bootstrap consensus tree from the MIN_TWO_GENES analysis to be the most reliable current inference of relationships within Cladobranchia based on molecular data, and present it in Figure 1. Phylogenetic trees based on the other data sets are presented in Appendix B, and all trees showed a lack of resolution among most branches. The genes that seem to have contributed the most information are 16 S and H 3 , due to a larger amount of resolution in the topology of these gene trees, followed by 18 S and 28 S , which had low taxon representation but a considerable amount of resolution, and COI, in which the topology was simply poorly resolved.

The tree generated using the MIN_TWO_GENES matrix (Figure 1) supported the monophyly of Cladobranchia (bootstrap value $=97$ ), including $\bar{M}$ elibe (Tethyidae). Relationships within Cladobranchia, however, were still largely unresolved; our tree included a massive polytomy at the base of Cladobranchia consisting of 41 small clades and 40 individual taxa. Many of these taxa and groups form non-monophyletic assemblages of species at all levels, including genus, family, superfamily and infraorder according to current taxonomic divisions. Five families were well supported (bootstrap value $\geq$ 80) as monophyletic: Bornellidae (bootstrap value $=100$ ), Hancockiidae (bootstrap value $=96$ ), Tergipedidae (bootstrap value $=93$ ), Dotidae (bootstrap value $=93$ ) and Dendronotidae (bootstrap value $=93$ ). One family had relatively low support ( $80>$ bootstrap value $\geq 70$ ) : Lomanotidae (bootstrap value $=$ 76). Two families in the analysis were represented by only one taxon (Dironidae and Charcotiidae), and these were well supported as sister taxa in our analysis (bootstrap value $=97$ ). All other taxa included in this analysis were from families that were not supported as monophyletic, with as yet unresolved evolutionary histories and taxonomic disarray.


Figure 1. The $70 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the MIN_TWO_GENES data matrix. Bootstrap values are provided above each branch.

## Discussion

In this section, we review our findings on the bootstrap support for both shallow and deep divergences in Cladobranchia. We then discuss the importance of our results and how they affect the current understanding of the group relationships themselves.

## Support levels

In our trees, the bootstrap support values are highly varied. A major point of interest is the high support for the monophyly of Cladobranchia itself, including Melibe, a genus excluded from Cladobranchia in Pola \& Gosliner [1]. However, the backbone of the tree within the group is rife with polytomies and low bootstrap values. Some genera and a few families are well supported in our analysis, but the majority of support and resolution comes at a very shallow phylogenetic level. Additionally, specific placement of roughly $15 \%$ of the taxa had had bootstrap values of less than $50 \%$, thus forming a comb along the backbone of our majority-rule consensus tree.

There are several explanations for this lack of resolution and low support in our analyses. One possibility is that our relatively small amount of data and sparse data matrices (with at most $78.0 \%$ completeness) may have prevented our likelihood analyses from performing well [20]. The problems may concern the specific number or type of genes that were sampled for each taxon and included in the analyses. For example, although species of the same genus were included in the analysis, there may be only one gene for one or more of those taxa. If these genes are different, there can be no comparison of similar characters to place them together on the phylogeny. There are a number of cases in our analyses where this could potentially be an issue, including multiple species of Eubranchus (E. exiguous, 76182; Eubranchus sp., 252571; E. rustyus, 763125; and E. sanjuanensis, 763126) and one species of Protaeolidiella (P. atra, 1154746).

Prior research suggests that this missing data may not be as much of a problem as previously suspected. In Cho et al. [21], a data matrix with $45 \%$ intentionally missing data yielded no signs of the contradictory groupings that missing data would supposedly produce. This result is consistent with those of three other studies from across a broad taxonomic range, including frogs [22], angiosperms [23], and an entire phylum of eukaryotes [24]. Other literature has also indicated that missing data is not always a substantial problem [25]. From Wiens and Morrill [25]: "Overall, our results confirm previous simulation and empirical studies showing that taxa with extensive missing data can be accurately placed in phylogenetic analyses and that adding characters with missing data can be beneficial (at least under some conditions)." In support of this, the tree obtained from the analysis of our MIN_149_TAXA matrix ( $78 \%$ complete) is actually less resolved than any of the other analyses (Table 1, Appendix Figure B20). This indicates that missing data are not the major issue, at least in this case, but rather that the available data are insufficient for the problem.

An alternative to the sparse data matrix hypothesis for the lack of resolution in our trees is possible contamination or specimen misidentification. Based on the location of certain taxa in the tree, either some identifications may be incorrect in GenBank, or these taxa may have been routinely placed in the wrong genus or family, including: Caloria indica (376200), Piseinotecus sp. (797203), Pinufius rebus (797256), Flabellina baetica (934968), Calma glaucoides (1154735), Flabellina cacaotica (1287503), Piseinotecus gabinierei (1287625), and Fiona pinnata (1287638). These taxa can be found in unexpected locations on both the ALL_TAXA and MIN_TWO_GENES phylogenies, often some distance from others within the same genus or family. The exact reasons for these instances of taxonomic discord are unknown, but may be due to misidentifications, contamination, or taxonomic misplacement. One point to note, however, is that not a single taxon on this list is associated with a higher proportion of ambiguous characters. As such, their placements are likely not artifacts of the consensus procedure.

A third possible explanation for a lack of resolution is that conflicting gene tree topologies may be confounding the true species tree in our analyses. This is a common problem in phylogenetic studies [26]. Our individual gene trees (COI, H3, 16S, 18S and 28S) all resolve different topologies (Figures S812), which would be consistent with this hypothesis. However, most of our trees are poorly resolved (both
those based on single gene and those based on multiple genes) and include a large number of polytomies. This, in turn, indicates not that the data are necessarily inconsistent, but that they are insufficient to resolve relationships within Cladobranchia.

Another plausible reason for the low bootstrap support and lack of resolution is that certain taxa are particularly troublesome and can negatively affect bootstrap support values [27]. The genus Melibe is a possible example, with long branches for COI and 16 S and absent sequences for 18 S and 28 S . Taxa with vastly elevated rates of evolution, such as Melibe, tend to move around, eroding support in bootstrap analyses. To address these concerns, analyses were run excluding Melibe from all data matrices where it was formerly included. These tended to have slightly higher support values for most nodes as compared to when Melibe was included. However, the exclusion of Melibe did not affect the overall resolution for each tree (Figures S13-19), and thus inclusion of this taxon is not likely a strong contributing factor to poor resolution.

Alternatively, gene sequences for a few taxa in our analyses contained slightly more ambiguous characters in their consensus sequence compared to other taxa, including those from Glaucus marginatus (1154738, 16S and COI), Doto coronata (154624, COI), Aeolidia papillosa (195873, 16S), Favorinus elenalexarium (797222, H3), Dondice banyulensis (869980, 16S), Spurilla neapolitana (929453, 16S and COI) and Phyllodesmium macphersonae (869973, 16S and COI). This could also have an effect on both the resolution and support for phylogenetic trees estimated by likelihood [28]. However, none of these taxa appear to be in an unexpected place on the MIN_TWO_GENES tree. This is most likely because the percentage of ambiguous characters in all of our matrices is extremely small: between $0.1 \%$ and $0.51 \%$ (Table 1). This indicates that for the majority of our taxa that have multiple sequences, there are few differences between those sequences. Indeed, one thing we note above is that the taxa identified as potential problems are not the same taxa with a larger percentage of ambiguity in their consensus sequence. On a final note, the taxon with the highest percentage of ambiguous characters in the full matrix, Spurilla neapolitana $(9.7 \%)$, was still appropriately placed within a clade with other members of the genus Spurilla in all analyses, affirming that our consensus procedure had little to no deleterious effects on our results.

As has been suggested in Regier et al. [19], a final possibility is that insufficient search effort on each bootstrap pseudo-replicate may have played a role in the low bootstrap values found in our tree. However, this is unlikely in our case, as our analysis included a total of five GARLI searches on each pseudo-replicate, in contrast to the single search replicate used in the analyses in Regier et al. [19]. As suggested in Debry \& Olmstead [29], this type of resampling results in more precise estimates of bootstrap values, likely because insufficient search effort during bootstrapping has been shown to artificially lower bootstrap values.

In summary, we conclude that the most likely reason for our lack of resolution is simply that the data do not have sufficient phylogenetic signal to successfully reconstruct deep phylogenetic relationships. These might be obtained, however, using high-throughput sequencing for greater genomic data sampling; i.e., "phylogenomics." Rather than yielding only a few genes, these sequencing assays often provide hundreds of genes and have been successfully used to resolve relationships within many groups [30-34]. Thus, a phylogenomics study may be more successful at mapping the evolutionary history of Cladobranchia.

## Current understanding of the phylogeny of Cladobranchia

Our results indicate that there is presently a severe lack of data useful for addressing deep evolutionary divergences within Cladobranchia. A result novel to this study, however, is the monophyly of Cladobranchia, which was recovered as paraphyletic (if the genus Melibe was included) in the only previous molecular phylogeny of this group [1]. Our analyses resolve Cladobranchia (including Melibe) as monophyletic, with high bootstrap support (bootstrap value $=97$, MIN_TWO_GENES). However, much like the study of Pola \& Gosliner [1], we find little resolution of relationships within Cladobranchia.

Six families within Cladobranchia were resolved as monophyletic, including Bornellidae, Hancockiidae, Tergipedidae, Dotidae, Dendronotidae and Lomanotidae. Three of these families
(Bornellidae, Hancockiidae and Dendronotidae) are consistent with the results from a previous study [1], while the monophyly of Tergipedidae, Dotidae and Lomanotidae are novel results. However, given the relatively low support of the monophyly of Lomanotidae, greater taxon and or gene sampling within this family is probably necessary to better establish its position and status. The lower bootstrap support for this clade could be the result of including only two species from Lomanotidae in the analysis. The remaining families within Cladobranchia that were included in the analysis appear as non-monophyletic species assemblages. The monophyly of some of these families, such as Aeolidiidae [7,35], was previously determined by morphological and molecular evidence, while the monophyly of other families, such as Arminidae [36] or Scyllaeidae [6], was determined using only morphological characters. Still other families in Cladobranchia have been weakly supported by morphological data, including Tritoniidae [8]. The lack of support for these clades in this analysis could be due to low taxon sampling in some cases. In other cases, the molecular data may have simply revealed paraphyly or polyphyly within groups previously well supported by morphology.

It is abundantly clear that the evolutionary history of Cladobranchia remains to be understood. Our results provided some additional for the relationships in this group, but the majority of the relationships in our trees remain unresolved (Table 1). In order to better understand evolution within this diverse group, as with any group of organisms, a well-resolved and well-supported phylogenetic tree is necessary. The recent advances in phylogenomic approaches may hold the key to our understanding of taxonomic relationships within Cladobranchia.

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## Appendix A.

Table of taxa, including GenBank taxon ID number, species name and GenBank accession number for each sequence used in the analyses.

Appendix A. Table of taxa, including GenBank taxon ID number, species name and GenBank accession number for each sequence used in the analyses.

| Taxon ID Taxon Name | COI GenBank IDs | H3 GenBank IDs | 16S GenBank IDs | 18S GenBank IDs | 28S GenBank IDs |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 195873 Aeolidia papillosa | JQ997042.1 | JQ996934.1 | JX087464.1 | GU227371.1 | JQ699293.1 |
|  | JQ997039.1 | JX087596.1 | JQ996836.1 |  |  |
|  | JX087536.1 | JQ996935.1 | JQ996834.1 |  |  |
|  | JQ699565.1 | JX087597.1 | JQ996835.1 |  |  |
|  | GQ292049.1 | JX087598.1 | JX087462.1 |  |  |
|  | JQ997038.1 | JQ996936.1 | JQ996833.1 |  |  |
|  | JQ997041.1 | JQ996937.1 | JQ699475.1 |  |  |
|  | JX087535.1 | JQ699385.1 | JX087463.1 |  |  |
|  | JX087534.1 |  |  |  |  |
|  | JQ997040.1 |  |  |  |  |
|  | AY345028.1 |  |  |  |  |
| 1290779 Aeolidia sp. A | JX087532.1 | JX087593.1 | JX087459.1 |  |  |
|  | JX087533.1 | JQ996933.1 | JQ996832.1 |  |  |
|  | JQ997037.1 | JX087594.1 | JX087460.1 |  |  |
|  | JX087531.1 | JX087595.1 | JX087461.1 |  |  |
| 1290780 Aeolidia sp. B | JQ997035.1 | JQ996931.1 | JQ996831.1 |  |  |
|  | JQ997036.1 | JQ996932.1 | JQ996830.1 |  |  |
| 1154711 Aeolidiella alba |  | JQ699386.1 |  |  | JQ699294.1 |
| 934974 Aeolidiella alderi | HQ616766.1 | HQ616794.1 | HQ616729.1 |  |  |
|  | HQ616765.1 | HQ616795.1 | JQ996811.1 |  |  |
|  |  | JQ996910.1 | HQ616728.1 |  |  |
| 1287571 Aeolidiella sanguinea | JX087538.1 | JX087599.1 | JX087465.1 |  |  |
|  | JX087537.1 | JX087600.1 | JX087466.1 |  |  |
| 1287507 Aeolidiella stephanieae | JQ997044.1 | JQ996940.1 | JQ996839.1 |  |  |
| 1288050 Aeolidiidae gen. sp. 'alba' | JQ997016.1 | JQ996909.1 | JQ996806.1 |  |  |
|  | JQ997017.1 | JQ996908.1 | JQ996805.1 |  |  |
|  | JQ997013.1 | JQ996907.1 | JQ996810.1 |  |  |
|  | JQ997015.1 | JQ996905.1 | JQ996808.1 |  |  |
|  | JQ997012.1 | JQ996906.1 | JQ996809.1 |  |  |
|  | JQ997014.1 | JQ996904.1 |  |  |  |
| 1288051 Aeolidiidae gen. sp. 'japonica' | JQ997033.1 | JQ996929.1 | JQ996828.1 |  |  |
| 1290799 Aeolidiidae gen. sp. A | JQ997011.1 | JQ996902.1 | JQ996803.1 |  |  |
|  |  | JQ996903.1 | JQ996804.1 |  |  |
| 1290800 Aeolidiidae gen. sp. B | JQ997022.1 | JQ996917.1 | JQ996818.1 |  |  |
|  | JQ997023.1 | JQ996916.1 | JQ996817.1 |  |  |


| 1394325 Aeolidiidae sp. | KC706903.1 |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 1287505 Aeolidiopsis ransoni | JQ997043.1 | JQ996938.1 | JQ996837.1 |  |
|  |  | JQ996939.1 | JQ996838.1 |  |
| 1287503 Anteaeolidiella cacaotica | JX087528.1 | JQ996926.1 | JX087455.1 |  |
|  | JQ997030.1 | JX087590.1 | JQ996825.1 |  |
|  |  |  | JX087457.1 |  |
| 1330516 Anteaeolidiella lurana | JQ997027.1 | JQ996922.1 | JQ996822.1 |  |
|  | JQ997031.1 | JQ996923.1 | JQ996821.1 |  |
|  |  | JQ996927.1 | JQ996826.1 |  |
| 1287504 Anteaeolidiella oliviae | JQ997034.1 | JQ996930.1 | JQ996829.1 |  |
| 1291184 Anteaeolidiella saldanhensis | JQ997032.1 | JQ996928.1 | JQ996827.1 |  |
| 1290782 Anteaeolidiella sp. A | JQ997029.1 | JQ996924.1 | JQ996823.1 |  |
|  | JQ997028.1 | JQ996925.1 | JQ996824.1 |  |
| 1290783 Anteaeolidiella sp. B | JQ997020.1 | JQ996914.1 | JQ996815.1 |  |
| 1330517 Anteaeolidiella takanosimensis | JX087529.1 | JX087592.1 | JX087458.1 |  |
|  | JX087530.1 | JX087591.1 | JX087456.1 |  |
| 763115 Armina californica | GQ292055.1 |  |  | GQ326884.1 |
| 71480 Armina lovenii | AF249781.1 |  | AF249243.1 | AF249196.1 |
| 1400840 Armina maculata | KF369111.1 |  |  |  |
| 431601 Armina neapolitana |  | EF133469.1 |  |  |
| 797211 Armina semperi | HM162696.1 | HM162512.1 | HM162606.1 |  |
| 797168 Armina sp. 3 |  | HM162513.1 | HM162607.1 |  |
| 797169 Armina sp. 9 |  | HM162514.1 | HM162608.1 |  |
| 930957 Armina sp. | HQ010504.1 | HQ010473.1 | HQ010539.1 |  |
| 869979 Austraeolis ornata | GQ403774.1 |  | GQ403752.1 |  |
| 1154718 Austraeolis stearnsi | JQ699571.1 | JQ699395.1 | JQ699483.1 |  |
| 934965 Babakina anadoni | HQ616767.1 | HQ616796.1 | HQ616710.1 |  |
|  | HQ616746.1 | HQ616776.1 | HQ616730.1 |  |
|  | HQ616747.1 | HQ616806.1 | HQ616711.1 |  |
|  | HQ616748.1 | HQ616775.1 | HQ616742.1 |  |
|  |  | HQ616807.1 | HQ616743.1 |  |
|  |  | HQ616805.1 | HQ616744.1 |  |
|  |  | HQ616777.1 | HQ616709.1 |  |


| 934966 Babakina festiva | HM162754.1 | HQ616802.1 | $\begin{aligned} & \text { HQ616736.1 } \\ & \text { HQ616735.1 } \end{aligned}$ | GU227367.1 |
| :---: | :---: | :---: | :---: | :---: |
|  |  | HQ616801.1 |  |  |
|  |  | HQ616803.1 |  |  |
| 797244 Babakina indopacifica |  | HM162587.1 | HM162678.1 |  |
| 929452 Baeolidia australis |  |  |  |  |
| 1287509 Baeolidia japonica | JQ997058.1 | JQ996954.1 | JQ996856.1 |  |
|  | JQ997059.1 | JQ996956.1 | JQ996855.1 |  |
|  | JQ997057.1 | JQ996957.1 | JQ996854.1 |  |
|  |  | JQ996955.1 | JQ996853.1 |  |
| 1287510 Baeolidia moebii | JX087550.1 | JX087619.1 | JQ996857.1 |  |
|  | HQ616771.1 | JQ996958.1 | JQ996858.1 |  |
|  | JQ997060.1 | JX087618.1 | HQ616733.1 |  |
|  | JQ997061.1 | JQ996959.1 | JX087481.1 |  |
|  | JX087551.1 | HQ616800.1 | HQ616734.1 |  |
|  | HQ616770.1 | HQ616799.1 | JX087482.1 |  |
| 934972 Baeolidia nodosa | JQ997080.1 | JX087629.1 | HQ616731.1 | GU339155.1 |
|  | JX087560.1 | JQ996992.1 | JQ996886.1 |  |
|  | HQ616768.1 | JX087630.1 | JX087493.1 |  |
|  | JX087559.1 | HQ616797.1 | JX087527.1 |  |
|  | JQ997081.1 | JQ996991.1 | JX087494.1 |  |
| 1290784 Baeolidia sp. A | JQ997056.1 | JQ996953.1 | JQ996851.1 |  |
|  | JQ997051.1 | JQ996948.1 | JQ996850.1 |  |
|  | JQ997054.1 | JQ996952.1 | JQ996852.1 |  |
|  | JQ997055.1 | JQ996951.1 | JQ996847.1 |  |
| 1290785 Baeolidia sp. B | JQ997046.1 | JQ996943.1 | JQ996842.1 |  |
| 1290786 Baeolidia sp. C | JQ997045.1 | JQ996941.1 | JQ996840.1 |  |
| 1287537 Berghia cf. salaamica | JQ997048.1 | JQ996945.1 | JQ996843.1 |  |
|  | JQ997047.1 | JQ996944.1 | JQ996844.1 |  |
| 1287511 Berghia coerulescens | JQ997049.1 | JQ996946.1 | JQ996845.1 |  |
|  |  | JX087604.1 | JX087470.1 |  |
| 1287631 Berghia columbina | JX087543.1 | JX087608.1 | JX087473.1 |  |
|  | JX087545.1 | JX087609.1 | JX087472.1 |  |
|  | JX087544.1 | JX087605.1 | JX087474.1 |  |
|  | JX087542.1 | JX087606.1 | JX087471.1 |  |
|  |  | JX087607.1 |  |  |
| 1287632 Berghia rissodominguezi | JX087552.1 | JX087621.1 | JX087484.1 |  |
|  |  | JX087620.1 | JX087483.1 |  |


| 1287506 Berghia salaamica | JQ997062.1 | JQ996962.1 | JQ996860.1 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | JQ996960.1 | JQ996859.1 |  |  |
|  |  |  | JQ996862.1 |  |  |
| 1290787 Berghia sp. A | JX087549.1 | JX087617.1 | JX087480.1 |  |  |
| 929456 Berghia verrucicornis | HQ616750.1 | JX087623.1 | HQ616713.1 | GU227364.1 |  |
|  | JX087553.1 | HQ616779.1 | JX087487.1 |  |  |
|  | JX087554.1 | JX087624.1 | JX087486.1 |  |  |
|  | HQ616749.1 | JX087610.1 | JX087485.1 |  |  |
|  |  | JX087622.1 | HQ616712.1 |  |  |
|  |  | HQ616778.1 | JX087488.1 |  |  |
| 797246 Bonisa nakaza | HM162746.1 | HM162579.1 | HM162670.1 |  |  |
| 1170240 Bornella anguilla |  | JN869424.1 |  |  |  |
|  |  | JN869425.1 |  |  |  |
| 797212 Bornella calcarata | HM162707.1 | HM162533.1 | HM162627.1 |  |  |
|  |  | JN869427.1 |  |  |  |
| 797213 Bornella hermanni | JN869448.1 | JN869421.1 | JN869403.1 |  |  |
|  | JN869446.1 | JN869422.1 | JN869404.1 |  |  |
|  | HM162705.1 | JN869423.1 | JN869402.1 |  |  |
|  | JN869447.1 | HM162531.1 | HM162625.1 |  |  |
|  |  | JN869420.1 |  |  |  |
| 797214 Bornella johnsonorum | HM162704.1 | JN869419.1 | HM162624.1 |  |  |
|  | JN869445.1 | HM162530.1 | JN869401.1 |  |  |
| 1170241 Bornella sarape |  | JN869428.1 |  |  |  |
| 219659 Bornella stellifer | HM162703.1 | JN869418.1 | JN869400.1 | AY165756.1 |  |
|  |  | JN869417.1 | HM162623.1 | AY165755.1 |  |
|  |  | HM162529.1 |  |  |  |
| 797215 Bornella valdae | JN869449.1 | HM162532.1 | HM162626.1 |  |  |
|  | HM162706.1 | JN869426.1 | JN869405.1 |  |  |
| 71296 Cadlina laevis | AY345034.1 |  | AY345034.1 |  |  |
| 1154735 Calma glaucoides | JQ699567.1 | JQ699388.1 | JQ699477.1 |  | JQ699296.1 |
| 929458 Calmella cavolini | HQ616772.1 |  | HQ616737.1 | GU227361.1 |  |
| 929450 Caloria elegans | HQ616751.1 | HQ616780.1 | HQ616714.1 | GU227363.1 |  |
|  |  |  | HQ616738.1 |  |  |
| 376200 Caloria indica | DQ417325.1 | JQ699389.1 | DQ417273.1 |  | JQ699297.1 |
| 1287538 Caloria sp. 4 | JQ997064.1 | JQ996966.1 | JQ996865.1 |  |  |
|  | JQ997063.1 | JQ996965.1 | JQ996864.1 |  |  |


| 1291185 Catriona sp. A | JQ997021.1 | JQ996915.1 | JQ996816.1 |  |
| :--- | :--- | :--- | :--- | :--- |
| 1290775 Catriona sp. B | JQ997024.1 | JQ996919.1 | JQ996819.1 |  |
| 929460 Cerberilla affinis |  | JQ996918.1 |  | JQ996863.1 | GU227366.1


| 763535 Cuthona sp. 3 | GQ292066.1 |  |  | $\begin{aligned} & \text { GQ326902.1 } \\ & \text { GQ326903.1 } \end{aligned}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1287545 Cuthona sp. 35 | JQ997026.1 | JQ996921.1 | JQ996820.1 |  |  |
| 763536 Cuthona sp. 4 | GQ292069.1 |  |  | GQ326901.1 |  |
| 763537 Cuthona sp. 5 | GQ292067.1 |  |  | GQ326900.1 |  |
| 763538 Cuthona sp. 6 | GQ292070.1 |  |  | GQ326896.1 |  |
|  |  |  |  | GQ326895.1 |  |
|  |  |  |  | GQ326897.1 |  |
|  |  |  |  | GQ326894.1 |  |
| 763539 Cuthona sp. 7 | GQ292074.1 |  |  | GQ326892.1 |  |
| 763540 Cuthona sp. 8 | GQ292073.1 |  |  | GQ326891.1 |  |
| 763541 Cuthona sp. 9 | GQ292076.1 |  |  | GQ326906.1 |  |
|  | GQ292075.1 |  |  |  |  |
|  | GQ292077.1 |  |  |  |  |
| 1290791 Cuthona sp. A | JQ997019.1 | JQ996913.1 | JQ996814.1 |  |  |
| 763120 Dendronotus albopunctatus | GQ292064.1 |  |  | GQ326861.1 |  |
| 904359 Dendronotus albus |  | HQ267088.1 | GU339186.1 |  |  |
|  |  |  | GU339185.1 |  |  |
| 154605 Dendronotus dalli | AF249800.1 |  | AF249252.1 | AY165757.1 | AY427450.1 |
| 71302 Dendronotus frondosus | JN869450.1 | HQ267089.1 | JN869406.1 | GQ326860.1 |  |
|  | GQ292063.1 | JN869429.1 | AF249251.1 | AF249206.1 |  |
|  | AY345041.1 |  | GU339187.1 |  |  |
|  |  |  | AY345041.1 |  |  |
| 219661 Dendronotus iris | GQ292062.1 | HM162537.1 | HM162631.1 | AY165758.1 |  |
|  |  | HQ267090.1 | GU339188.1 |  |  |
|  |  | JN869431.1 | GU339189.1 |  |  |
|  |  |  | GU339190.1 |  |  |
| 797216 Dendronotus lacteus | HM162710.1 | HM162538.1 |  |  |  |
| 1170242 Dendronotus orientalis |  | JN869432.1 |  |  |  |
| 1054385 Dendronotus patricki | HQ225828.1 |  | HQ225829.1 | HQ225830.1 |  |
| 797217 Dendronotus regius | JN869451.1 | JN869430.1 | HM162629.1 |  |  |
|  | HM162708.1 | HM162535.1 | JN869407.1 |  |  |
| 904360 Dendronotus rufus |  | HQ267091.1 | GU339191.1 |  |  |
| 763135 Dendronotus sp. 1 | GQ292061.1 |  |  |  |  |



| 1154717 Dondice occidentalis | JQ699570.1 | KC526527.1 | KC526513.1 |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  | KC526536.1 | KC526510.1 |  |
|  |  | KC526528.1 | KC526509.1 |  |
|  |  | KC526530.1 | KC526517.1 |  |
|  |  | KC526533.1 | KC526518.1 |  |
|  |  | KC526526.1 | KC526514.1 |  |
|  |  | KC526524.1 | KC526512.1 |  |
|  |  | KC526529.1 | KC526508.1 |  |
|  |  | JQ699394.1 | JQ699482.1 |  |
|  |  | KC526532.1 | KC526507.1 |  |
|  |  | KC526534.1 | KC526506.1 |  |
|  |  | KC526531.1 | KC526515.1 |  |
|  |  | KC526525.1 | KC526511.1 |  |
|  |  | KC526523.1 | KC526519.1 |  |
|  |  |  | KC526516.1 |  |
| 1353478 Dondice parguerensis |  | KC526535.1 | KC526521.1 |  |
|  |  |  | KC526522.1 |  |
|  |  |  | KC526520.1 |  |
| 763123 Doto antarctica | GQ292025.1 |  |  | GQ326882.1 |
| 763124 Doto columbiana | GQ292026.1 |  |  | GQ326881.1 |
| 154624 Doto coronata | $\begin{aligned} & \text { HM162734.1 } \\ & \text { AF249794.1 } \end{aligned}$ | HM162566.1 | HM162657.1 | AF249203.1 |
| 154610 Doto eireana |  |  | AF249248.1 | AF249204.1 |
| 154645 Doto floridicola | AF249820.1 |  |  | AY165759.1 |
| 154611 Doto koenneckeri | HM162735.1 | HM162567.1 | HM162658.1 | AF249205.1 |
|  | AF249797.1 |  | AF249249.1 |  |
| 154612 Doto pinnatifida | AF249793.1 |  | AF249250.1 | AF249202.1 |
| 797176 Doto sp. 15 | HM162739.1 | HM162571.1 | HM162662.1 |  |
| 797177 Doto sp. 2 | HM162737.1 | HM162569.1 | HM162660.1 |  |
| 797178 Doto sp. 7 | HM162738.1 | HM162570.1 | HM162661.1 |  |
| 797179 Doto sp. H | HM162740.1 | HM162572.1 | HM162663.1 |  |
| 797180 Doto sp. I | HM162741.1 | HM162573.1 | HM162664.1 |  |
| 797181 Doto sp. J | HM162742.1 | HM162574.1 | HM162665.1 |  |
| 797182 Doto sp. K | HM162743.1 | HM162575.1 | HM162666.1 |  |
| 797221 Doto ussi | HM162736.1 | HM162568.1 | HM162659.1 |  |


| 76182 Eubranchus exiguus | AF249792.1 |  | AF249246.1 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 763125 Eubranchus rustyus | GQ292065.1 |  |  | GQ326905.1 |  |
| 763126 Eubranchus sanjuanensis | GQ292079.1 |  |  | GQ326909.1 |  |
| 252571 Eubranchus sp. A | AY345046.1 |  | AY345046.1 |  |  |
| 76181 Eubranchus sp. B | AF249791.1 |  |  |  |  |
| 1287513 Facelina annulicornis | JQ997076.1 | JQ996986.1 | JQ996881.1 |  |  |
|  |  | JQ996987.1 |  |  |  |
| 219665 Facelina bostoniensis | AY345031.1 |  | AY345031.1 | $\begin{aligned} & \text { AY165763.1 } \\ & \text { GU339157.1 } \end{aligned}$ |  |
|  |  |  |  |  |  |
| 154649 Facelina punctata | AF249816.1 |  |  |  |  |
| 1291186 Facelina sp. A | JQ997052.1 | JQ996949.1 | JQ996848.1 |  |  |
| 1290792 Facelina sp. B | JQ997066.1 | JQ996972.1 | JQ996868.1 |  |  |
|  | JQ997067.1 | JQ996971.1 | JQ996871.1 |  |  |
|  |  | JQ996970.1 | JQ996870.1 |  |  |
|  |  | JQ996969.1 | JQ996869.1 |  |  |
|  |  |  | JQ996882.1 |  |  |
| 1290793 Facelina sp. C | JQ997072.1 | JQ997004.1 | JQ996898.1 |  |  |
|  | JQ997093.1 | JQ996981.1 | JQ996877.1 |  |  |
|  | JQ997073.1 | JQ996982.1 | JQ996876.1 |  |  |
|  | JQ997092.1 | JQ997005.1 | JQ996897.1 |  |  |
| 1290794 Facelina sp. D | JQ997074.1 | JQ996983.1 | JQ996878.1 |  |  |
| 1287568 Facelinidae sp. 2 | JQ997071.1 | JQ996985.1 | JQ996880.1 |  |  |
|  | JQ997075.1 | JQ996984.1 | JQ996879.1 |  |  |
| 1290781 Facelinidae sp. A | JQ997025.1 | JQ996920.1 |  |  |  |
| 934967 Favorinus brachialis | $\begin{array}{ll} \text { HQ616761.1 } & \text { HQ616790.1 } \\ \text { AY345042.1 } & \end{array}$ |  | HQ616724.1 |  |  |
|  |  |  | HQ616741.1 |  |  |
|  |  |  | AY345042.1 |  |  |
| 797222 Favorinus elenalexiarum | HM162755.1 | JQ699396.1 | HM162679.1 |  | JQ699304.1 |
|  |  | HM162588.1 | JQ699484.1 |  |  |
| 929454 Favorinus sp. |  |  |  | GU227369.1 |  |
| 1287638 Fiona pinnata | JX087558.1 | JX087628.1 | JX087492.1 |  |  |
| 154626 Flabellina affinis | HQ616753.1 | HQ616782.1 | HQ616716.1 | AY165767.1 |  |
|  | AF249783.1 |  | AY345055.1 |  |  |  |
|  | AY345055.1 |  |  |  |  |  |
| 763127 Flabellina amabilis | GQ292022.1 |  |  | GQ326912.1 |  |


| 219672 Flabellina babai | HQ616754.1 | HQ616783.1 | HQ616717.1 | AY165768.1 | AY427449.1 |
| :--- | :--- | :--- | :--- | :--- | :--- |
| 934968 Flabellina baetica | HQ616755.1 | HQ616784.1 | HQ616718.1 |  |  |
| 929451 Flabellina bilas |  |  |  | GU227368.1 |  |
| 1287634 Flabellina confusa | JX087556.1 | JX087627.1 | JX087490.1 |  |  |
| 1154719 Flabellina exoptata | JX087557.1 | JX087626.1 | JX087491.1 |  |  |
| 1154720 Flabellina fusca | JQ699572.1 | JQ699397.1 | JQ699485.1 |  | JQ699305.1 |
| 154627 Flabellina ischitana | JQ699573.1 | JQ699398.1 | JQ699486.1 |  | JQ699306.1 |
|  | HQ616757.1 | HQ616785.1 | HQ616719.1 |  |  |
| 76183 Flabellina pedata | HQ616756.1 | HQ616808.1 | HQ616720.1 |  |  |
|  | AF249814.1 | HQ616786.1 | HQ616745.1 |  |  |
| 219673 Flabellina sp. | HQ616758.1 | HQ616787.1 | AF249247.1 |  |  |
| 763128 Flabellina trilineata | AF249817.1 |  | HQ616721.1 | AY165769.1 |  |
| 763129 Flabellina trophina | GQ292024.1 | JQ699399.1 | JQ699487.1 | GQ326911.1 | JQ699307.1 |
| 154613 Flabellina verrucosa | GQ292023.1 |  |  | AF249245.1 | AF249198.1 |


| 1154737 Glaucus atlanticus | JQ699595.1 | JQ699403.1 | JQ699510.1 | JQ699312.1 |
| :--- | :--- | :--- | :--- | :--- |
|  | JQ699590.1 | JQ699400.1 | JQ699514.1 | JQ699314.1 |
|  | JQ699598.1 | JQ699415.1 | JQ699511.1 | JQ699327.1 |
|  | JQ699576.1 | JQ699414.1 | JQ699508.1 | JQ699337.1 |
|  | JQ699594.1 | JQ699427.1 | JQ699492.1 | JQ699336.1 |
|  | JQ699596.1 | JQ699406.1 | JQ699500.1 | JQ699317.1 |
|  | JQ699588.1 | JQ699416.1 | JQ699495.1 | JQ699326.1 |
|  | JQ699586.1 | JQ699401.1 | JQ699496.1 | JQ699324.1 |
|  | JQ699581.1 | JQ699422.1 | JQ699499.1 | JQ699329.1 |
|  | JQ699583.1 | JQ699409.1 | JQ699490.1 | JQ699318.1 |
|  | JQ699575.1 | JQ699426.1 | JQ699512.1 | JQ699330.1 |
|  | JQ699578.1 | JQ699425.1 | JQ699489.1 | JQ699321.1 |
|  | JQ699574.1 | JQ699412.1 | JQ699513.1 | JQ699335.1 |
|  | JQ699585.1 | JQ699407.1 | JQ699497.1 | JQ699322.1 |
|  | JQ699600.1 | JQ699421.1 | JQ699506.1 | JQ699308.1 |
|  | JQ699602.1 | JQ699405.1 | JQ699507.1 | JQ6993333.1 |
|  | JQ699601.1 | JQ699428.1 | JQ699517.1 | JQ699332.1 |
|  | JQ699589.1 | JQ699413.1 | JQ699491.1 | JQ699315.1 |
|  | JQ699580.1 | JQ699410.1 | JQ699494.1 | JQ699325.1 |
|  | JQ699587.1 | JQ699417.1 | JQ699501.1 | JQ699323.1 |
|  | JQ699597.1 | JQ699402.1 | JQ699505.1 | JQ699334.1 |
|  | JQ699599.1 | JQ699419.1 | JQ699488.1 | JQ699313.1 |
|  | JQ699603.1 | JQ699423.1 | JQ699503.1 | JQ699331.1 |
|  | JQ699579.1 | JQ699408.1 | JQ699498.1 | JQ699311.1 |
|  | JQ699592.1 | JQ699429.1 | JQ699502.1 | JQ699309.1 |
|  | JQ699584.1 | JQ699404.1 | JQ699509.1 | JQ699319.1 |
|  | JQ699593.1 | JQ699411.1 | JQ699516.1 | JQ699320.1 |
|  | JQ699591.1 | JQ699418.1 | JQ699504.1 | JQ699328.1 |
|  | JQ699577.1 | JQ699420.1 | JQ699493.1 | JQ699515.1 |


| 1154738 Glaucus marginatus | JQ699607.1 | JQ699459.1 | JQ699552.1 |  | JQ699339.1 |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | JQ699618.1 | JQ699442.1 | JQ699519.1 |  | JQ699347.1 |
|  | JQ699613.1 | JQ699446.1 | JQ699523.1 |  | JQ699354.1 |
|  | JQ699616.1 | JQ699451.1 | JQ699518.1 |  | JQ699342.1 |
|  | JQ699623.1 | JQ699438.1 | JQ699551.1 |  | JQ699344.1 |
|  | JQ699625.1 | JQ699450.1 | JQ699550.1 |  | JQ699349.1 |
|  | JQ699605.1 | JQ699435.1 | JQ699529.1 |  | JQ699370.1 |
|  | JQ699608.1 | JQ699445.1 | JQ699533.1 |  | JQ699368.1 |
|  | JQ699609.1 | JQ699441.1 | JQ699541.1 |  | JQ699357.1 |
|  | JQ699604.1 | JQ699436.1 | JQ699549.1 |  | JQ699338.1 |
|  | JQ699622.1 | JQ699454.1 | JQ699544.1 |  | JQ699343.1 |
|  | JQ699612.1 | JQ699457.1 | JQ699545.1 |  | JQ699364.1 |
|  | JQ699610.1 | JQ699432.1 | JQ699543.1 |  | JQ699346.1 |
|  | JQ699611.1 | JQ699461.1 | JQ699548.1 |  | JQ699358.1 |
|  | JQ699606.1 | JQ699448.1 | JQ699539.1 |  | JQ699371.1 |
|  | JQ699627.1 | JQ699433.1 | JQ699521.1 |  | JQ699345.1 |
|  | JQ699624.1 | JQ699439.1 | JQ699540.1 |  | JQ699361.1 |
|  | JQ699620.1 | JQ699465.1 | JQ699546.1 |  | JQ699348.1 |
|  | JQ699619.1 | JQ699463.1 | JQ699528.1 |  | JQ699362.1 |
|  | JQ699626.1 | JQ699434.1 | JQ699542.1 |  | JQ699372.1 |
|  | JQ699614.1 | JQ699460.1 | JQ699537.1 |  | JQ699351.1 |
|  | JQ699615.1 | JQ699447.1 | JQ699530.1 |  | JQ699366.1 |
|  | JQ699628.1 | JQ699453.1 | JQ699522.1 |  | JQ699353.1 |
|  | JQ699629.1 | JQ699458.1 | JQ699532.1 |  | JQ699359.1 |
|  | JQ699617.1 | JQ699464.1 | JQ699553.1 |  | JQ699341.1 |
|  | JQ699621.1 | JQ699430.1 | JQ699535.1 |  | JQ699340.1 |
|  |  | JQ699455.1 | JQ699526.1 |  | JQ699373.1 |
|  |  | JQ699440.1 | JQ699547.1 |  | JQ699355.1 |
|  |  | JQ699449.1 | JQ699524.1 |  | JQ699350.1 |
|  |  | JQ699437.1 | JQ699525.1 |  | JQ699369.1 |
|  |  | JQ699462.1 | JQ699520.1 |  | JQ699365.1 |
|  |  | JQ699452.1 | JQ699527.1 |  | JQ699363.1 |
|  |  | JQ699443.1 | JQ699538.1 |  | JQ699360.1 |
|  |  | JQ699431.1 | JQ699531.1 |  | JQ699352.1 |
| 869980 Godiva banyulensis | GQ403773.1 | HQ616804.1 | GQ403751.1 | AY165764.1 |  |
|  | AF249782.1 |  | HQ616740.1 |  |  |
| 797223 Godiva quadricolor | HM162756.1 | HM162589.1 | HM162680.1 |  |  |


| 797263 Hancockia californica | HM162702.1 | HM162527.1 | JN869408.1 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | JN869452.1 | JN869433.1 | HM162621.1 |  |  |
| 797208 Hancockia cf. uncinata |  | HM162528.1 | HM162622.1 |  |  |
| 252574 Hancockia uncinata | AY345047.1 |  | AY345047.1 |  |  |
| 205593 Hermissenda crassicornis | $\begin{aligned} & \text { JQ699630.1 } \\ & \text { GQ292054.1 } \end{aligned}$ | JQ699466.1 | JQ699554.1 |  | JQ699374.1 |
| 1154740 Hermosita hakunamatata | JQ699631.1 | JQ699467.1 | JQ699555.1 |  | JQ699375.1 |
| 797226 Janolus barbarensis | HM162747.1 | HM162580.1 | HM162671.1 |  |  |
| 797227 Janolus capensis | HM162748.1 | HM162581.1 | HM162672.1 |  |  |
| 154651 Janolus cristatus | AF249813.1 |  |  | AF249194.1 |  |
| 763130 Janolus fuscus | GQ292048.1 |  |  | GQ326887.1 |  |
| 797228 Janolus longidentatus | HM162749.1 | HM162582.1 | HM162673.1 |  |  |
| 797229 Janolus mirabilis | HM162750.1 | HM162583.1 | HM162674.1 |  |  |
| 797183 Janolus sp. 1 | HM162751.1 | HM162584.1 | HM162675.1 |  |  |
| 797184 Janolus sp. 2 | HM162752.1 | HM162585.1 | HM162676.1 |  |  |
| 797185 Janolus sp. 7 | HM162753.1 | HM162586.1 | HM162677.1 |  |  |
| 1154742 Learchis poica | JQ699632.1 | JQ699468.1 | JQ699556.1 |  | JQ699376.1 |
| 797250 Leminda millecra | HM162745.1 | HM162578.1 | HM162669.1 |  |  |
| 1287514 Limenandra fusiformis | JQ997077.1 | JQ996988.1 | JQ996883.1 |  |  |
|  | JQ997078.1 | JQ996989.1 | JQ996884.1 |  |  |
| 1290776 Limenandra sp. A | JQ997082.1 | HQ616798.1 | JQ996887.1 |  |  |
|  | HQ616769.1 | JQ996993.1 | HQ616732.1 |  |  |
| 1291187 Limenandra sp. B | JX087540.1 | JQ996947.1 | JQ996846.1 |  |  |
|  | JX087539.1 | JX087601.1 | JX087469.1 |  |  |
|  | JX087541.1 | JX087602.1 | JX087468.1 |  |  |
|  | JQ997050.1 | JX087603.1 | JX087467.1 |  |  |
| 1290795 Limenandra sp. C | JQ997079.1 | JQ996990.1 | JQ996841.1 |  |  |
|  |  | JQ996942.1 | JQ996885.1 |  |  |
| 797197 Lomanotus sp. E | HM162715.1 | HM162547.1 | HM162640.1 |  |  |
| 1170243 Lomanotus sp. | JN869453.1 | JN869434.1 | JN869409.1 |  |  |
| 1170244 Lomanotus vermiformis |  | JN869435.1 |  |  |  |
| 797254 Marianina rosea | HM162733.1 | HM162565.1 | HM162656.1 |  |  |
| 797230 Marionia arborescens | HM162722.1 | HM162554.1 | HM162646.1 |  |  |



| 1171429 Notobryon thompsoni | JN869455.1 | JN869438.1 | JN869412.1 |
| :---: | :---: | :---: | :---: |
|  | JN869456.1 | JN869439.1 | JN869413.1 |
| 797237 Notobryon wardi | JN869454.1 | HM162546.1 | HM162637.1 |
|  | HM162714.1 | JN869436.1 | HM162639.1 |
|  |  | HM162545.1 | JN869411.1 |
|  |  | JN869437.1 | JN869410.1 |
|  |  | HM162544.1 | HM162638.1 |
| 1287519 Noumeaella isa | JQ997084.1 | JQ996995.1 | JQ996889.1 |
| 1290774 Noumeaella rehderi |  | JQ996961.1 | JQ996861.1 |
| 1287556 Noumeaella sp. 3 | JQ997087.1 | JQ996999.1 | JQ996893.1 |
|  | JQ997088.1 | JQ996998.1 | JQ996892.1 |
| 1287557 Noumeaella sp. 4 | JQ997085.1 | JQ997003.1 | JQ996894.1 |
|  | JQ997090.1 | JQ997000.1 | JQ996890.1 |
|  | JQ997091.1 | JQ997001.1 | JQ996891.1 |
|  | JQ997086.1 | JQ996997.1 | JQ996896.1 |
|  | JQ997089.1 | JQ996996.1 | JQ996895.1 |
|  |  | JQ997002.1 |  |
| 1290796 Noumeaella sp. A | JQ997053.1 | JQ996950.1 | JQ996849.1 |
| 1290797 Noumeaella sp. B | JX087548.1 | JX087616.1 | JX087479.1 |
| 376196 Phestilla lugubris | DQ417299.1 |  | DQ417253.1 |
|  | DQ417300.1 |  | DQ417252.1 |
|  | DQ417298.1 |  | DQ417254.1 |
| 376192 Phestilla melanobrachia | DQ417281.1 |  | DQ417228.1 |
|  | DQ417277.1 |  | DQ417233.1 |
|  | DQ417274.1 |  | DQ417231.1 |
|  | DQ417280.1 |  | DQ417236.1 |
|  | DQ417282.1 |  | DQ417235.1 |
|  | DQ417278.1 |  | DQ417230.1 |
|  | DQ417279.1 |  | DQ417232.1 |
|  | DQ417275.1 |  | DQ417229.1 |
|  | DQ417276.1 |  | DQ417234.1 |


| 376197 Phestilla minor | DQ417311.1 | DQ417263.1 |
| :---: | :---: | :---: |
|  | DQ417301.1 | DQ417257.1 |
|  | DQ417307.1 | DQ417258.1 |
|  | DQ417308.1 | DQ417262.1 |
|  | DQ417310.1 | DQ417264.1 |
|  | DQ417305.1 | DQ417260.1 |
|  | DQ417303.1 | DQ417256.1 |
|  | DQ417304.1 | DQ417261.1 |
|  | DQ417313.1 | DQ417259.1 |
|  | DQ417309.1 | DQ417255.1 |
|  | DQ417302.1 |  |
|  | DQ417312.1 |  |
|  | DQ417306.1 |  |
| 376195 Phestilla sibogae | DQ417297.1 | DQ417245.1 |
|  | DQ417292.1 | DQ417249.1 |
|  | DQ417293.1 | DQ417242.1 |
|  | DQ417296.1 | DQ417251.1 |
|  | DQ417288.1 | DQ417248.1 |
|  | DQ417287.1 | DQ417241.1 |
|  | DQ417294.1 | DQ417246.1 |
|  | DQ417291.1 | DQ417247.1 |
|  | DQ417290.1 | DQ417244.1 |
|  | DQ417295.1 | DQ417250.1 |
|  | DQ417289.1 | DQ417243.1 |
| 376198 Phestilla sp. 1 | DQ417320.1 | DQ417272.1 |
|  | DQ417324.1 | DQ417270.1 |
|  | DQ417322.1 | DQ417269.1 |
|  | DQ417314.1 | DQ417266.1 |
|  | DQ417316.1 | DQ417268.1 |
|  | DQ417315.1 | DQ417267.1 |
|  | DQ417319.1 | DQ417271.1 |
|  | DQ417323.1 | DQ417265.1 |
|  | DQ417318.1 |  |
|  | DQ417317.1 |  |
|  | DQ417321.1 |  |


|  | DQ417286.1 |  | DQ417238.1 |  |
| :--- | :--- | :--- | :--- | :--- |
|  | DQ417285.1 |  | DQ417237.1 |  |
|  | DQ417284.1 |  | DQ417239.1 |  |
| 1154721 Phidiana hiltoni | DQ417283.1 |  | DQ417240.1 |  |
| 219669 Phidiana lynceus |  | JQ699470.1 | JQ699558.1 |  |
|  |  | JX087562.1 | JX087633.1 | JX087497.1 | AY165765.1 $\quad$ JQ699378.1


| 869971 Phyllodesmium lembehensis | GQ403780.1 |  | GQ403758.1 |  |
| :--- | :--- | :--- | :--- | :--- |
|  |  |  | GQ403771.1 |  |
| 674072 Phyllodesmium lizardensis | HQ010505.1 | HQ010464.1 | GQ403772.1 |  |
|  | HQ010496.1 | HQ010474.1 | HQ010540.1 | HQ010532.1 |
| 869972 Phyllodesmium longicirrum |  |  | GQ403761.1 | JQ699559.1 |


| 934978 Pruvotfolia longicirrha | HQ616760.1 | HQ616789.1 | HQ616723.1 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 934979 Pruvotfolia pselliotes | HQ616762.1 | HQ616791.1 | HQ616725.1 |  |  |
| 1290777 Pruvotfolia sp. A |  | JQ997008.1 |  |  |  |
| 1290778 Pruvotfolia sp. B |  | JQ996980.1 |  |  |  |
| 797258 Pseudobornella orientalis |  | HM162534.1 | HM162628.1 |  |  |
| 929462 Pteraeolidia ianthina |  | JQ699473.1 | JQ699562.1 | GU227370.1 | JQ699382.1 |
|  |  | JQ997006.1 |  |  |  |
| 797260 Sakuraeolis enosimensis | HQ010503.1 | HQ010472.1 | HQ010538.1 |  |  |
|  | HM162758.1 | HM162591.1 | HM162682.1 |  |  |
| 797262 Scyllaea pelagica | HM162711.1 | HM162540.1 | JN869415.1 |  |  |
|  | JN869458.1 | JN869441.1 | JN869414.1 |  |  |
|  | JN869459.1 | JN869442.1 | HM162633.1 |  |  |
|  | JN869457.1 | JN869443.1 | JN869416.1 |  |  |
| 1449875 Spurilla braziliana | JX087575.1 | JQ997009.1 | JX087503.1 |  |  |
|  | JX087568.1 | JX087639.1 | JQ996900.1 |  |  |
|  | JQ997095.1 | JX087638.1 | JX087508.1 |  |  |
|  | JQ997097.1 | JX114844.1 | JX087511.1 |  |  |
|  | JX087567.1 | JX087644.1 |  |  |  |
|  | JX087578.1 | JX087647.1 |  |  |  |
|  | JQ997096.1 |  |  |  |  |
| 1154712 Spurilla chromosoma | JQ699566.1 | JQ996912.1 | JQ996812.1 |  | JQ699295.1 |
|  | JQ997018.1 | JQ699387.1 | JQ699476.1 |  |  |
|  |  | JQ996911.1 | JQ996813.1 |  |  |
| 1287630 Spurilla creutzbergi | JX087547.1 | JX087614.1 | JX087475.1 |  |  |
|  | JX087546.1 | JX087613.1 | JX087477.1 |  |  |
|  |  | JX087615.1 | JX087478.1 |  |  |
|  |  | JX087612.1 | JX087476.1 |  |  |
| 910326 Spurilla major |  |  |  | GU227365.1 |  |


| 929453 Spurilla neapolitana | JX087574.1 | JX087655.1 | JX087509.1 | GU227362.1 | JQ699383.1 |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | JX087583.1 | JX087656.1 | HQ616726.1 |  |  |
|  | JX087586.1 | JX087646.1 | JX087502.1 |  |  |
|  | JX087566.1 | JX087643.1 | HQ616727.1 |  |  |
|  | JX087576.1 | JX087645.1 | JX087517.1 |  |  |
|  | JX087570.1 | JX087640.1 | JX087521.1 |  |  |
|  | JQ699636.1 | JX087661.1 | JX087523.1 |  |  |
|  | JX087569.1 | JX087651.1 | JX087520.1 |  |  |
|  | HQ616764.1 | JX087642.1 | JX087504.1 |  |  |
|  | JX087572.1 | HQ616792.1 | JX087519.1 |  |  |
|  | JX087571.1 | JX087652.1 | JX087518.1 |  |  |
|  | JX087581.1 | JX087658.1 | JX087506.1 |  |  |
|  | JX087577.1 | JX087635.1 | JX087499.1 |  |  |
|  | HQ616763.1 | JX087650.1 | JX087516.1 |  |  |
|  | JX087564.1 | JX114845.1 | JX087510.1 |  |  |
|  | JX087585.1 | JX087654.1 | JX087524.1 |  |  |
|  | JX087584.1 | JX087660.1 | JX114843.1 |  |  |
|  | JX087582.1 | JX087637.1 | JX087507.1 |  |  |
|  | JX087587.1 | JX087641.1 | JX087522.1 |  |  |
|  | JX087573.1 | JX087659.1 | JX087514.1 |  |  |
|  |  | JX087657.1 | JX087515.1 |  |  |
|  |  | JQ699474.1 | JQ699563.1 |  |  |
|  |  | JX087662.1 | JX087500.1 |  |  |
|  |  | JX087653.1 | JX087505.1 |  |  |
|  |  | HQ616793.1 | JX114842.1 |  |  |
| 1154722 Spurilla sargassicola | JX087589.1 | JX087663.1 | JQ996901.1 |  | JQ699384.1 |
|  | JX087588.1 | JX087664.1 | JX087525.1 |  |  |
|  | JQ997098.1 | JQ997010.1 | JQ699564.1 |  |  |
|  |  | JX087665.1 | JX087526.1 |  |  |
| 1290798 Spurilla sp. A | JX087565.1 | JX087649.1 | JX087501.1 |  |  |
|  | JX087580.1 | JX087636.1 | JX087513.1 |  |  |
|  | JX087579.1 | JX087648.1 | JX087512.1 |  |  |
| 530585 Tergipes antarcticus | EU727251.1 |  | KF713480.1 |  |  |
|  | EU727252.1 |  |  |  |  |
|  | EU727253.1 |  |  |  |  |
|  | GU227106.1 |  |  |  |  |
|  | EU727250.1 |  |  |  |  |
| 157144 Tergipes tergipes | AY345032.1 |  | AY345032.1 | AF249197.1 |  |


| 252556 Tethys fimbria |  | EF133468.1 |  |  |
| :--- | :--- | :--- | :--- | :--- |
| 797240 Tritonia antarctica | HM162718.1 | HM162550.1 | HM162643.1 |  |
| 763132 Tritonia challengeriana | GQ292052.1 |  |  | GQ326904.1 |
| 70853 Tritonia diomedea | GQ292050.1 |  | GU339203.1 | GQ326890.1 |
| 763133 Tritonia festiva | GQ292051.1 | HM162551.1 |  | GQ326889.1 |
| 157146 Tritonia nilsodhneri | HM162719.1 |  |  | AF249200.1 |
| 797241 Tritonia pickensi | HM162716.1 | HM162548.1 | HM162641.1 | HM162717.1 |
| 797192 Tritonia sp. 3 | HM162549.1 | HM162642.1 |  |  |
| 797193 Tritonia sp. 4 | HM162731.1 | HM162563.1 | HM162654.1 |  |
| 797194 Tritonia sp. F | HM162720.1 | HM162552.1 | HM162644.1 |  |
| 797195 Tritonia sp. G | HM162730.1 | HM162562.1 | HM162653.1 |  |
| 157148 Tritoniella belli | GQ292056.1 |  | GU227002.1 | AF249201.1 |
|  | GU227111.1 |  |  | GQ326883.1 |

## Appendix B.

This appendix contains the legends for 20 additional tree figures, as cited in the text.

## Figure B1.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the ALL_TAXA data matrix. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure $\mathbf{B 2}$.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S), partitioned, in the ALL_TAXA data matrix. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure 33.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S), partitioned, in the MIN_TWO_GENES data matrix. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B4.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the MIN_THREE_GENES data matrix. The outgroup is Discodoris atromaculata.

## Figure $\mathbf{B 5}$.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S), partitioned, in the MIN_THREE_GENES data matrix. The outgroup is Discodoris atromaculata.

## Figure B6.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from three genes (COI, H3, 16S) in the THREE_GENES data matrix. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B7.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from three genes (COI, H3, 16S), partitioned, in the THREE_GENES data matrix. The outgroup is containing Discodoris atromaculata.

## Figure 88.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the mitochondrial Cytochrome Oxidase I (COI) gene. The outgroup is Discodoris atromaculata.

## Figure $\mathbf{B 9}$.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the nuclear Histone 3 (H3) gene. The outgroup is Discodoris atromaculata.

## Figure B10.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the mitochondrial 16S rRNA gene. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B11.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the nuclear 28 S rRNA gene. The outgroup is Discodoris atromaculata.

## Figure B12.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the nuclear 18S rRNA gene. The outgroup is Discodoris atromaculata.

## Figure B13.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the ALL_TAXA data matrix, excluding all sequences from the genus Melibe. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B14.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the MIN_TWO_GENES data matrix, excluding all sequences from the genus Melibe. Bootstrap values are provided above each branch.

## Figure B15.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the MIN_THREE_GENES data matrix, excluding all sequences from the genus Melibe. The outgroup is Discodoris atromaculata.

## Figure $B 16$.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from three genes (COI, H3, 16S) in the THREE_GENES data matrix, excluding all sequences from the genus Melibe. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B17.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the mitochondrial Cytochrome Oxidase I (COI) gene, excluding all sequences from the genus Melibe. The outgroup is Discodoris atromaculata.

## Figure $B 18$.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the nuclear Histone 3 (H3) gene, excluding all sequences from the genus Melibe. The outgroup is Discodoris atromaculata.

Figure $B 19$.
The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from the mitochondrial 16 S rRNA gene, excluding all sequences from the genus Melibe. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

## Figure B20.

The $50 \%$ majority-rule bootstrap consensus tree of Cladobranchia using sequence data from five genes (COI, H3, 16S, 18S, 28S) in the MIN_149_TAXA data matrix. The outgroup is the clade containing Discodoris atromaculata and Cadlina laevis.

