- 1 RUNNING HEAD: Phylogenomics of Tunicata
- 3 Phylogenomics offers resolution of major tunicate relationships
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Summary

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Tunicata, a diverse clade of approximately 3,000 described species of marine, filter-feeding chordates, is of great interest to researchers because tunicates are the closest living relatives of vertebrates and they facilitate comparative studies of our own biology. The group also includes numerous invasive species that cause considerable economic damage and some species of tunicates are edible. Despite their diversity and importance, relationships among major lineages of Tunicata are not completely resolved. Here, we supplemented public data with transcriptomes from seven species spanning the diversity of Tunicata and conducted phylogenomic analyses on data sets of up to 798 genes. Sensitivity analyses were employed to examine the influences of reducing compositional heterogeneity and branch-length heterogeneity. All analyses maximally supported a monophyletic Tunicata within Olfactores (Vertebrata + Tunicata). Within Tunicata, all analyses recovered Appendicularia sister to the rest of Tunicata and confirmed (with maximal support) that Thaliacea is nested within Ascidiacea. Stolidobranchia is the sister taxon to all other tunicates except Appendicularia. In most analyses, phlebobranch tunicates were recovered paraphyletic with respect to Aplousobranchia. Support for this topology varied but was strong in some cases. However, when only the 50 best genes based on compositional heterogeneity were analysed, we recovered Phlebobranchia and Aplousobranchia reciprocally monophyletic with strong support, consistent with most traditional morphology-based hypotheses. Examination of internode certainty also cast doubt on results of phlebobranch paraphyly, which may be due to limited taxon sampling. Taken together, these results provide a higher-level phylogenetic framework for our closest living invertebrate relatives.

1. Introduction

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Tunicata (Lamarck, 1816) is a diverse clade of approximately 3,000 described species of marine, filter-feeding chordates (Shenkar and Swalla, 2011). This morphologically plastic group, which may be benthic or pelagic and solitary or colonial, has intrigued researchers for a number of reasons. Its position as the sister lineage of vertebrates (e.g., Bourlat et al., 2006; Delsuc et al., 2005, 2006) makes it an import resource for studying the evolution of developmental mechanisms (e.g., Racioppi et al., 2017; Suzuki et al., 2016; Swalla and Jeffery, 1996; Taketa and De Tomaso, 2015). Additionally, Tunicata includes some of the most invasive benthic marine animals known (e.g., Kaplan et al., 2017; Reem et al., 2013), which can cause catastrophic damage if left unchecked. Lastly, tunicates are eaten in some parts of the world, thus supporting fishery industries (Lambert et al., 2016). Despite their importance, evolutionary relationships within Tunicata remain uncertain, hindering understanding of the evolutionary history and ancestral character states of its constituent lineages and Chordata as a whole. Tunicates date back to the Early Cambrian (Chen et al., 2003; Shu et al., 2001). Lamarck (1816) first described modern tunicates for their hard, leathery outer covering, the "tunic," and included ascidians, pyrosomes and salps within the group. Tunicates were subsequently divided into three recognized classes: Ascidiacea (sea squirts), Thaliacea (pelagic salps, doliolids, and pyrosomes; Berrill, 1936), and Appendicularia (larvaceans), which have been associated with a suite of gross morphological and life history features (Table 1). Of these classes, Ascidiacea, comprised of sessile tunicates, is the most species rich (~3,000 species), but a growing body of evidence indicates that the pelagic Thaliacea (~100 species) is nested within Ascidiacea (Swalla et al., 2000; Stach et al., 2002; Tsagkogeorga et al., 2009; Winchell et al., 2002; Zeng et al., 2006).

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Appendicularia is a group of small, pelagic tunicates characterized by a putatively paedomorphic adult body plan that resembles the tadpole larva, very short generation times, and secretion of a unique mucous-like "house" used in feeding (Stach et al., 2008). Most appendicularian genes studied to date exhibit high rates of nucleotide substitution leading to long branches in molecular phylogenetic analyses (e.g., Delsuc et al., 2006, 2008; Govindarajan et al., 2011; Stach and Turbeville, 2002; Swalla et al., 2000; Wada, 1998). Previous studies have recovered appendicularians as either sister to the rest of Tunicata or within Ascidiacea (reviewed by Tsagkogeorga et al., 2009). Higher-level taxonomy of Ascidiacea is based primarily on morphological characters of the branchial sac, the organ used to collect particles from the water column, and as such they are divided into three traditionally recognized orders: Aplousobranchia (simple branchial sac), Phlebobranchia (vascular branchial sac), and Stolidobranchia (folded branchial sac; Lahille, 1886). However, relationships among these clades remain uncertain and, as noted above, Thaliacea and Appendicularia may be nested within this clade of otherwise benthic tunicates. Stolidobranchia is a large and morphologically heterogeneous clade of solitary, social, and colonial tunicates (Zeng and Swalla, 2005; Zeng et al., 2006). Despite this, molecular phylogenetic analyses based on 18S rDNA and mitochondrial genes have shown convincingly that Molgulidae, a group of solitary ascidians with various larval morphologies, is sister to all other stolidobranchs whereas relationships within Pyuridae and Styelidae, the two other major lineages of Stolidobranchia, have generally not been well-resolved (e.g., Stach et al., 2002; Swalla et al., 2000; Tsagkogeorga et al., 2009; Winchell et al., 2002; Zeng and Swalla, 2005; Zeng et al. 2006). Aplousobranchia has been recovered as unambiguously monophyletic in most

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molecular analyses to date (e.g., Stach and Turbeville, 2002; Tsagkogeorga et al., 2009; Turon and López-Legentil, 2004), but these colonial tunicates tend to have high rates of nucleotide substitution and support for their position relative to other tunicates has generally been weak. Phlebobranchia is a traditionally recognized group of mostly solitary tunicates, but the composition of this clade has been debated. For example, Cionidae, which includes the widelystudied species Ciona intestinalis, C. robusta and C. savignyi, was originally included within Phlebobranchia (Berrill, 1936). However, this genus of important model tunicate species has also been viewed as a subclade of Aplousobranchia (e.g., Kott, 1990, 1969). Although molecular phylogenetic studies of tunicates conducted to date (Govindarajan et al., 2011; Shenkar et al., 2016; Singh et al., 2009; Stach et al., 2010; Stach and Turbeville, 2002; Swalla et al., 2000; Tsagkogeorga et al., 2009; Turon and López-Legentil, 2004; Zeng et al., 2006; Zeng and Swalla, 2005) have greatly advanced understanding of relationships within some clades, tunicate higher-level phylogeny has been difficult to reconstruct. Evolutionary history of Tunicata has likely been a particularly challenging question in invertebrate systematics because several tunicate lineages (Appendicularia, Thaliacea, and many species within Aplousobranchia) exhibit long branch lengths for 18S and at least some other genes (Stach and Turbeville, 2002; Swalla et al., 2000; Tsagkogeorga et al., 2009; Winchell et al., 2002; Yokobori et al., 2006; Zeng et al., 2006). Phylogenomic analyses have been important to our understanding of chordate evolutionary history by showing that tunicates and not cephalochordates are the sister taxon of the vertebrates (Bourlat et al., 2006; Delsuc et al. 2006, 2008; Dunn et al. 2008; Putnam et al. 2008), but no

study to date has had the necessary taxon sampling to address long-standing questions about evolutionary relationships among the major lineages of tunicates. To this end, we supplemented publicly available tunicate and outgroup genome and transcriptome data with transcriptomes from taxa spanning the diversity of Tunicata and re-evaluated the higher-level evolutionary history of this important group.

2. Methods

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We sampled publicly available and newly generated transcriptome and/or genome data from all extant tunicate orders with the exception of Doliolida, which was previously shown to be nested within the otherwise well-sampled taxon Thaliacea (Govindarajan et al., 2011). Available tunicate data were augmented with new transcriptomes from specimens collected from Antarctica, the Northeastern Pacific, and the Northwestern Atlantic (Table 2). With the exception of the unidentified Ascidia sp. (?) from Antarctica, all of the newly sequenced species are wellknown species from their respective collection localities that were easily identified based on habitus and structure of the branchial basket and gut. RNA was extracted and purified from RNAlater-preserved or frozen tissue samples using the Omega Bio-Tek Mollusc RNA kit or the Qiagen RNeasy kit. In either case, an on-column DNAse digestion was performed. RNA concentration was measured using a Qubit (Thermo Fisher) with the RNA High Sensitivity kit, RNA purity was assessed by measuring the 260/280 nm absorbance ratio using a Nanodrop Lite (Thermo Fisher), and RNA integrity was evaluated using a 1% SB agarose gel or a TapeStation (Agilent). At least 1 µg of total RNA for each specimen was sent to Macrogen (Cambridge, MA, USA) for Illumina TruSeq RNA v2 library preparation (polyA enrichment) and sequencing on the Illumina HiSeq 2500 system using the HiSeq SBS V4 chemistry with 100 bp paired-end

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sequencing. For Styela gibbsii, two separate libraries from material from the same individual were prepared and sequenced separately, but the raw reads were combined prior to transcriptome assembly. Dataset processing followed the general approach of Kocot et al. (2017). Publicly available genomic data were downloaded as predicted proteins if available (Table 3). Otherwise, predicted transcripts from genomes or assembled transcriptomes were downloaded. Publicly available transcriptomes available only as raw read data and our new transcriptome data were assembled using Trinity 2.2.0 with the --trimmomatic and --normalize reads flags (Grabherr et al., 2011). Transcripts were translated with TransDecoder 2.0.0 or 2.0.1 (Haas et al., 2014) using the UniProt SwissProt database (accessed on September 20th, 2016; The Uniprot Consortium, 2014) and PFAM (Pfam-A.hmm) version 27 (Finn et al., 2015). For orthology inference, we employed HaMStR 13 (Ebersberger et al., 2009) with the "model organsisms" core-ortholog set. Translated transcripts for all taxa except Ciona intestinalis and human were searched against the 1,031 profile hidden Markov models (pHMMs) using the default options. The "representative" flag was not used because it is not compatible with PhyloTreePruner (Kocot et al., 2013; see below). Sequences matching a pHMM were compared to the proteome of *Ciona* using BLASTP with the default search settings of HaMStR. If the Ciona amino acid sequence contributing to the pHMM was the best BLASTP hit in each of these back-BLASTs, the sequence was then assigned to that putative orthology group (simply referred to as "gene" henceforth). Redundant sequences that were identical (including partial sequences that were identical at least where they overlapped) were then removed with

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UniqHaplo (http://raven.iab.alaska.edu/~ntakebay/), leaving only unique sequences for each taxon. Each gene was then aligned with MAFFT 7.273 using the automatic alignment strategy with a "maxiterate" value of 1,000 (Katoh and Standley, 2013). Alignments were then trimmed with Aliscore (Misof and Misof, 2009) and Alicut (Kück et al., 2010) with the default options to remove ambiguously aligned regions. Lastly, we deleted sequences that did not overlap with all other sequences in the alignment by at least 20 amino acids, starting with the shortest sequences not meeting this criterion. This step was necessary for downstream single-gene tree reconstruction. Finally, genes sampled for fewer than half of the 28 taxa after these steps were discarded. In some cases, a taxon was represented in an alignment by two or more sequences (splice variants, lineage-specific gene duplications [=inparalogs], overlooked paralogs, or exogenous contamination). To screen for evidence of paralogy or contamination and select just one sequence for each taxon, an approximately maximum likelihood tree was inferred for each remaining alignment using FastTree 2 (Price et al., 2010) using the -slow and -gamma options. PhyloTreePruner (Kocot and Citarella et al., 2013) was then employed to use a tree-based approach to screen each single-gene alignment for evidence of paralogy or contamination. First, nodes with support values below 0.95 were collapsed into polytomies. Next, the maximally inclusive subtree was selected where each taxon was represented by no more than one sequence or, in cases where more than one sequence was present for any taxon, all sequences from that taxon formed a clade or were part of the same polytomy. Putative paralogs and contaminants (sequences falling outside of this maximally inclusive subtree) were then deleted from the input

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alignment. In cases where multiple sequences from the same taxon formed a clade or were part of the same polytomy, all sequences except the longest were deleted. In order to further screen for genes or taxa with paralogy or contamination issues, genes that passed PhyloTreePruner screening and were still sampled for at least 15 of the 28 taxa were retained and used for single-gene tree building in RAxML 8.2.8 (Stamatakis, 2014) with the PROTGAMMAAUTOF model. The tree with the best likelihood score after 10 random addition sequence replicates was retained and topological robustness (i.e., nodal support) was assessed with rapid bootstrapping with the number of replicates determined by the autoMRE criterion. Concatenation of remaining sequences to assemble the data matrix henceforth referred to as the "original full dataset" was performed using FASconCAT-G (Kück and Longo, 2014). Because compositional heterogeneity (Delsuc et al., 2005; Jermiin et al., 2004; Kocot et al., 2017; Nesnidal et al., 2010; Rodríguez-Ezpeleta et al., 2007) and branch-length heterogeneity (Kocot et al., 2017; Struck et al., 2014) have been shown to be potential sources of systematic error in phylogenomics, we calculated relative composition frequency variability (RCFV; Zhong et al., 2011) and branch-length heterogeneity score (LB; Struck et al., 2014) for each gene in the original full dataset and assembled data matrices corresponding to the best 50, 100, 200, and 500 genes according to RCFV and LB. This allowed us to examine effects of excluding genes with relatively high compositional heterogeneity or branch-length heterogeneity. Average RCFV was calculated for each gene based on per-taxon RCFV scores calculated in BaCoCa 1.104.r with a subclade definition file that divided the taxa into

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Ambulacraria (Hemichordata + Echinodermata), Vertebrata, Cephalochordata, and Tunicata. LB was calculated for each gene with TreSpEx 1.1 (Struck, 2014) using RAxML single-gene trees generated as described above. As above, concatenation was performed using FASconCAT-G (Kück and Longo, 2014). For brevity, these matrices are referred to using abbreviated names such as LB 50, which represents the best 50 genes according to branchlength heterogeneity or RCFV 200, which represents the best 200 genes according to RCFV. Maximum likelihood (ML) analyses were conducted for all data matrices in RAxML 8.2.8 (Stamatakis 2014). Matrices were partitioned by gene and the PROTGAMMAAUTOF model was specified for all partitions. The tree with the best likelihood score after 10 random addition sequence replicates was retained and nodal support was assessed with rapid bootstrapping with the number of replicates determined by the autoMRE criterion. We also conducted Bayesian inference (BI) analyses in Phylobayes MPI 1.6j (Lartillot et al., 2013) using a site-heterogeneous mixture model. Specifically, the CAT+GTR+Γ4 model (Lartillot and Philippe, 2004) was used to account for site-specific rate heterogeneity (-cat -gtr dgam 4). Because of the computationally intensive nature of Phylobayes analyses using this model, BI was only conducted for the RCFV 50 and LB 50 data sets. For the BI analysis of RCFV 50, five parallel chains were run for roughly 15,000-18,000 cycles with the first 10,000 discarded as burn-in. For the BI analysis of LB 50, four parallel chains were run for roughly 17,000-28,000 cycles with the first 10,000 discarded as burn-in. The bycomp maxdiff values (0.0039 for RCFV 50 and 0.0697 for LB 50) were used to assess convergence of chains.

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Because the CAT mixture model implemented in Phylobayes could not be feasibly applied to all datasets, we also conducted ML analyses in IQ-TREE using the posterior mean site frequency (PMSF) model (Wang et al. 2017). PMSF is a rapid approximation of the time- and memory-intensive profile mixture model of Lee et al. (2008), which is a variant of the Phylobayes CAT model. Specifically, the LG+C60+G+F model was specified. Because this approach requires a guide tree to infer the site frequency model, we used the previously generated RAxML tree for each PMSF analysis. For the PMSF analyses of RCFV 50 and LB 50, we additionally tested the effect of using the consensus trees recovered by Phylobayes as the guide tree. Nodal support was assessed with 1000 replicates of ultrafast bootstrapping (bb 1000). To screen for outlier genes and taxa (genes or taxa that have contamination and/or paralogy issues), single-gene RAxML trees were analyzed in Phylo-MCOA (De Vienne et al., 2012) finding successive decomposition axes from individual ordinations (derived from distance matrices) that maximize a covariance function. For detection of "complete outliers" (genes or taxa most likely to have contamination and/or paralogy issues) we used values of k=1.5 and thres=0.5. After the above analyses were conducted, transcriptome data became available for two additional tunicate species: Clavelina lepadiformis (Aplousobranchia) and Salpa thompsoni (Thaliacea). We assembled an additional dataset (Full dataset+2) following an identical approach to that described above to produce the original full dataset except for the addition of these two taxa. The minimum number of taxa required to keep a gene was kept at fifteen (>50%

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of the original 28 sampled taxa). We also assembled datasets corresponding to the best 50 genes according to RCFV (RCFV 50+2) and LB (LB 50+2) identified as described above that were retained by our pipeline after the addition of these two taxa (47 and 48 genes, respectively). Maximum likelihood analyses were conducted on these datasets in RAxML as described above. We examined tree certainty (TC), relative tree certainty (RTC), and internode certainty (IC) using the approaches of Salichos and Rokas (2013) and Salichos et al. (2014) as calculated in RAxML 8.2.4. We calculated TC and RTC for the original full dataset and each of the submatrices based on this dataset. Trees resulting from the RAxML analysis of each dataset (provided to RAxML with "-t") and trees based on the corresponding RAxML single-gene trees (provided with "-z") were used. IC was calculated for the original full dataset based on the corresponding RAxML single-gene trees (provided with "-z"). TC, RTC, and IC were calculated under stochastic bipartition adjustment (excluding conflicting bipartitions). Finally, we sought to confirm species identification by extracting cytochrome c oxidase subunit I (COI) sequences from all of our newly generated transcriptomes analyzed herein (when present) and conducting a phylogenetic analysis with publicly available tunicate COI sequences. Sequences were manually put in the proper open reading from and were translated to amino acids using the ascidian mitochondrial code in MEGA 7.0.14 (Kumar et al., 2016). Amino acids were aligned with MUSCLE (Edgar, 2004) as implemented in MEGA 7.0.14. A phylogenetic analysis was conducted in RAxML using the approach described above for our concatenated data matrices except the alignment was not partitioned by gene.

3. Results

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Our bioinformatic pipeline resulted in a matrix ("original full dataset") of 798 genes totaling 254,865 amino acid (AA) positions in length (Table 4). The average Alicut-trimmed alignment length was 319 AAs with the longest being 1,326 AAs and the shortest being 55 AAs. All genes were sampled for at least 15 taxa but some were sampled for all 28 taxa with an average of 24 taxa sampled per gene. Missing data in the original full dataset was 22.57% (i.e., 77.43% matrix occupancy). We conducted ML analyses on all twelve data sets (Figure 1, Supplementary Figures 1-23) and BI analyses on the two smallest data sets: RCFV 50 and LB 50 (Figure 1). All ML and BI analyses recovered Olfactores (Vertebrata + Tunicata) and Tunicata with maximal support (bootstrap support, bs = 100 and posterior probability, pp = 1.00). Within Tunicata, all analyses recovered the appendicularian Oikopleura dioica as the sister taxon to the rest of Tunicata with maximal bootstrap support. Of significance, all ML and BI analyses recovered Thaliacea within "Ascidiacea" with maximal support. Specifically, we consistently recovered Stolidobranchia sister to a clade in which Thaliacea was sister to Phlebobranchia and Aplousobranchia. However, a monophyletic Phlebobranchia was only recovered in the analyses of RCFV 50 (Figure 1, Supplementary Figures 1-3) and LB 100 (Supplementary Figures 9-10). Bootstrap support for Phlebobranchia was weak to moderate in the ML analyses recovering it, but this group was strongly supported by posterior probabilities in the BI analysis of RCFV 50 (pp = 0.99). In the BI analysis of LB 50 and all other ML analyses, phlebobranch tunicates were recovered paraphyletic with respect to Aplousobranchia. BI analysis of LB 50 recovered Corella sister to Aplousobranchia with strong support (pp = 0.99) but the ML analysis of this matrix weakly

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supported this relationship. Most other ML analyses recovered Ascidia + Corella sister to Aplousobranchia. ML Bootstrap support for relationships among phlebobranchs and Aplousobranchia varied, but as more genes (with increasingly poor average per-taxon RCFV or LB scores) were sampled, support for phlebobranch paraphyly increased. Aside from nodes dealing with placement of and relationships among phlebobranch tunicates, all other major tunicate clades as well as relationships within them were consistently recovered and maximally supported in all analyses. Molgulididae was recovered sister to the rest of Stolidobranchia with *Halocynthia* (Pyuridae) sister to Styelidae. Within Styelidae, *Styela* was recovered sister to Botryllus + Botrylloides. All analyses recovered Thaliacea monophyletic with Pyrosomella sister to a clade consisting of the salps Iasis (=Weelia) and Salpa. Likewise, all analyses recovered Aplousobranchia monophyletic. ML analyses of a data matrix assembled in the same manner as the original full dataset but with the addition of transcriptome data from Clavelina lepadiformis (Aplousobranchia) and Salpa thompsoni (Thaliacea; "full dataset+2"; Supplementary Figure 21) resulted in the same general branching order as the analysis of the original full dataset. C. lepadiformis was recovered sister to the remaining Aplousobranchia with maximal support, and Aplousobranchia was recovered within Phlebobranchia. S. thompsoni was recovered sister to S. fusiformis with maximal support. Likewise, analyses of datasets with reduced compositional heterogeneity (RCFV 50+2; Supplementary Figure 22) and branch-length heterogeneity (LB 50+2; Supplementary Figure 23) including these taxa also reflected the topologies recovered in our original analyses without the addition of these data.

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ML analyses based on reduced subsets of genes had higher TC and RTC scores than the analysis of the original full dataset (Table 4), which had a TC of 8.76 and an RTC of 0.35. The dataset with the highest tree certainty and relative tree certainty, LB 100, had a TC of 16.14 an RTC of 0.65. Among the smallest two data sets, RCFV 50 (TC = 13.81, RC = 0.55) had slightly lower values than LB 50 (TC = 13.95 and RC = 0.56). We also examined internode certainty (IC) values for the original full dataset (Supplementary Figure 24). IC values were generally low to very low. Notably, the node nesting Aplousobranchia within Phlebobranchia received zero support. To search for evidence of overlooked paralogy or contamination that could explain the inconsistencies observed among analyses, we screened the original full dataset (without the addition of Clavelina lepadiformis or Salpa thompsoni) with Phylo-MCOA. This software did not identify any taxa or genes as "complete outliers" (i.e., taxa with contamination or genes with overlooked paralogs) and no individual sequences were identified as outliers (i.e., paralogs; Supplementary Figures 25-26). Our taxonomic identifications were generally confirmed to at least the genus-level by comparing COI sequences derived from our transcriptomes to publicly available data. Although COI was not recovered in all of our transcriptomes, placement of COI sequences in the tree (Supplementary Figure 27; also see Supplementary Data on Dryad) was consistent with our identifications and the current taxonomy of the sampled taxa in most cases. The only exception was for the Antarctic tunicate we identified as Ascidia sp. in the field. The COI sequence derived from from this tunicate clustered with *Phallusia*, which is in the same family as *Ascidia*, but it is unclear if we misidentified the sampled specimen or if there is a taxonomic problem with these genera. Unfortunately the specimen was destroyed in the field to sample internal tissues for molecular work.

4. Discussion

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4.1 Higher-level tunicate phylogeny

Our phylogenomic analyses recovered Appendicularia sister to all other tunicates and confirm that thaliaceans are derived ascidians. Stolidobranchia is monophyletic and sister to a clade that encompasses all other ascidians and thaliaceans. However, monophyly of Phlebobranchia is ambiguous in our analyses. Based on these results, the traditional groupings of higher level tunicate taxa should be revisited as it is not surprising that features such as benthic versus pelagic lifestyle, solitary versus colonial habit, or structure of feeding apparatuses are evolutionarily plastic. Importantly, our results show that "Ascidiacea" is not monophyletic as traditionally defined. Thus, this term should be abandoned as a formal taxonomic name as it represents a paraphyletic group of benthic tunicates, or it should be redefined to also include Thaliacea. Of particular interest, the taxonomic composition of Phlebobranchia has not been well circumscribed. Cionidae, which includes the widely-studied model species Ciona intestinalis, Ciona robusta and Ciona savignyi, was originally included within Phlebobranchia (Berrill, 1936), but this family is viewed as a subclade of Aplousobranchia by some taxonomists (e.g., Kott, 1969, 1990). This family was recovered sister to all other Aplousobranchia in an analysis of mitochondrial cytochrome c oxidase subunit I (COI) by Turon and López-Legentil (2004) and

biochemical analyses of vanadium oxidation state by Hawkins et al. (1983) also suggest a close relationship of Cionidae to Aplousobranchia. In contrast, none of our analyses recovered Cionidae within or sister to Aplousobranchia, even though Phlebobranchia was recovered paraphyletic with respect to Aplousobranchia in some analyses. Conclusions about the monophyly of Phlebobranchia are difficult to make based on the analyses presented herein as some trees strongly support the paraphyly of this group and others recover it monophyletic. Interestingly, the dataset with the highest TC and RTC, LB_100, was one of just two data sets that resulted in trees recovering Phlebobranchia monophyletic.

One challenge of previous molecular phylogenetic studies of ascidians has been that aplousobranchs show elevated rates of nucleotide substitution in ribosomal and mitochondrial genes when compared to most other tunicates, leading to concerns about long branch artifacts

aplousobranchs show elevated rates of nucleotide substitution in ribosomal and mitochondrial genes when compared to most other tunicates, leading to concerns about long branch artifacts (e.g., Turon and López-Legentil, 2004; Tsagkogeorga et al., 2009). Many species of aplousobranchs have rapidly-evolving 18S genes with large insertions in multiple parts of the molecule when compared to other tunicates. However, in all of our reconstructed trees based on nuclear protein-coding genes, the sampled aplousobranchs have comparable branch lengths to most other tunicates. Notably, species of *Clavelina* and *Distaplia* sampled in the analysis of 18S by Tsagkogeorga et al. (2009) were among the shortest-branched aplousobranch ascidians in that study. However, they also sampled a species of *Cystodites*, which was a rather long-branched taxon in that analysis, suggesting that evolutionary rates of 18S and nuclear protein-coding genes differ in this lineage.

4.2 Thaliaceans evolved from a benthic ancestor

Traditionally, tunicates were classified among three classes with the pelagic Thaliacea (pyrosomes, salps, and doliolids) considered a distinct clade from Ascidiacea (e.g., Ruppert et al. 2004, Brusca et al., 2016). All of our results strongly support placement of Thaliacea within the traditional class Ascidiacea as the sister group of Phlebobranchia + Aplousobranchia as recovered by Swalla et al. (2000) and Stach and Turbeville (2002) with 18S rDNA and as hinted at by analyses of 18S by Tsagkogeorga et al. (2009). These results suggest a greater degree of lability in the evolution of benthic versus pelagic lifestyles than traditionally recognized. Given that early cephalochordates, early vertebrates, and larvaceans were swimming organisms (Mallatt and Chen, 2003), a benthic lifestyle must have evolved in the last common ancestor of the ascidian-thaliacean clade. Subsequent to this change in lifestyle, Thaliacea, which is nested wellwithin a clade of otherwise benthic ascidians, reacquired a pelagic lifestyle, supporting previous assertions that these pelagic tunicates evolved from a benthic ancestor (Swalla et al. 2000). Historically, Ascidiacea was classified on the basis of the relative position of the gonads with Enterogona including Aplousobranchia and Phlebobranchia, who have gonads closely associated with the gut, and Pleurogona consisting of Stolidobranchia, who generally have gonads distinct from the gut (Garstang, 1928; Perrier, 1898). Thaliacea have gonads associated with the gut, like Aplousobranchia and Phelobobranchia, reinforces the utility of this morphological character that defined Enterogona as noted by Tsagkogeorga et al. (2009).

4.3 The phylogenetic position of Appendicularia

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Our results are consistent with previous studies recovering Appendicularia outside of "Ascidiacea" (Govindarajan et al., 2011; Stach and Turbeville, 2002; Swalla et al., 2000; Wada,

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1998). In studies based on markers such as 18S rDNA (e.g., Govindarajan et al., 2011; Swalla et al., 2000; Tsagkogeorga et al., 2009) these unusual pelagic tunicates tend to have somewhat elevated nucleotide substitution rates and in, phylogenomic analyses (e.g., Delsuc et al., 2006, 2008; this study), Oikopleura, the only larvacean from which genomic or transcriptomic data are available, is an extremely long-branched taxon. Thus, recovery of Appendicularia as the sister group of all other tunicates has been questioned as a possible result of long-branch attraction (Swalla et al. 2000). The most recent study examining tunicate phylogeny with the broadest taxon sampling of 18S to date recovered this unusual group within Ascidiacea as the sister group of Stolidobranchia (Tsagkogeorga et al., 2009). However, to further complicate the issue, Appendicularia and Molgulidae tend to have AT-rich 18S sequences, and this shared compositional heterogeneity could be causing an artefactual attraction of these two taxa (Tsagkogeorga et al., 2009). The branch leading to *Oikopleura* was noticeably longer from root to tip than all other taxa in our analyses of all genes retained by our pipeline and data sets with reduced compositional heterogeneity. However, as data sets with fewer but 'better' genes according to branch-length heterogeneity were analyzed, the branch leading to Oikopleura decreased in length relative to other sampled taxa, and was even shorter than the branches leading to Salpa and Iasis in both the ML and BI analyses of LB 50. Given our consistent recovery of Appendicularia as sister to the rest of Tunicata with maximal support even when compositional heterogeneity and long-branch attraction are reduced, we consider Appendicularia to be an early-branching tunicate lineage and not a derived ascidian clade as previously hypothesized.

4.4 Future directions

This study represents a first step towards resolving tunicate phylogeny using genomic data, but greatly improved taxon sampling will be needed to begin to gain a full picture of the evolutionary history of this diverse and important group. For example, Oikopleuridae is one of three families of Appendicularia but the only family included in any molecular phylogenetic investigation to date. Likewise, Sorberacea is an enigmatic clade of benthic, deep-sea tunicates that has been considered to be a separate class from other tunicates (Monniot et al., 1975). Whether this clade is indeed a distinct lineage of Tunicata or yet another derived ascidian lineage has never been tested with any source of molecular data, let alone phylogenomics. Moreover, many questions about tunicate evolutionary history at family level have been challenging to address, particularly within Aplousobranchia, which exhibits extreme branch-length heterogeneity for 18S rDNA. As most tunicates in the present study exhibit more-or-less comparable branch lengths, especially when steps are taken to exclude genes with exceptional branch-length heterogeneity scores, phylogenomics appears well-suited to address these evolutionary questions.

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Figure legends Figure 1. Phylogeny of Tunicata as inferred in the present study. A. Consensus phylogram from the Bayesian inference analysis of RCFV 50 with bootstrap support values from RAxML and IQ-TREE ML analyses of RCFV 50, RCFV 100, RCFV 200, RCFV 500, and the original full dataset shown. **B.** Consensus phylogram from the Bayesian inference analysis of LB 50 with bootstrap support values from RAxML and IQ-TREE ML analyses of LB 50, LB 100, LB 200, LB 500, and the original full dataset shown. Nodes without support matrices received maximal support in all BI and ML analyses. For the IQ-TREE analyses of RCFV 50 and LB 50, both the Phylobayes (PB) and RAxML (ML) topologies were tested as guide trees and bootstrap support values resulting from these analyses are presented above and below the diagonal line in the bottom left cells of the support matrices, respectively. Dashes in support matrices indicate that a relationship was not recovered. Scale bars represent 0.1 substitutions per site. Corresponding ML tree topologies are presented in Supplementary Figures 1-22. **Supplementary Figure 1.** Phylogeny of Tunicata based on the RAxML analysis of the best 50 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 2.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 50 genes according to RCFV with the RAxML tree used as the guide tree. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site.

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Supplementary Figure 3. Phylogeny of Tunicata based on the IQ-TREE analysis of the best 50 genes according to RCFV with the Phylobayes tree used as the guide tree. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 4.** Phylogeny of Tunicata based on the RAxML analysis of the best 50 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 5.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 50 genes according to LB with the RAxML tree used as the guide tree. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 6.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 50 genes according to LB with the Phylobayes tree used as the guide tree. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 7.** Phylogeny of Tunicata based on the RAxML analysis of the best 100 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 8.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 100 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site.

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Supplementary Figure 9. Phylogeny of Tunicata based on the RAxML analysis of the best 100 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 10.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 100 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 11.** Phylogeny of Tunicata based on the RAxML analysis of the best 200 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. Supplementary Figure 12. Phylogeny of Tunicata based on the IQ-TREE analysis of the best 200 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 13.** Phylogeny of Tunicata based on the RAxML analysis of the best 200 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 14.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 200 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site.

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Supplementary Figure 15. Phylogeny of Tunicata based on the RAxML analysis of the best 500 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 16.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 500 genes according to RCFV. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 17.** Phylogeny of Tunicata based on the RAxML analysis of the best 500 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 18.** Phylogeny of Tunicata based on the IQ-TREE analysis of the best 500 genes according to LB. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 19.** Phylogeny of Tunicata based on the RAxML analysis of the original full dataset (798 genes). Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site.

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Supplementary Figure 20. Phylogeny of Tunicata based on the IQ-TREE analysis of the original full dataset (798 genes). Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 21.** Phylogeny of Tunicata based on the RAxML analysis of the subset of the best 50 genes in the original full dataset according to RCFV retained by our pipeline after the addition of *Clavelina lepadiformis* and *Salpa thompsoni* (47 genes). Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 22.** Phylogeny of Tunicata based on the RAxML analysis of the subset of the best 50 genes in the original full dataset according to LB retained by our pipeline after the addition of Clavelina lepadiformis and Salpa thompsoni (48 genes). Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 23.** Phylogeny of Tunicata based on the RAxML analysis of all genes retained by our pipeline (788) after the addition of *Clavelina lepadiformis* and *Salpa thompsoni*. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. **Supplementary Figure 24.** Phylogeny of Tunicata based on the RAxML analysis of all 798 genes with internode certainty scores presented at each node. **Supplementary Figure 25.** Phylo-MCOA matrix.

Supplementary Figure 26. Phylo-MCOA charts. Supplementary Figure 27. Phylogenetic analysis of COI. Bootstrap support values are presented at each node. Scale bar represents 0.1 substitutions per site. Tree presented as rooted by RAxML but should be interpreted as unrooted.

Table 1. Gross morphology and life history features of major tunicate clades

Taxon	Benthic/Pelagic	Solitary/Colonial	Branchial sac	Gonad position
Appendicularia	Pelagic	Solitary	None	Dorsal
Phlebobranchia	Benthic	Usually solitary	Vascular	Enterogona ¹
Aplousobranchia	Benthic	Colonial	Simple	Enterogona ¹
Stolidobranchia	Benthic	Solitary or Colonial	Folded	Pleurogona ²
Thaliacea	Pelagic	Solitary or Colonial	Simple	Enterogona ¹

¹Unpaired gonads are situated on one side or behind the intestinal loop. ²Gonads are in the lateral mantle wall on both sides.

Table 2. Specimen collection data.

Species	Collection locality	Latitude	Longitude	Tissue extracted
Ascidia sp.	Ross Sea, Antarctica	78°03'47.7"S	169°59'28.1"W	Gonad
Corella willmeriana	Roche Harbor, San Juan Island, WA	48°36'34.6"N	123°09'18.8"W	Gonad and branchial sac
Distaplia occidentalis	Roche Harbor, San Juan Island, WA	48°36'34.6"N	123°09'18.8"W	Entire small colony
Pyrosomella verticillata	Northwestern Atlantic	37°45'46.8"N	73°38'37.8"W	Several entire zooids
Salpa fusiformis	Northwestern Atlantic	39°15'07"N	71°56'24"W	Gonad and branchial sac
Styela gibbsii	Friday Harbor, San Juan Island, WA	48°32'43.2"N	123°00'43.2"W	Gonad
Iasis (=Weelia) cylindrica	Northwestern Atlantic	37°41'05"N	73°37'07"W	Gonad and branchial sac

Table 3. Taxon sampling, number of HaMStR orthologous groups (OGs) recovered for each taxon (out of 1,031), and sources of data used in phylogenomic analyses.

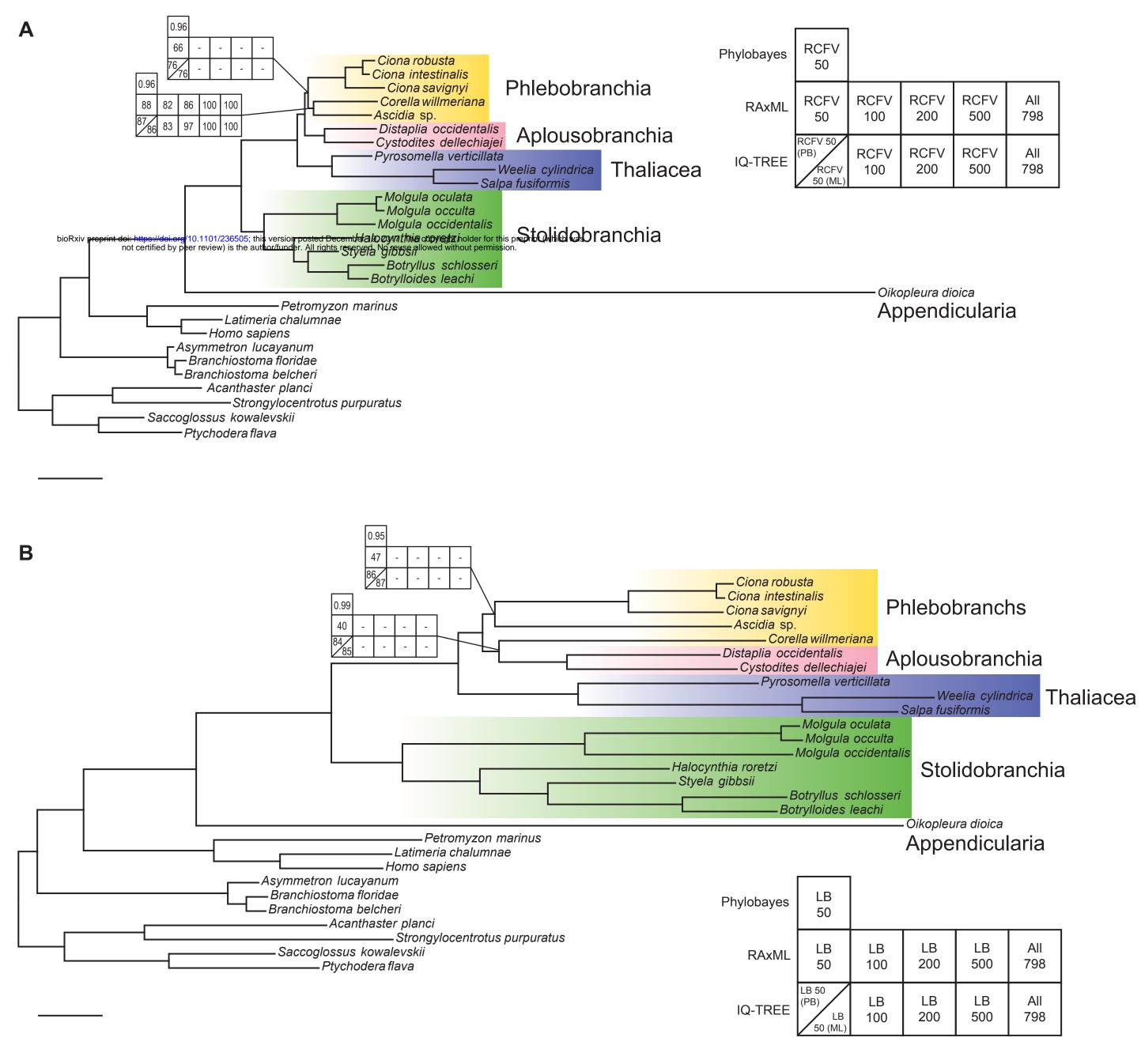
HaMStR				
Species	Clade	Abbrev.	OGs	Data source (Accession/URL/etc.)
Acanthaster planci	Echinodermata	AAUS	991	http://marinegenomics.oist.jp/cots/download/gbr-

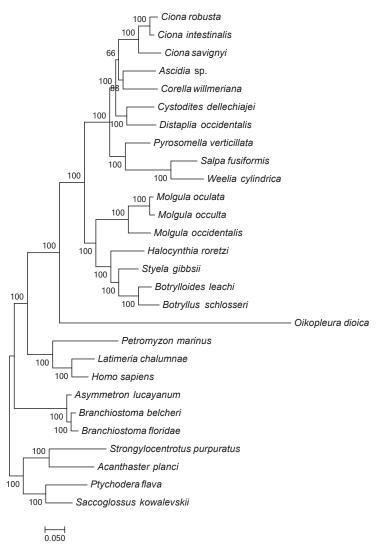
				cotsv1.0.EVM2.prot.gz
Ascidia sp.	Tunicata	ASCI	961	SRR6363557
Asymmetron lucayanum	Cephalochordata	ALUC	995	NCBI TSA GESY00000000.1
Botrylloides leachi	Tunicata	BLEA	941	NCBI SRA SRR2641167, SRR2729871, & SRR2729872
Botryllus schlosseri	Tunicata	BSCH	824	http://botryllus.stanford.edu/botryllusgenome/download/start_st op_transcripts30.fa
Branchiostoma belcheri	Cephalochordata	BBEL	1008	http://genome.bucm.edu.cn/lancelet/download_data.php
Branchiostoma floridae	Cephalochordata	BFLO	1006	http://genome.jgi.doe.gov/Brafl1/Brafl1.home.html
Ciona intestinalis	Tunicata	CINT	1031	HaMStR model organisms core ortholog set
Ciona robusta	Tunicata	CROB	987	NCBI SRA SRR3953074, SRR3953075, & SRR3953076
Ciona savignyi	Tunicata	CSAV	933	http://www.aniseed.cnrs.fr/aniseed/download/?file=data%2Fcs%2Fciona savignyi transcripts gff3 fasta.zip
Corella willmeriana	Tunicata	CWIL	958	SRR6363558
Cystodites dellechiajei	Tunicata	CDEL	996	NCBI SRA SRR1324903
Distaplia occidentalis	Tunicata	DIST	993	SRR6363555
Halocynthia roretzi	Tunicata	HROR	853	http://www.aniseed.cnrs.fr/aniseed/download/?file=data%2Fhr %2Fhalocynthia roretzi est fasta.zip
Homo sapiens	Vertebrata	HSAP	1031	HaMStR model organisms core ortholog set
Latimeria chalumnae	Vertebrata	LCHA	995	ftp://ftp.ensembl.org/pub/release- 87/fasta/latimeria chalumnae/pep/
Molgula occidentalis	Tunicata	OCCI	383	http://www.aniseed.cnrs.fr/aniseed/download/download data
Molgula occulta	Tunicata	OCCU	876	http://www.aniseed.cnrs.fr/aniseed/download/download data
Molgula oculata	Tunicata	OCUL	915	http://www.aniseed.cnrs.fr/aniseed/download/?file=data ⁶ / ₂ Fmo ocul%2Fmolgula oculata transcripts gff3 fasta.zip
Oikopleura dioica	Tunicata	ODIO	924	http://oikoarrays.biology.uiowa.edu/Oiko/Downloads.html
Petromyzon marinus	Vertebrata	PMAR	739	ftp://ftp.ensembl.org/pub/release- 87/fasta/petromyzon marinus/pep/
Ptychodera flava	Hemichordata	PFLA	870	http://octopus.unit.oist.jp/HEMIDATA/pfl.prot
Pyrosomella verticillata	Tunicata	PVER	979	SRR6363556
Saccoglossus kowalevskii	Hemichordata	SKOW	999	http://octopus.unit.oist.jp/HEMIDATA/sko.prot
Salpa fusiformis	Tunicata	SFUS	980	SRR6363561

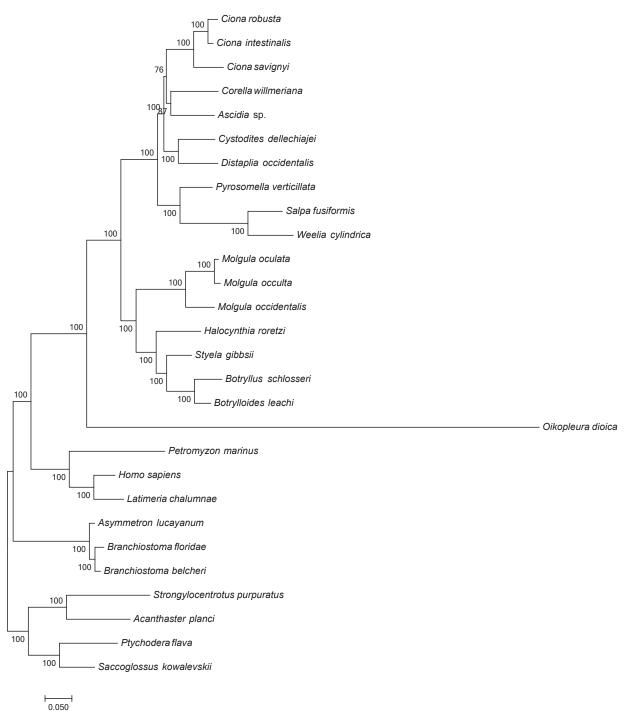
Strongylocentrotus purpuratus	Echinodermata	SPUR	1016	http://www.echinobase.org/Echinobase/SpDownloads/SPU_pep tide.fasta.zip
Styela gibbsii	Tunicata	SGIB	1010	SRR6363562
Iasis (=Weelia) cylindrica	Tunicata	WCYL	984	SRR6363560

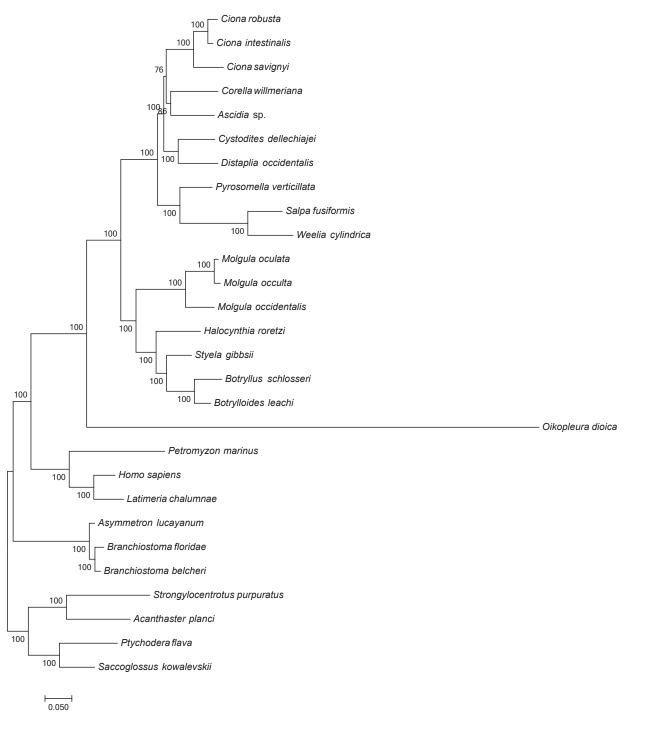
Table 4. Length, percent missing data, tree certainty (TC), and relative tree certainty (RTC) for each data matrix analyzed.

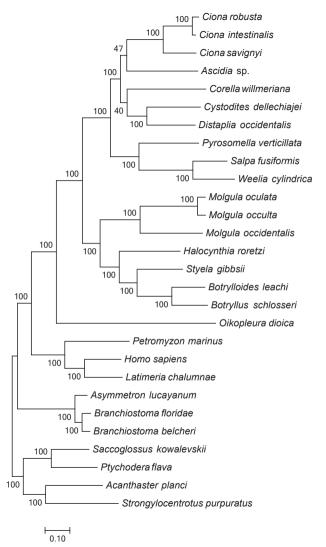
	Genes	Taxa	Length	Missing	TC	RTC
Data matrix			(AAs)	data		
Original full dataset	798	28	256,609	22.6%	8.76	0.35
RCFV_50	50	28	31,260	23.0%	13.81	0.55
RCFV_100	100	28	54,689	22.0%	14.22	0.57
RCFV_200	200	28	99,576	21.9%	10.14	0.41
RCFV 500	500	28	196,200	21.9%	9.78	0.39
LB_50	50	28	15,203	24.6%	13.95	0.56
LB_100	100	28	32,776	23.4%	16.14	0.65
LB_200	200	28	66,032	22.6%	16.02	0.64
LB 500	500	28	166,734	22.4%	11.73	0.47
Full dataset+2	788	30	258,910	22.6%		
RCFV_50+2	47	30	29,831	22.6%		
LB_50+2	48	30	14,990	24.4%		

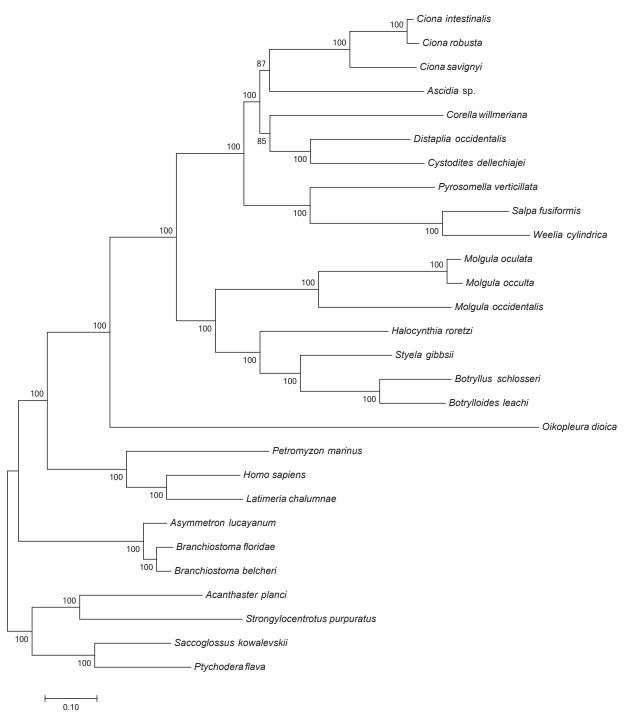


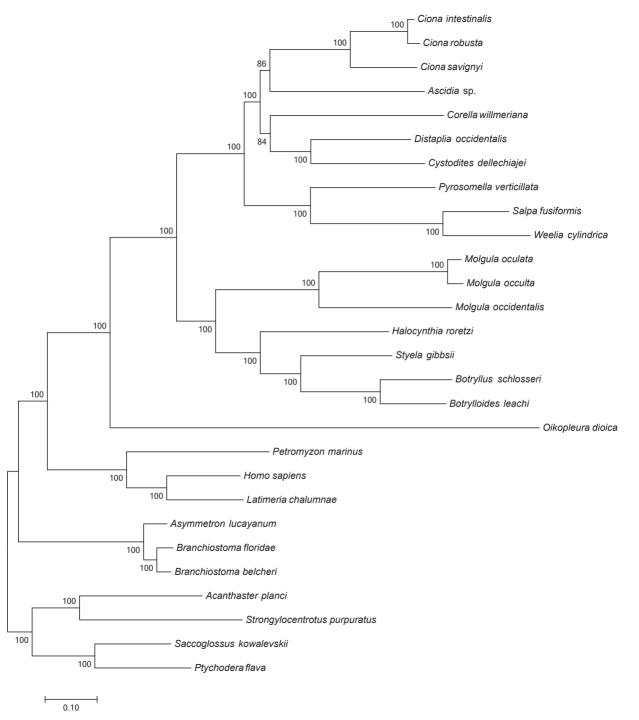


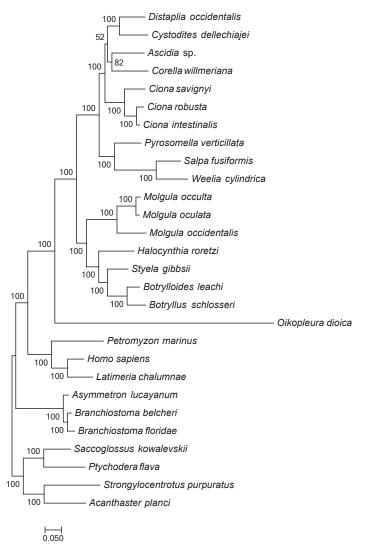


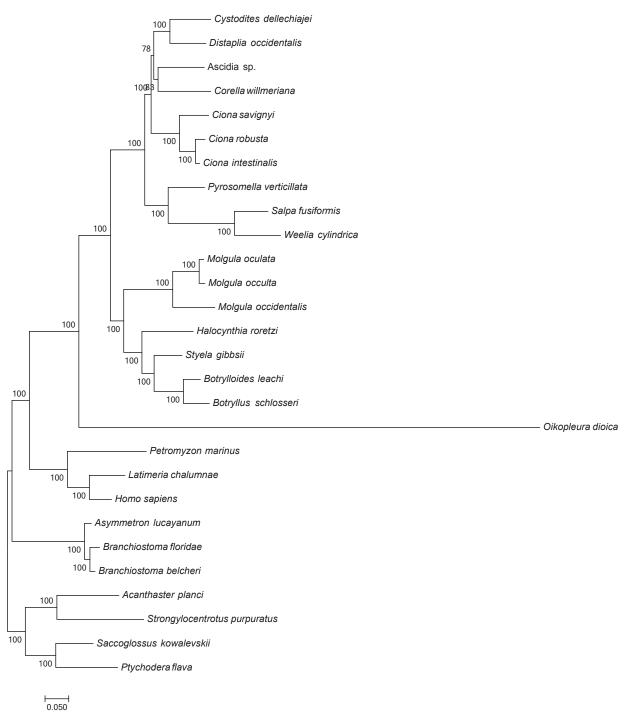


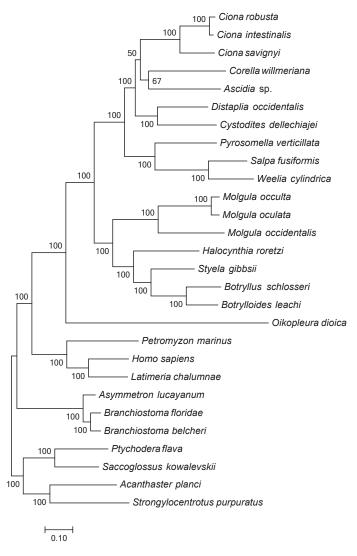


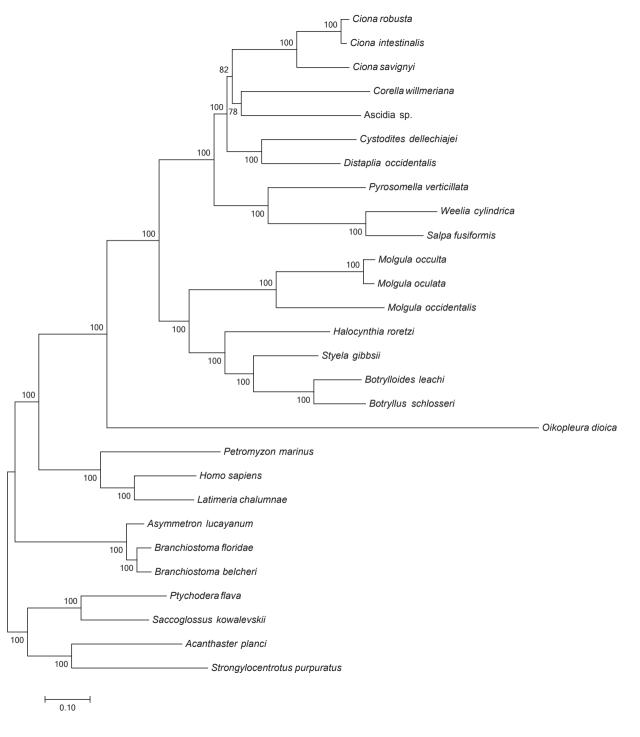


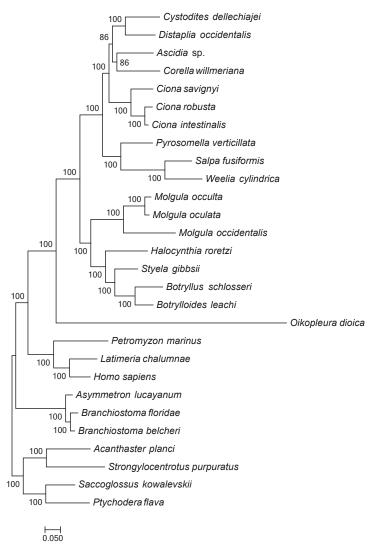


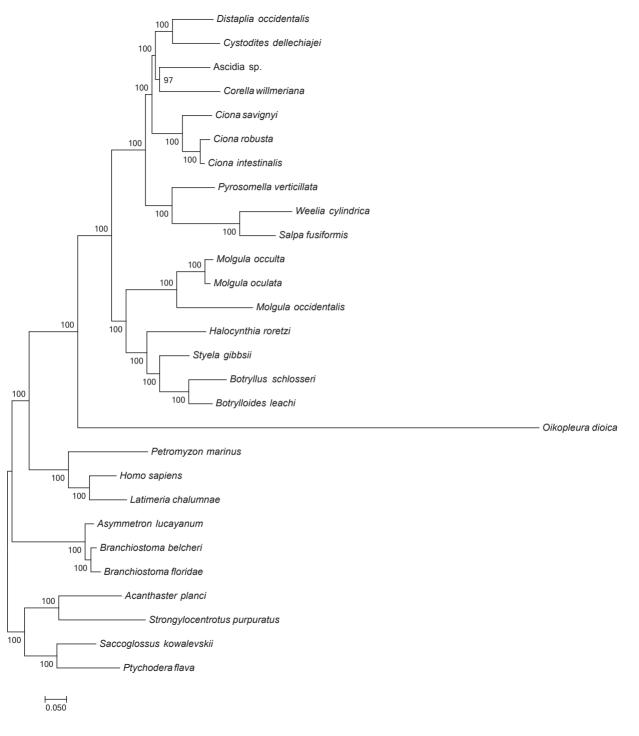


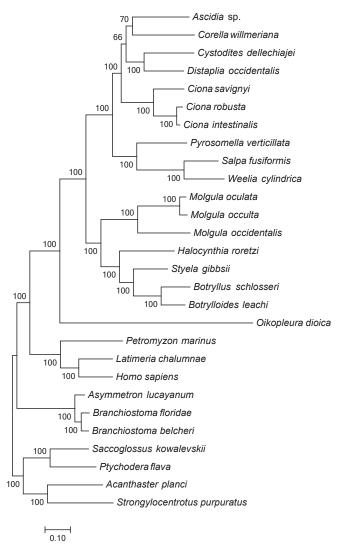


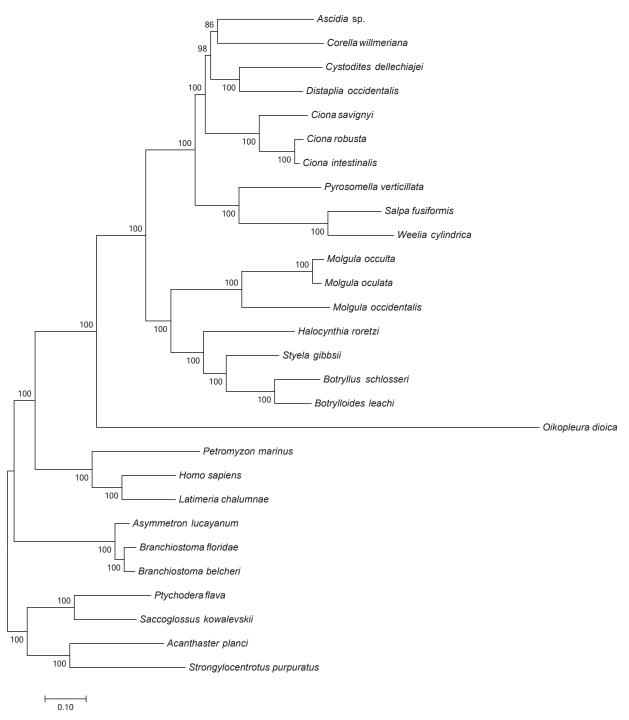


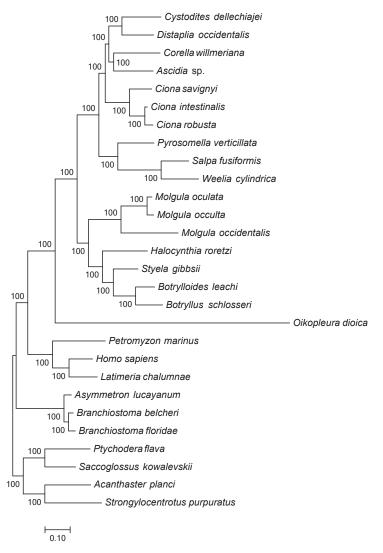


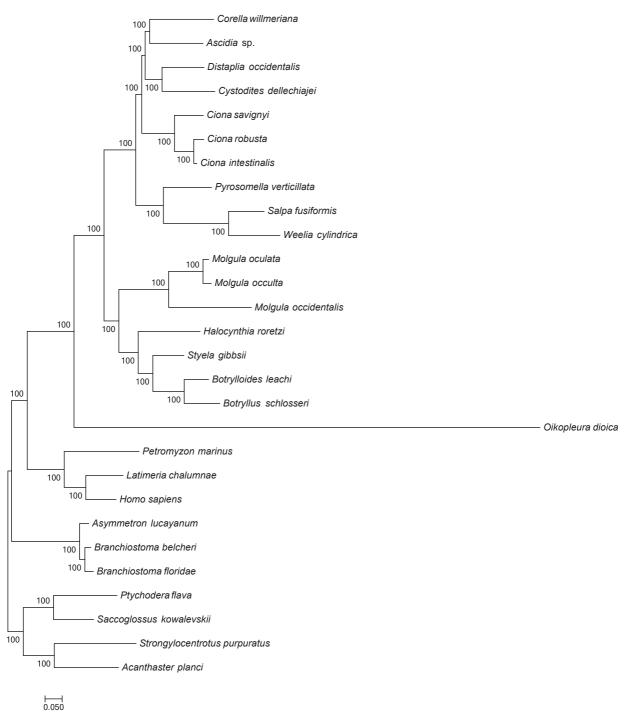


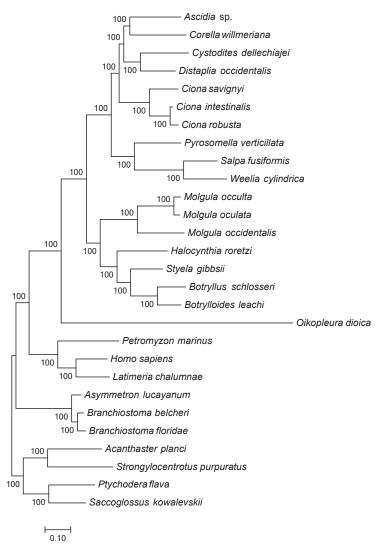


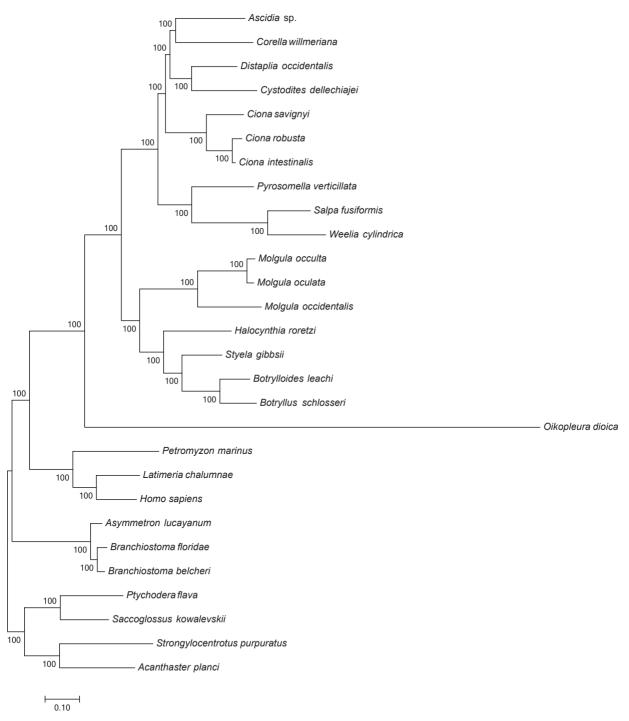


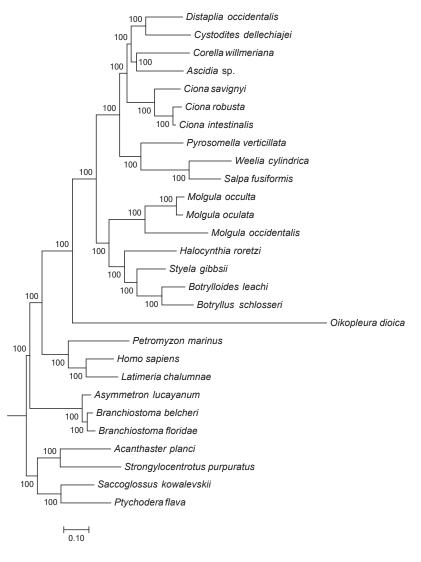


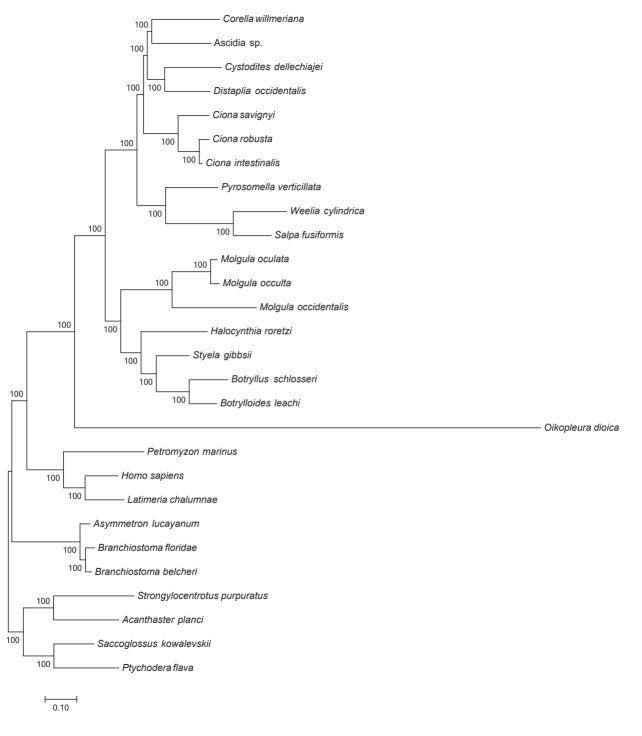


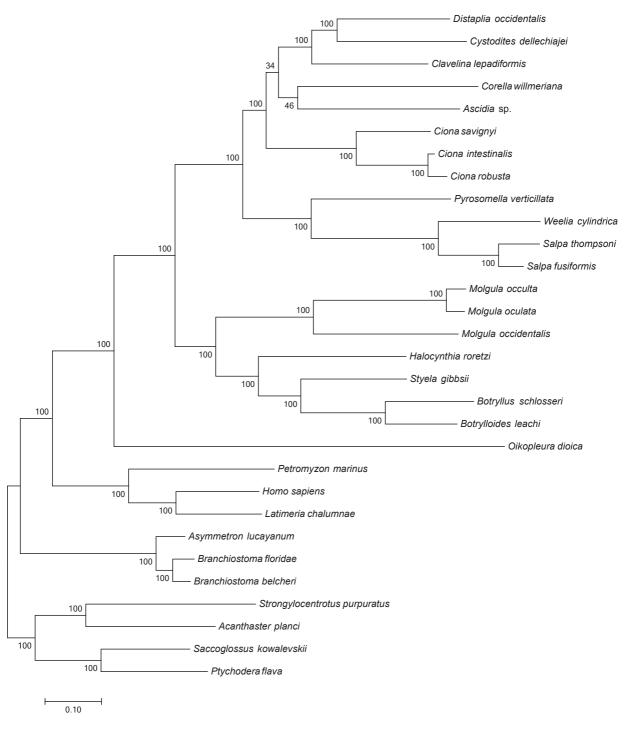


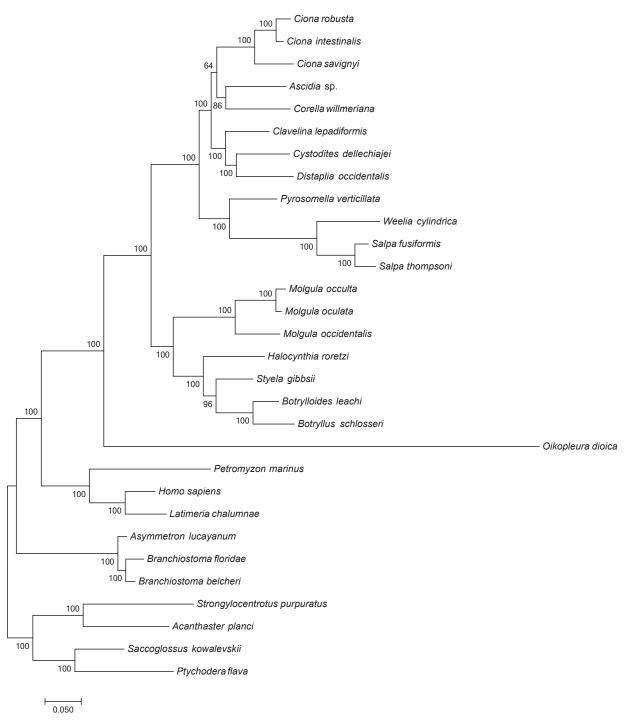


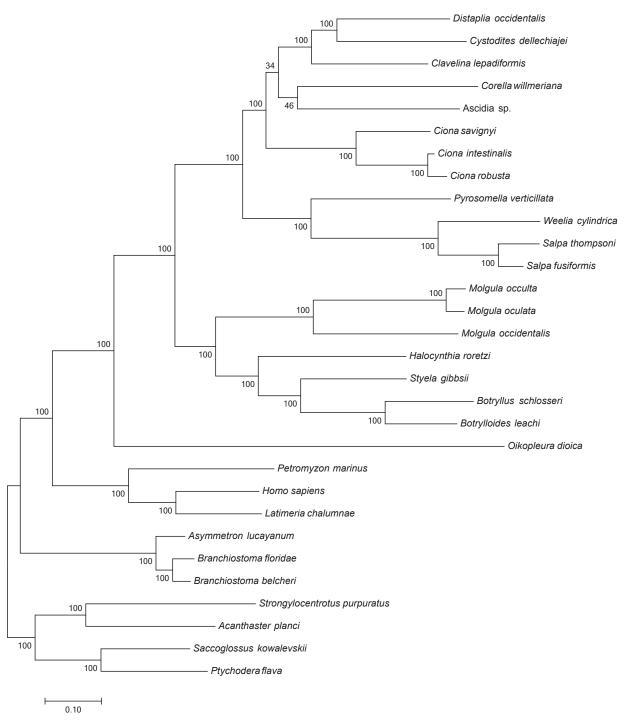


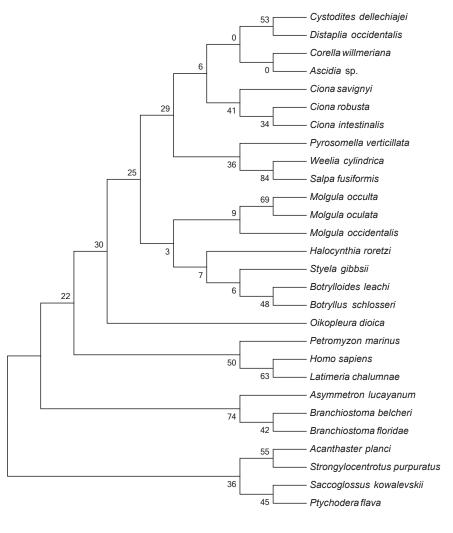


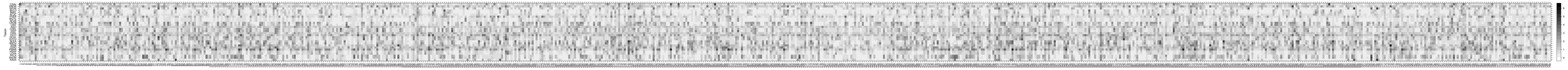


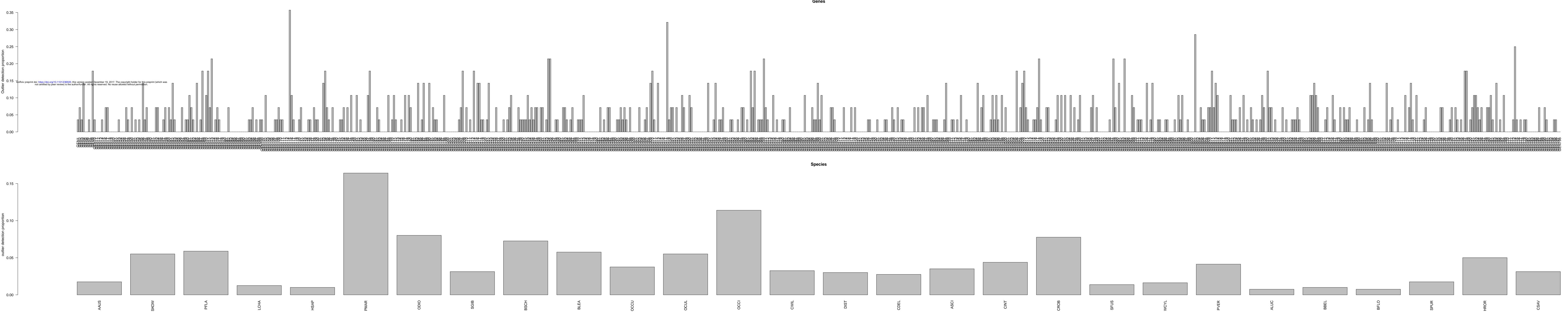












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PRE-AP cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 — KJ725153.1 Diplosoma simile isolate GAASC2 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 42 L KU221218.1 Diplosoma simile voucher UF 697 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221184.1 Diplosoma sp. 1 AO-2016 voucher ColBIO TL 729 cytochrome oxidase subunit I COI gene partial cds mitochondrial 48 99 F KU221228.1 Diplosoma sp. 3 AO-2016 voucher UF 680 cytochrome oxidase subunit I COI gene partial cds mitochondrial L KU221226.1 Diplosoma sp. 3 AO-2016 voucher UF 759 cytochrome oxidase subunit I COI gene partial cds mitochondrial 11 - KU221220.1 Diplosoma sp. 2 AO-2016 voucher UF 644 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KJ725166.1 Diplosoma spongiforme isolate GAASC15 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KF309624.1 Diplosoma spongiforme isolate DS-DL cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600972.1 Diplosoma spongiforme cytochrome c oxidase subunit I COI gene partial cds mitochondrial KX650775.1 Polyclinum indicum voucher DBTIC155 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650777.1 Polyclinum indicum voucher DBTIC157 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT345963.1 Polyclinum indicum voucher DBTIC48 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU220954.1 Polyclinum madrasensis voucher DBTIC15 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT276223.1 Polyclinum indicum voucher DBTIC27 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KP072781.1 Polyclinum indicum voucher DBTIC28 cytochrome oxidase subunit I COI gene partial cds mitochondrial 63 KX650778.1 Polyclinum indicum voucher DBTIC158 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650774.1 Polyclinum indicum voucher DBTIC154 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650780.1 Polyclinum indicum voucher DBTIC160 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650776.1 Polyclinum indicum voucher DBTIC156 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ944391.1 UNVERIFIED Polyclinum indicum voucher DBTIC 002 cytochrome oxidase subunit I-like COI gene partial sequence mitochondrial KX650786.1 Polyclinum indicum voucher DBTIC166 cytochrome oxidase subunit I COI gene partial cds mitochondrial · KX650785.1 Polyclinum indicum voucher DBTIC165 cytochrome oxidase subunit I COI gene partial cds mitochondrial 33 KX650781.1 Polyclinum indicum voucher DBTIC161 cytochrome oxidase subunit I COI gene partial cds mitochondrial — KX650783.1 Polyclinum indicum voucher DBTIC163 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX650784.1 Polyclinum indicum voucher DBTIC164 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650782.1 Polyclinum indicum voucher DBTIC162 cytochrome oxidase subunit I COI gene partial cds mitochondrial 89 F KU667271.1 Diplosoma sp. 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AO-2016 voucher ColBIO TL 538 cytochrome oxidase subunit I COI gene partial cds mitochondrial L KU221206.1 Trididemnum fetia voucher UF 629 cytochrome oxidase subunit I COI gene partial cds mitochondrial 32 KJ725158.1 Trididemnum cyanophorum isolate GAASC7 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 77` KJ632947.1 Trididemnum cyanophorum strain MH-JMC3 cytochrome c oxidase subunit I COI gene partial cds mitochondrial JF506187.1 Trididemnum cyanophorum clone 30May08-2-1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KU221183.1 Trididemnum maragogi voucher ColBIO TL 234 cytochrome oxidase subunit I COI gene partial cds mitochondrial JF506186.1 Trididemnum solidum clone 9Jun08-2-1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial ⁷⁹ KR604728.1 Trididemnum maragogi voucher ColBIO TL438 cytochrome oxidase subunit I COI gene partial cds mitochondrial ^r AY600982.1 Polycitor adriaticum cytochrome c oxidase subunit I COI gene partial cds mitochondrial 100 [|] AY523042.1 Cystodytes dellechiajei haplotype S1 cytochrome oxidase subunit I COI gene partial cds mitochondrial - AM709504.1 Polycitorella coronaria mitochondrial partial COI gene for cytochrome-c oxidase 100 | KR703628.1 Eudistoma malum voucher DBTIC 35 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU360793.1 Eudistoma malum isolate ASC08 cytochrome oxidase subunit I COI gene partial cds mitochondrial — KR703627.1 Eudistoma tumidum voucher DBTIC 33 cytochrome oxidase subunit I COI gene partial cds mitochondrial 99 100 KU360797.1 Eudistoma tumidum isolate ASC12 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR815818.1 Symplegma brakenhielmi voucher DBTIC 08 cytochrome oxidase subunit I COI gene partial cds mitochondrial 11 KJ944392.1 Eudistoma viride voucher DBTIC 003 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ944393.1 Eudistoma viride voucher DBTIC 004 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ710709.1 Eudistoma viride voucher ICBT005 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX650769.1 Eudistoma gilboviride voucher DBTIC149 cytochrome oxidase subunit I COI gene partial cds mitochondrial HG005369.1 Eudistoma elongatum mitochondrial partial COI gene for cytochrome oxidase subunit 1 99 EF619346.1 Eudistoma elongatum isolate 3Houhora cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 | AY600979.1 Pseudodistoma crucigaster cytochrome c oxidase subunit I COI gene partial cds mitochondrial AJ748708.1 Pseudodistoma crucigaster mitochondrial partial coi gene for cytochrome oxidase subunit 1 haplotype IV - AY600970.1 Pseudodistoma cyrnusense cytochrome c oxidase subunit I COI gene partial cds mitochondrial AY600977.1 Eudistoma planum cytochrome c oxidase subunit I COI gene partial cds mitochondrial 100 Lugardian JQ403421.1 Eudistoma viride cytochrome c oxidase subunit I COI gene partial cds mitochondrial AY600978.1 Eudistoma plumbeum cytochrome c oxidase subunit I COI gene partial cds mitochondrial 72 KY111422.1 Eudistoma capsulatum isolate 24Jun15
Ep1 Ep2 Ep4 Ep5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111423.1 Eudistoma capsulatum isolate 24Jun15 Ey3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111420.1 Eudistoma capsulatum isolate 24Jun15 Ey4 Ey5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111421.1 Eudistoma capsulatum isolate 22Jul14-1-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600974.1 Eudistoma posidoniarum cytochrome c oxidase subunit I COI gene partial cds mitochondrial 100 LAY600973.1 Eudistoma banyulensis cytochrome c oxidase subunit I COI gene partial cds mitochondrial KX650768.1 Eudistoma angolanum voucher DBTIC148 cytochrome oxidase subunit I COI gene partial cds mitochondrial KM411614.1 Eudistoma microlarvum voucher DBTIC18 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU360794.1 Eudistoma microlarvum isolate ASC09 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KU667266.1 Eudistoma microlarvum voucher DBTIC76 cytochrome oxidase subunit I COI gene partial cds mitochondrial 18 – KX138484.1 Eudistoma amplum voucher DBTIC111 cytochrome oxidase subunit I COI gene partial cds mitochondrial — KX138482.1 Eudistoma amplum voucher DBTIC109 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138480.1 Eudistoma amplum voucher DBTIC107 cytochrome oxidase subunit I COI gene partial cds mitochondrial 56 KU667265.1 Eudistoma amplum voucher DBTIC75 cytochrome oxidase subunit I COI gene partial cds mitochondrial 62 KM411609.1 Eudistoma amplum voucher DBTIC17 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX138483.1 Eudistoma amplum voucher DBTIC110 cytochrome oxidase subunit I COI gene partial cds mitochondrial — KX138481.1 Eudistoma amplum voucher DBTIC108 cytochrome oxidase subunit I COI gene partial cds mitochondrial 91 | KM411615.1 Eudistoma laysani voucher DBTIC19 cytochrome oxidase subunit I COI gene partial cds mitochondrial — KU667263.1 Eudistoma laysani voucher DBTIC73 cytochrome oxidase subunit I COI gene partial cds mitochondrial 59 KU667264.1 Eudistoma laysani voucher DBTIC74 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138476.1 Eudistoma ovatum voucher DBTIC103 cytochrome oxidase subunit I COI gene partial cds mitochondrial ── KX650771.1 Eudistoma ovatum voucher DBTIC151 cytochrome oxidase subunit I COI gene partial cds mitochondrial 60 L KU667259.1 Eudistoma ovatum voucher DBTIC69 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667260.1 Eudistoma ovatum voucher DBTIC70 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667262.1 Eudistoma ovatum voucher DBTIC72 cytochrome oxidase subunit I COI gene partial cds mitochondrial KM411610.1 Eudistoma ovatum voucher DBTIC20 cytochrome oxidase subunit I COI gene partial cds mitochondrial _{56 I} KU667261.1 Eudistoma ovatum voucher DBTIC71 cytochrome oxidase subunit I COI gene partial cds mitochondrial L KR867634.1 Eudistoma ovatum voucher DBTIC41 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU715280.1 Eudistoma ovatum voucher DBTIC96 cytochrome oxidase subunit I COI gene partial cds mitochondrial – KX138475.1 Eudistoma ovatum voucher DBTIC102 cytochrome oxidase subunit I COI gene partial cds mitochondrial 63 KX138477.1 Eudistoma ovatum voucher DBTIC104 cytochrome oxidase subunit I COI gene partial cds mitochondrial _{100 ⊏} KU697732.1 Morchellium argus isolate A50 cytochrome c oxidase subunit I COI gene partial cds mitochondrial ^l KF309621.1 Morchellium argus isolate DS-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KF309620.1 Morchellium argus isolate DS-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600971.1 Aplidium elegans cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309663.1 Aplidium cf. accarense TO-AP cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309654.1 Aplidium cf. accarense SIT-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309657.1 Aplidium cf. accarense TA-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309571.1 Aplidium cf. accarense ESC-6 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309558.1 Aplidium cf. accarense CA-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309655.1 Aplidium cf. accarense SIT-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309584.1 Aplidium cf. accarense FOR-AP cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309601.1 Aplidium cf. accarense MAT-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309599.1 Aplidium cf. accarense MAT-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309618.1 Aplidium cf. accarense PB-7 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309625.1 Aplidium cf. accarense POG-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309598.1 Aplidium cf. accarense MAS-AB cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309629.1 Aplidium cf. accarense POM-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309646.1 Aplidium cf. accarense SCR-6 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309656.1 Aplidium cf. accarense SIT-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309640.1 Aplidium cf. accarense SA-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309626.1 Aplidium cf. accarense POG-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309585.1 Aplidium cf. accarense GAR-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309597.1 Aplidium cf. accarense MAS-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309586.1 Aplidium cf. accarense GAR-AB cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309574.1 Aplidium cf. accarense ESC-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309553.1 Aplidium cf. accarense BLA-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309630.1 Aplidium cf. accarense POM-AB cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309627.1 Aplidium cf. accarense POG-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial - KJ725161.1 Synoicum castellatum isolate GAASC10 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KJ632946.1 Aplidium conicum strain MH-JMC2 cytochrome c oxidase subunit I COI gene partial cds mitochondrial AY600969.1 Aplidium conicum cytochrome c oxidase subunit I COI gene partial cds mitochondrial Aplidium conicum NC 013584.1 cds YP 003331401.1 9 KY111412.1 Aplidium stellatum isolate 21Jul14-3-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111414.1 Aplidium stellatum isolate 22Jul14-1-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111413.1 Aplidium stellatum isolate 21Jul14-3-5 Grey cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111411.1 Aplidium stellatum isolate 17Jul14-1-3 White cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600967.1 Aplidium pseudolobatum cytochrome c oxidase subunit I COI gene partial cds mitochondrial KJ725160.1 Aplidium fuscum isolate GAASC9 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 100 AY600975.1 Aplidium fuscum cytochrome c oxidase subunit I COI gene partial cds mitochondrial 10 AY600983.1 Rophalaea neapolitana cytochrome c oxidase subunit I COI gene partial cds mitochondrial AM706464.1 Clavelina australis coi gene for cytochrome c oxidase AM706465.1 Clavelina breve coi gene for cytochrome c oxidasehaplotype Cbv1 KF309535.1 Clavelina sp. AM-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309645.1 Clavelina sp. SCR-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309638.1 Clavelina lepadiformis isolate ROS-CLY cytochrome oxidase subunit I COI gene partial cds mitochondrial AJ884573.1 Clavelina gemmae mitochondrial partial COI gene for cytochrome oxidase 1 AJ866716.1 Clavelina gemmae mitochondrial partial coi gene for cytochrome oxidase subunit 1 Clavelina lepadiformis FJ839918.1 cds ACO40300.1 1 AY603104.1 Clavelina lepadiformis cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309563.1 Clavelina lepadiformis isolate CF-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial – AM706468.1 Clavelina meridionalis coi gene for cytochrome c oxidasehaplotype Cme1 AY603105.1 Clavelina delavallei cytochrome c oxidase subunit I COI gene partial cds mitochondrial JN703738.1 Clavelina oblonga haplotype 1 cytochrome c oxidase subunit I gene partial cds mitochondrial Clavelina phlegraea NC 024105.1 cds YP 009029840.1 4 JN703739.1 Clavelina oblonga haplotype 2 cytochrome c oxidase subunit I gene partial cds mitochondrial KJ725165.1 Clavelina oblonga isolate GAASC14 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KY111417.1 Clavelina oblonga isolate 22Jul14-1-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial 29 AY603106.1 Clavelina oblonga cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309648.1 Clavelina oblonga isolate SCR-CO cytochrome oxidase subunit I COI gene partial cds mitochondrial - JN859182.1 Clavelina oblonga isolate FL2 cytochrome c oxidase subunit I gene partial cds mitochondrial 59 AM706472.1 Clavelina moluccensis coi gene for cytochrome c oxidase JN703740.1 Clavelina picta cytochrome c oxidase subunit I gene partial cds mitochondrial AM706489.1 Nephtheis fascicularis coi gene for cytochrome c oxidase AM706462.1 Clavelina arafurensis coi gene for cytochrome c oxidase haplotype Car1 AM403684.1 Pycnoclavella atlantica mitochondrial coi gene for cytochrome c oxidase subunit I haplotype H1 AM706474.1 Pycnoclavella detorta coi gene for cytochrome c oxidase haplotype Pde2 AM706473.1 Pycnoclavella detorta coi gene for
cytochrome c oxidase haplotype Pde1 AM706484.1 Pycnoclavella tabella coi gene for cytochrome c oxidase haplotype Ptb1 AM706479.1 Pycnoclavella martae coi gene for cytochrome c oxidase haplotype Pma1 AM706481.1 Pycnoclavella martae coi gene for cytochrome c oxidase haplotype Pma3 - AM706480.1 Pycnoclavella martae coi gene for cytochrome c oxidase haplotype Pma2 KJ725156.1 Pycnoclavella diminuta isolate GAASC5 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KJ632945.1 Pycnoclavella diminuta strain MH-JMC1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial AM746343.1 Pycnoclavella communis mitochondrial partial coi gene for cytochrome c oxidase subunit I haplotype H1 29 AM403696.1 Pycnoclavella nana mitochondrial coi gene for cytochrome c oxidase subunit I haplotype H26 AM403690.1 Pycnoclavella aurilucens mitochondrial coi gene for cytochrome c oxidase subunit I haplotype H10 AY600988.1 Pycnoclavella sp. SLL-2004 cytochrome c oxidase subunit I COI gene partial cds mitochondrial AM706475.1 Clavelina flava coi gene for cytochrome c oxidase haplotype Pfl1 AM403686.1 Pycnoclavella brava mitochondrial coi gene for cytochrome c oxidase subunit I haplotype H2 AM403703.1 Pycnoclavella sp. RPP-2006 mitochondrial coi gene for cytochrome c oxidase subunit I haplotype H6 - AM706483.1 Clavelina producta coi gene for cytochrome c oxidase haplotype Ppd2 AM706482.1 Clavelina producta coi gene for cytochrome c oxidase haplotype Ppd1 ^l AY600966.1 Archidistoma aggregatum cytochrome c oxidase subunit I COI gene partial cds mitochondrial Distaplia occidentalis TRINITY DN13342 c0 g1 i1 · KU221227.1 Distaplia sp. AO-2016 voucher TL 02 cytochrome oxidase subunit I COI gene partial cds mitochondrial 57 - KY111419.1 Distaplia bermudensis isolate 1Mar16 3B-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 [KU221217.1 Polysyncraton poro voucher UF 664 cytochrome oxidase subunit I COI gene partial cds mitochondrial Light KU221222.1 Polysyncraton poro voucher UF 682 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600986.1 Polysyncraton lacazei cytochrome c oxidase subunit I COI gene partial cds mitochondrial 18 84 r KU221187.1 Polysyncraton sp. AO-2016 voucher ColBIO TL 432 cytochrome oxidase subunit I COI gene partial cds mitochondrial 79 99 KU221186.1 Polysyncraton sp. AO-2016 voucher ColBIO TL 810 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU906100.1 Molgula manhattensis voucher SERCINVERT0184 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KU905891.1 Molgula manhattensis voucher SERCINVERT0432 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KU905755.1 Molgula manhattensis voucher SERCINVERT0185 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial JQ742950.1 Molgula manhattensis clone SY011703 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial JQ742955.1 UNVERIFIED Molgula manhattensis clone SY011708 cytochrome c oxidase subunit 1-like COI gene partial sequence mitochondrial KY111424.1 Molgula manhattensis isolate 19Jul14-1-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 HM574376.1 Molgula provisionalis cytochrome oxidase subunit I COI gene partial cds mitochondrial HM574345.1 Molgula manhattensis haplotype 1 cytochrome oxidase subunit I COI gene partial cds mitochondrial HM574378.1 Molgula socialis haplotype HB cytochrome oxidase subunit I COI gene partial cds mitochondrial HM574379.1 Molgula socialis haplotype HC cytochrome oxidase subunit I COI gene partial cds mitochondrial HM574377.1 Molqula socialis haplotype HA cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111418.1 Didemnum lutarium isolate 17Jul14-1-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial 42 KU221202.1 Didemnum ligulum voucher UF 1387 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 KU221198.1 Didemnum ligulum voucher UF 613 cytochrome oxidase subunit I COI gene partial cds mitochondrial ^l KU221200.1 Didemnum ligulum voucher UF 694 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 KU221210.1 Didemnum ligulum voucher ColBIO TL796 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KJ725159.1 Didemnum albidum isolate GAASC8 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KJ725155.1 Didemnum granulatum isolate GAASC4 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 79 JQ013198.1 Didemnum granulatum strain Sri2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KP779901.1 Didemnum granulatum cytochrome oxidase subunit I COI gene partial cds mitochondrial KR612209.1 Didemnum vexillum isolate Fairlie11 cytochrome c oxidase Col gene partial cds mitochondrial Didemnum vexillum NC 026107.1 cds YP 009114980.1 11 KU221189.1 Didemnum psammatodes voucher ColBIO TL 27 cytochrome oxidase subunit I COI gene partial cds mitochondrial JN624758.1 Didemnum psammatode strain sri1 cytochrome C oxidase subunit I COI gene partial cds mitochondrial 66 KP779902.1 Didemnum psammatodes cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667268.1 Didemnum spadix voucher DBTIC78 cytochrome oxidase subunit I COI gene partial cds mitochondrial ∮9 KU667267.1 Didemnum spadix voucher DBTIC77 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR604727.1 Polysyncraton maurizeliae voucher ColBIO TL61 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 | KU221211.1 Didemnum apuroto voucher UF 813 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221212.1 Didemnum apuroto voucher UF 814 cytochrome oxidase subunit I COI gene partial cds mitochondrial 21 - KU221192.1 Didemnum cineraceum voucher ColBIO TL 839 cytochrome oxidase subunit I COI gene partial cds mitochondrial 13 91 KU221193.1 Didemnum cineraceum voucher ColBIO TL 230 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309573.1 Didemnum sp. ESC-10 cytochrome oxidase subunit I COI gene partial cds mitochondrial 3 LKF309622.1 Didemnum sp. DS-4 cytochrome oxidase subunit ICOI gene partial cds mitochondrial - KM411613.1 Didemnum sp. JAHA-2014 voucher DBTIC25 cytochrome oxidase subunit I COI gene partial cds mitochondrial 87 L KU667269.1 Didemnum sp. JAHA-2014 voucher DBTIC79 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221201.1 Didemnum fragile voucher UF 839 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221209.1 Didemnum fragile voucher UF 746 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221207.1 Didemnum mutabile voucher UF 689 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 LKU221223.1 Didemnum mutabile voucher UF 691 cytochrome oxidase subunit LCOI gene partial cds mitochondrial 44 KU221208.1 Didemnum sordidum voucher UF 734 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221195.1 Didemnum sordidum voucher UF 597 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221197.1 Didemnum sordidum voucher UF 612 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309576.1 Didemnum fulgens isolate ESC-DF cytochrome oxidase subunit I COI gene partial cds mitochondrial 🕆 🖟 NJ725152.1 Didemnum fulgens isolate GAASC1 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KU221199.1 Didemnum cuculliferum voucher UF 676 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221219.1 Didemnum cuculliferum voucher UF 724 cytochrome oxidase subunit I COI gene partial cds mitochondrial 98 | KU221191.1 Didemnum granulatum voucher ColBIO TL 686 cytochrome oxidase subunit I COI gene partial cds mitochondrial ⁶⁵∫ KU221190.1 Didemnum granulatum voucher ColBIO TL 671 cytochrome oxidase subunit I COI gene partial cds mitochondrial - JQ780668.1 Didemnum granulatum haplotype HAP01 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221196.1 Didemnum granulatum voucher UF 573 cytochrome oxidase subunit I COI gene partial cds mitochondrial JQ731741.1 Didemnum sp. BAOR-2012 haplotype 11 cytochrome oxidase subunit I COI gene partial cds mitochondrial JQ731742.1 Didemnum sp. BAOR-2012 haplotype 12 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221225.1 Didemnum membranaceum voucher UF 720 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221216.1 Didemnum membranaceum voucher UF 526 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221213.1 Didemnum membranaceum voucher UF 1389 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221215.1 Didemnum granulatum voucher UF 1408 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU221204.1 Didemnum granulatum voucher UF 601 cytochrome oxidase subunit I COI gene partial cds mitochondrial JQ692626.1 Didemnum incanum haplotype 8 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ725167.1 Didemnum sp. GA-2014 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial - JQ731736.1 Didemnum patulum haplotype 1 cytochrome oxidase subunit I COI gene partial cds mitochondrial · KX641024.1 Didemnum sp. IEAPMC1237 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138486.1 Didemnum perlucidum voucher DBTIC113 cytochrome oxidase subunit I COI gene partial cds mitochondrial _{0 I} KX138487.1 Didemnum perlucidum voucher DBTIC114 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138490.1 Didemnum perlucidum voucher DBTIC117 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667270.1 Didemnum perlucidum voucher DBTIC80 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR537439.1 Didemnum perlucidum voucher DBTIC 30 cytochrome oxidase subunit I COI gene partial cds mitochondrial 51 KR303626.1 Didemnum perlucidum voucher DBTIC 29 cytochrome oxidase subunit I COI gene partial cds mitochondrial JQ731735.1 Didemnum perlucidum isolate 2 cytochrome oxidase subunit I
COI gene partial cds mitochondrial KU883151.1 Didemnum perlucidum isolate JD781 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX650790.1 Didemnum perlucidum voucher DBTIC170 cytochrome oxidase subunit I COI gene partial cds mitochondrial 98 L KX650791.1 Didemnum perlucidum voucher DBTIC171 cytochrome oxidase subunit I COI gene partial cds mitochondrial AB104866.1 Megalodicopia hians COI for cytochrome c oxidase subunit 1 partial cds 54 ┌ - AB104867.1 Chelyosoma siboja COI for cytochrome c oxidase subunit I partial cds □ Corella willmeriana|TRINITY DN3184 c0 g1 i1 99 LEU140801.1 Corella eumyota haplotype H1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial 91 — KX138509.1 Ecteinascidia thurstoni voucher DBTIC134 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138508.1 Ecteinascidia venui voucher DBTIC133 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693190.1 Ecteinascidia venui voucher DBTIC50 cytochrome oxidase subunit I COI gene partial cds mitochondrial · EF643374.1 Ecteinascidia turbinata cytochrome c oxidase subunit I COI gene partial cds mitochondrial KY111425.1 Perophora viridis isolate 22Jul14-3-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial 95 JQ863390.1 Perophora japonica haplotype H 1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial 95 AY600968.1 Ecteinascidia herdmanni cytochrome c oxidase subunit I COI gene partial cds mitochondrial KU667277.1 Perophora multiclathrata voucher DBTIC88 cytochrome oxidase subunit I COI gene partial cds mitochondrial 88 - KU667278.1 Perophora multiclathrata voucher DBTIC89 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693196.1 Perophora multiclathrata voucher DBTIC56 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT276225.1 Perophora multiclathrata voucher DBTIC45 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KU360801.1 Perophora multiclathrata isolate ASC16 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667276.1 Perophora multiclathrata voucher DBTIC87 cytochrome oxidase subunit I COI gene partial cds mitochondrial └ KT693197.1 Perophora multiclathrata voucher DBTIC 57 cytochrome oxidase subunit I COI gene partial cds mitochondrial Ciona intestinalis NC 004447.2 cds NP 758778.1 12 KF309570.1 Ciona robusta isolate ESC-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309580.1 Ciona robusta isolate EST-CI cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309554.1 Ciona robusta isolate BLA-CI cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309587.1 Ciona robusta isolate HI-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309575.1 Ciona robusta isolate ESC-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309578.1 Ciona robusta isolate EST-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309532.1 Ciona robusta isolate AM-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309651.1 Ciona robusta isolate SFG-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial 87 KF309591.1 Ciona robusta isolate LLAN-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309662.1 Ciona robusta isolate TO-AA cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309658.1 Ciona robusta isolate TA-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309613.1 Ciona robusta isolate MP-CIT cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309628.1 Ciona robusta isolate POG-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309604.1 Ciona robusta isolate MAT-CI cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309614.1 Ciona robusta isolate PB-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309593.1 Ciona robusta isolate LLAN-CI cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309603.1 Ciona robusta isolate MAT-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309602.1 Ciona robusta isolate MAT-AP cytochrome oxidase subunit I COI gene partial cds mitochondrial Ciona robusta NC 034372.1 cds YP 009357192.1 12 Ciona intestinalis NC 017929.1 cds YP 006341036.1 12 KF309636.1 Ciona sp. ROS-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial l KF597196.1 Ciona savignyi isolate KCl00031 cytochrome c oxidase subunit I COI gene partial cds mitochondrial 100 Ciona savignyi NC 004570.1 cds NP 786952.1 1 KF414706.1 Phallusia arabica isolate ASP9 cytochrome c oxidase subunit I COI gene partial cds mitochondrial Phallusia mammillata NC 009833.1 cds YP 001481141.1 12 AY600980.1 Phallusia mammillata cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309607.1 Phallusia mammillata isolate MP-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KP779903.1 Phallusia arabica cytochrome oxidase subunit I COI gene partial cds mitochondrial KP779905.1 Phallusia mammillata cytochrome oxidase subunit I COI gene partial cds mitochondrial KP779904.1 Phallusia fumigata cytochrome oxidase subunit I COI gene partial cds mitochondrial 10 KF309548.1 Phallusia fumigata isolate AR-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF414698.1 Phallusia mammillata isolate ASP1 cytochrome c oxidase subunit I COI gene partial cds mitochondrial Phallusia fumigata NC 009834.1 cds YP 001481152.1 10 40 - Ascidia sp|TRINITY DN8074 c1 g1 i1 KX650762.1 Phallusia nigra voucher DBTIC142 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600976.1 Phallusia ingeria cytochrome c oxidase subunit I COI gene partial cds mitochondrial 961 KY111416.1 Ascidia interrupta isolate 21Jul14-2-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111415.1 Ascidia interrupta isolate 17Jul14-1-10 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR604726.1 Ascidia viridina voucher ColBIO TL524 cytochrome oxidase subunit I COI gene partial cds mitochondrial 12 KJ725162.1 Ascidia virginea isolate GAASC11 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 93 KF309647.1 Ascidia virginea isolate SCR-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial 46 KX650763.1 Ascidia gemmata voucher DBTIC143 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309529.1 Ascidiella scabra isolate AB-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309556.1 Ascidiella scabra isolate BU-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309572.1 Ascidiella scabra isolate ESC-9 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309560.1 Ascidiella scabra isolate CF-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309650.1 Ascidiella scabra isolate SFG-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ725163.1 Ascidiella aspersa isolate GAASC12 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial JQ742948.1 Ascidiella aspersa clone SY011701 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KF309559.1 Ascidiella aspersa isolate CA-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309631.1 Ascidiella aspersa isolate POM-SC cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309653.1 Ascidiella aspersa isolate SFG-SC cytochrome oxidase subunit I COI gene partial cds mitochondrial KU695283.1 Ascidiella aspersa isolate 91 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309594.1 Ascidiella aspersa isolate LLAN-SC cytochrome oxidase subunit I COI gene partial cds mitochondrial KF886702.1 Ascidiella aspersa isolate DNAS-54-45089 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309555.1 Ascidiella aspersa isolate BLA-SC cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309534.1 Ascidiella aspersa isolate AM-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309568.1 Ascidiella aspersa isolate EMB-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309533.1 Ascidiella aspersa isolate AM-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309562.1 Ascidiella aspersa isolate CF-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309661.1 Ascidiella aspersa isolate TO-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309606.1 Ascidiella aspersa isolate MAT-SC cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309637.1 Ascidiella aspersa isolate ROS-AS cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309617.1 Ascidiella aspersa isolate PB-6 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528625.1 Pyura squamulosa isolate 60 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528614.1 Pyura gibbosa isolate 20 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528613.1 Pyura gibbosa isolate 6 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528615.1 Pyura gibbosa isolate 21 cytochrome oxidase subunit I COI gene partial cds mitochondrial 78 FJ528617.1 Pyura australis isolate 22 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528616.1 Pyura australis isolate 13 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528612.1 Pyura spinifera isolate 23 cytochrome oxidase subunit I COI gene partial cds mitochondrial 99 FJ528611.1 Pyura spinifera isolate 4 cytochrome oxidase subunit I COI gene partial cds mitochondrial DQ658850.1 Pyura praeputialis isolate cyp6 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528624.1 Pyura praeputialis isolate 64 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528621.1 Pyura praeputialis isolate 55 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528623.1 Pyura praeputialis isolate 58 cytochrome oxidase
subunit I COI gene partial cds mitochondrial FJ528622.1 Pyura praeputialis isolate 57 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528620.1 Pyura praeputialis isolate 14 cytochrome oxidase subunit I COI gene partial cds mitochondrial GU065362.1 Boltenia echinata from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065361.1 Boltenia echinata from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065360.1 Boltenia echinata from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial 89 FJ528628.1 Boltenia ovifera isolate 18 cytochrome oxidase subunit I COI gene partial cds mitochondrial 5971 FJ528627.1 Boltenia ovifera isolate 16 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 | KF309582.1 Styelidae EST-MI cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309588.1 Polycarpa sp. HI-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309596.1 Pyura dura isolate MAS-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial 69 FJ528619.1 Pyura dura isolate 25 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528618.1 Pyura dura isolate 24 cytochrome oxidase subunit I COI gene partial cds mitochondrial GU220387.1 Botrylloides violaceus haplotype Bv6 cytochrome oxidase subunit I COI gene partial cds mitochondrial GQ365694.1 Botrylloides violaceus isolate Bv4 cytochrome oxidase subunit I COI gene partial cds mitochondrial GQ365695.1 Botrylloides violaceus isolate Bv5 cytochrome oxidase subunit I COI gene partial cds mitochondrial GQ365693.1 Botrylloides violaceus isolate Bv3 cytochrome oxidase subunit I COI gene partial cds mitochondrial GQ365691.1 Botrylloides violaceus isolate Bv1 cytochrome oxidase subunit I COI gene partial cds mitochondrial GU220388.1 Botrylloides violaceus haplotype Bv7 cytochrome oxidase subunit I COI gene partial cds mitochondrial GQ365692.1 Botrylloides violaceus isolate Bv2 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528644.2 Botrylloides violaceum isolate 67 cytochrome oxidase subunit I COI gene partial cds mitochondrial 33 GU065359.1 Botrylloides violaceus isolate 4 from USA cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065356.1 Botrylloides violaceus isolate 30 from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065357.1 Botrylloides violaceus isolate 6 from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065355.1 Botrylloides violaceus isolate 27 from Canada cytochrome c oxidase subunit I COI gene partial cds mitochondrial GU065358.1 Botrylloides violaceus isolate 3 from USA cytochrome c oxidase subunit I COI gene partial cds mitochondrial GQ365690.1 Botrylloides fuscus cytochrome oxidase subunit I COI gene partial cds mitochondrial - KT693201.1 Botrylloides nigrum voucher DBTIC61 cytochrome oxidase subunit I COI gene partial cds mitochondrial Botrylloides nigrum NC 021467.1 cds YP 008083006.1 8 $_{ extsf{ iny KT693200.1}}$ Botrylloides nigrum voucher DBTIC60 cytochrome oxidase subunit I COI gene partial cds mitochondrial 85 L KT693198.1 Botrylloides nigrum voucher DBTIC58 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309551.1 Botrylloides leachii isolate AR-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309549.1 Botrylloides leachii isolate AR-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309641.1 Botrylloides leachii isolate SCR-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309608.1 Botrylloides leachii isolate MP-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU711782.1 Botrylloides nigrum isolate IRAR2 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309610.1 Botrylloides leachii isolate MP-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KP254541.1 Botrylloides nigrum voucher FTP 0791 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KF309642.1 Botrylloides leachii isolate SCR-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309611.1 Botrylloides leachii isolate MP-6 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528645.1 Botrylloides leachi isolate 53 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309609.1 Botrylloides leachii isolate MP-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309644.1 Botrylloides leachii isolate SCR-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial Botrylloides leachii NC 024103.1 cds YP 009029811.1 1 20 LT908007.1 Botryllus schlosseri mitochondrial partial COI gene for cytochrome oxidase subunit 1 specimen voucher MSNVE-24197 isolate NeoA 100 | KX650765.1 Botrylloides chevalense voucher DBTIC145 cytochrome oxidase subunit I COI gene partial cds mitochondrial └ KX650764.1 Botrylloides chevalense voucher DBTIC144 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT276224.1 Symplegma brakenhielmi voucher DBTIC38 cytochrome c oxidase subunit I COI gene partial cds mitochondrial FJ528648.1 Symplegma rubra isolate 74 cytochrome oxidase subunit I COI gene partial cds mitochondrial · KU360802.1 Symplegma brakenhielmi isolate ASC17 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528651.1 Stolonica socialis isolate 69 cytochrome oxidase subunit I COI gene partial cds mitochondrial 36 - KX138510.1 Symplegma brakenhielmi voucher DBTIC136 cytochrome oxidase subunit I COI gene partial cds mitochondrial HF922625.1 Botrylloides giganteum mitochondrial partial COI gene for cytochrome oxidase subunit 1 isolate PE Botrylloides pizoni NC 024104.1 cds YP 009029824.1 1 · KF309623.1 Distomus variolosus isolate DS-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528652.1 Distomus variolosus isolate 84 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528653.1 Distomus variolosus isolate 85 cytochrome oxidase subunit I COI gene partial cds mitochondrial _l FJ528650.1 Dendrodoa grossularia isolate 79 cytochrome oxidase subunit I COI gene partial cds mitochondrial - FJ528649.1 Dendrodoa grossularia isolate 78 cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 г KY111429.1 Polyandrocarpa zorritensis isolate 17Jul14-2-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309643.1 Polyandrocarpa zorritensis isolate SCR-3 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528646.1 Polycarpa aurata isolate 40 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309583.1 Styelidae FOR-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309577.1 Styelidae EST-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial KJ725157.1 Styelidae sp. GA-2014 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial AY600984.1 Polycarpa pomaria cytochrome c oxidase subunit I COI gene partial cds mitochondrial KF309569.1 Styelidae ESC-1 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528655.1 Polycarpa tenera isolate 90 cytochrome oxidase subunit I COI gene partial cds mitochondrial - FJ528647.1 Polyzoa opuntia isolate 73 cytochrome oxidase subunit I COI gene partial cds mitochondrial r KY111427.1 Polyandrocarpa anguinea isolate 17Jul14-3-4 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111426.1 Polyandrocarpa anguinea isolate 17Jul14-1-1B cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111428.1 Polyandrocarpa anguinea isolate 22Jul14-1-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial 551 KU697729.1 Microcosmus squamiger isolate Ll3I cytochrome c oxidase subunit I COI gene partial cds mitochondrial - KX650804.1 Microcosmus helleri voucher DBTIC184 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF414709.1 Microcosmus sp. ASP-2013 cytochrome c oxidase subunit I COI gene partial cds mitochondrial - KX650803.1 Microcosmus helleri voucher DBTIC183 cytochrome oxidase subunit I COI gene partial cds mitochondrial | KX138507.1 Microcosmus exasperatus voucher DBTIC132 cytochrome oxidase subunit I COI gene partial cds mitochondrial 53 100 KX138506.1 Microcosmus exasperatus voucher DBTIC131 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX650767.1 Cnemidocarpa sp. AHJ-2016 voucher DBTIC147 cytochrome oxidase subunit I COI gene partial cds mitochondrial 60 13 – KX138513.1 Cnemidocarpa areolata voucher DBTIC137 cytochrome oxidase subunit I COI gene partial cds mitochondrial 75 39 KU667275.1 Cnemidocarpa sp. n. JAHA-2016 voucher DBTIC86 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528605.1 Microcosmus claudicans isolate 59 cytochrome oxidase subunit I COI gene partial cds mitochondrial - Microcosmus sulcatus NC 013752.1 cds YP 003406728.1 13 – KF297502.1 Halocynthia papillosa haplotype HP Hap 1 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial S54796.1 cytochrome oxidase subunit I Halocynthia roretzi ascidian Mitochondrial 1263 nt Halocynthia roretzi NC 002177.1 cds NP 038239.1 1 FJ528610.1 Halocynthia pyriformis isolate 30 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528607.1 Halocynthia papillosa isolate 76 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528606.1 Halocynthia papillosa isolate 75 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528608.1 Halocynthia papillosa isolate 77 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528609.1 Halocynthia papillosa isolate 62 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600981.1 Halocynthia papillosa cytochrome c oxidase subunit I COI gene partial cds mitochondrial 86 | JX312279.1 Asterocarpa humilis haplotype H2 cytochrome c oxidase subunit I COI gene partial cds mitochondrial JX312278.1 Asterocarpa humilis haplotype H1 cytochrome c oxidase subunit I COI gene partial cds
mitochondrial FJ528634.1 Styela plicata isolate 34 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528633.1 Styela plicata isolate 32 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528626.1 Boltenia ovifera isolate 1 cytochrome oxidase subunit I COI gene partial cds mitochondrial – FJ528640.1 Styela sp. RPP-2008 isolate 91 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528639.1 Styela sp. RPP-2008 isolate 51 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528604.1 Microcosmus polymorphus isolate 54 cytochrome oxidase subunit I COI gene partial cds mitochondrial Herdmania momus NC 013561.1 cds YP 003331094.1 1 HM490289.1 Herdmania momus haplotype 1 cytochrome oxidase subunit I COI gene partial cds mitochondrial 94 | FJ528629.1 Herdmania grandis isolate 26 cytochrome oxidase subunit I COI gene partial cds mitochondrial 94 FJ528630.1 Herdmania grandis isolate 27 cytochrome oxidase subunit I COI gene partial cds mitochondrial r KU667272.1 Herdmania momus voucher DBTIC82 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR867633.1 Herdmania momus voucher DBTIC42 cytochrome oxidase subunit I COI gene partial cds mitochondrial 26 67 KM411616.1 Herdmania momus voucher DBTIC22 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX138500.1 Styela canopus voucher DBTIC127 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309557.1 Styelidae CA-2 cytochrome oxidase subunit I COI gene partial cds mitochondrial - KX138501.1 Styela canopus voucher DBTIC128 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309589.1 Styelidae HI-5 cytochrome oxidase subunit I COI gene partial cds mitochondrial KF309590.1 Styela canopus isolate HI-6 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693195.1 Styela canopus voucher DBTIC55 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX138498.1 Styela canopus voucher DBTIC125 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693194.1 Microcosmus curvus voucher DBTIC54 cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528637.1 Styela gibbsii isolate 47 cytochrome oxidase subunit I COI gene partial cds mitochondrial Styela gibbsii|TRINITY DN32987 c0 g1 i1 ^l HQ916447.1 Styela gibbsii cytochrome oxidase subunit I COI gene partial cds mitochondrial FJ528638.1 Styela montereyensis isolate 48 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU905887.1 Styela canopus voucher SERCINVERT0909 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KU714784.1 Styela clava isolate L3C3 cytochrome c oxidase subunit I COI gene partial cds mitochondrial · KU714785.1 Styela plicata isolate G3B5 cytochrome c oxidase subunit I COI gene partial cds mitochondrial FJ528631.1 Styela plicata isolate 10 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111432.1 Styela plicata isolate BIO585 S11 cytochrome oxidase subunit I COI gene partial cds mitochondrial KY111430 1 Styela plicata isplate BIO585 S2 cytochrome oxidase subunit I COI gene partial cds mitochondrial bioRxiv preprint doi: https://doi.org/10.1 not certified by peer re meaunorrunder au rights reserved. No reuse allowed without permission 585 S6 cytochrome oxidase subunit I COI gene partial cds mitochondrial AY600985.1 Styela plicata cytochrome c oxidase subunit I COI gene partial cds mitochondrial JQ742957.1 Styela plicata clone SY011710 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial FJ528632.1 Styela plicata isolate 31 cytochrome oxidase subunit I COI gene partial cds mitochondrial Styela plicata NC 013565.1 cds YP 003331150.1 5 AB104868.1 Styela plicata COI for cytochrome c oxidase subunit I partial cds HQ916425.1 Styela plicata haplotype 1 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT988335.1 Styela plicata cytochrome oxidase subunit I COI gene partial cds mitochondrial ┌ KX871693.1 Styela plicata isolate B1 20 jgLCO-1490 cytochrome oxidase subunit I COI gene partial cds mitochondrial ─ KX871692.1 Styela plicata isolate B5 Asc1 jgLCO-1490 cytochrome oxidase subunit I COI gene partial cds mitochondrial KX871694.1 Styela plicata isolate B5 Asc4 jgLCO-1490 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693192.1 Eudistoma sp. AHJ-2015 voucher DBTIC52 cytochrome oxidase subunit I COI gene partial cds mitochondrial KT693193.1 Eudistoma sp. AHJ-2015 voucher DBTIC53 cytochrome oxidase subunit I COI gene partial cds mitochondrial KR030569.1 Macropsis tunicata voucher CNC*HEM305577 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial - KU667285.1 Ascidia sydneiensis voucher DBTIC85 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667273.1 Ascidia sydneiensis voucher DBTIC83 cytochrome oxidase subunit I COI gene partial cds mitochondrial KU667274.1 Ascidia sydneiensis voucher DBTIC84 cytochrome oxidase subunit I COI gene partial cds mitochondrial 33 ^I KR815822.1 Ascidia sydneiensis voucher DBTIC 13 cytochrome oxidase subunit I COI gene partial cds mitochondrial 52 KC974532.1 Amauronematus tunicatus voucher DEIGISHym18102 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KC976922.1 Amauronematus tunicatus voucher DEIGISHym18903 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KC974460.1 Amauronematus tunicatus voucher DEIGISHym18905 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial KR630374.1 Eutrichota tunicata voucher BIOUG17280-B08 cytochrome oxidase subunit 1 COI gene partial cds mitochondrial 100 | KJ725172.1 Ascidia ahodori isolate GAPB6 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 19 77 AB104870.1 Ascidia ahodori COI for cytochrome c oxidase subunit I partial cds AB104869.1 Perophora sagamiensis COI for cytochrome c oxidase subunit I partial cds AJ830012.1 Cnemidocarpa verrucosa mitochondrial partial COI gene for cytochrome c oxidase subunit I 40 KJ725168.1 Didemnum candidum isolate GAPB2 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 100 KJ725164.1 Didemnum candidum isolate GAASC13 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KJ725170.1 Didemnum candidum isolate GAPB4 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial 69 KJ725169.1 Didemnum candidum isolate GAPB3 cytochrome c oxidase subunit 1 COI gene partial cds mitochondrial KC429151.1 Corbula tunicata voucher BivAToL-85 cytochrome c oxidase subunit I COI gene partial cds mitochondrial KC706853.1 Salpa sp. BMOO-06976 cytochrome c oxidase subunit I COI gene partial cds mitochondrial 30 100 | Salpa fusiformis|TRINITY DN12252 c0 g1 i1 100 Salpa fusiformis|TRINITY DN12252 c0 g2 i1 · lasia cylindrica|TRINITY DN5006 c0 g2 i1 KT818685.1 Brooksia lacromae dev-stage oozooid cytochrome oxidase subunit I COI gene partial cds mitochondrial 100 KT818686.1 Brooksia lacromae dev-stage blastozooid cytochrome oxidase subunit I COI gene partial cds mitochondrial

— KX650801.1 Lissoclinum patella voucher DBTIC181 cytochrome oxidase subunit I COI gene partial cds mitochondrial

— 0.10

KF977307.1 Oikopleura intermedia isolate DNZ117 cytochrome oxidase subunit I COI gene partial cds mitochondrial