Submitted, accepted and published by Elsevier, Molecular Phylogenetics and Evolution 107: 142-151 (2017).

Beyond *Conus*: phylogenetic relationships of Conidae based on complete mitochondrial genomes

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Abstract

Understanding how the extraordinary taxonomic and ecological diversity of cone snails (Caenogastropoda: Conidae) evolved requires a statistically robust phylogenetic framework, which thus far is not available. While recent molecular phylogenies have been able to distinguish several deep lineages within the family Conidae, including the genera *Profundiconus*, *Californiconus*, *Conasprella*, and *Conus* (and within this one, several subgenera), phylogenetic relationships among these genera remain elusive. Moreover, the possibility that additional deep lineages may exist within the family is open. Here, we reconstructed with probabilistic methods a molecular phylogeny of Conidae using the newly sequenced complete or nearly complete (mt) mitochondrial genomes of the following nine species that represent all main Conidae lineages and potentially new ones: *Profundiconus teramachii, Californiconus californicus*, *Conasprella wakayamaensis*, *Lilliconus sagei*, *Pseudolilliconus traillii*, *Conus* (*Kalloconus*) *venulatus*, *Conus* (*Lautoconus*) *ventricosus*, *Conus* (*Lautoconus*) *hybridus*, and *Conus* (*Eugeniconus*) *nobilis*. To test the monophyly of the family, we also sequenced the nearly complete mt genomes of the following three species representing closely related conoidean families: *Benthomangelia* sp. (Mangeliidae), *Tomopleura* sp. (Borsoniidae), and *Glyphostoma* sp. (Clathurellidae). All newly sequenced conoidean mt genomes shared a relatively constant gene order with rearrangements limited to tRNA genes. The reconstructed phylogeny recovered with high statistical support the monophyly of Conidae and phylogenetic relationships within the family. The genus *Profundiconus* was placed as sister to the remaining genera. Within these, a clade including *Californiconus* and *Lilliconus* + *Pseudolilliconus* was the sister group of *Conasprella* to the exclusion of *Conus*. The phylogeny included a new lineage whose relative phylogenetic position was unknown (*Lilliconus*) and uncovered thus far hidden

diversity within the family (*Pseudolilliconus*). Moreover, reconstructed phylogenetic relationships allowed inferring that the peculiar diet of *Californiconus* based on worms, mollusks, crustaceans and fish is derived, and reinforce the hypothesis that the ancestor of Conidae was a worm hunter. A chronogram was reconstructed under an uncorrelated relaxed molecular clock, which dated the origin of the family shortly after the Cretaceous-Tertiary boundary (about 59 million years ago) and the divergence among main lineages during the Paleocene and the Eocene (56-30 million years ago).

Key words: Conidae, *Californiconus*, *Conasprella*, *Lilliconus*, *Conus*, mtDNA.

1. Introduction

With more than 830 described species (WoRMS, 2016), cone snails (Family Conidae, Fleming, 1822 *sensu lato*) constitute a major component of the biodiversity of tropical and subtropical oceans (Tucker and Tenorio, 2013). The species diversity of cones is highest in the Indo-West Pacific region (Röckel et al., 1995) but notably about 10% of the species radiated in the Cape Verde archipelago (Cunha et al., 2005; Duda and Rolan, 2005). While some species show widespread distributions (e.g., *Conus litteratus* Linnaeus, 1758 throughout the Indo-West Pacific region), others are narrowly restricted to an island or a bay (e.g., *Conus verdensis* Trovão, 1979 from Santiago Island in Cape Verde). Cones are found from deep waters to the intertidal zone, associated to rocky shores, coral reefs, and sandy bottoms (Kohn, 1959). These marine gastropods are predatory carnivores feeding mostly on marine worms, snails and fishes (Duda et al., 2001), and have evolved a sophisticated mechanism to capture preys, which are paralyzed thanks to harpoon-like radular teeth coated with a cocktail of toxins produced in a venom gland (Olivera, 2002). Interestingly, recent transcriptomic studies have shown that predation- and defense-evoked venoms are produced in the distal and proximal regions of the venom duct, respectively (Dutertre et al., 2014; Prashanth et al., 2016). Moreover, conotoxins are of important medical and pharmaceutical interest since they are potent and have very specific inhibitors of ion channels in the human brain (Terlau and Olivera, 2004). Reconstructing a statistically robust phylogeny of Conidae is mandatory for understanding how the great species diversity of the family was generated and addressing other important evolutionary open questions in the group such as the origin of the different diet specializations or how did predation and defense venoms appeared and evolved (Duda et al., 2001; Puillandre et al., 2014a). Moreover, current discovery of pharmacologically important conotoxins could be enhanced and improved by using a concerted discovery strategy that takes into account robustly inferred phylogenetic relationships to target most divergent and poorly studied groups (Holford et al., 2009; Puillandre and Holford, 2010).

All cones share a typical conical shell of different sizes (mm to about 20 cm), often brightly colored, and with diverse banding patterns that is highly appreciated by collectors (Tucker and Tenorio, 2013). The inner walls of the shell are re-absorbed during growth, and this is considered a synapomorphy of the family Conidae (Tucker and Tenorio, 2009). In general, the shell is helpful for species identification but has limited utility for discrimination of higher taxonomic levels, at which other characters such as the shape of the radula and DNA sequences are used (Tucker and Tenorio, 2009; Puillandre et al., 2014a). The family Conidae belongs to the superfamily Conoidea (Caenogastropoda: Neogastropoda) together with closely related families such as e.g., Conorbidae, Raphitomidae, Mangeliidae, Borsoniidae, Clathurellidae, and Mitromorphidae (See Puillandre et al. 2011, and references therein). Traditionally, most authors assumed that the family Conidae contained only the genus *Conus* (e.g., Röckel et al., 1995; but see Cotton, 1945; Walls, 1978; Da Motta, 1991; Taylor et al., 1993). However, two recent studies have proposed considerable changes to the classification of the family. One study (Tucker and Tenorio, 2009) was based on cladistic analysis of radular teeth and shell characters and proposed to recognize some previously introduced genera in addition to *Conus*, to raise some previously known subgenera to the genus level, and to erect completely new genera. The proposed classification distinguished up to four living families (including Conidae, Conilithidae, Conorbidae, and Taranteconidae) and 86 extant genera. Later, Conorbidae was tentatively maintained as a separate family (Bouchet et al., 2011) and species within Taranteconidae were found to be closely related to *Conus* (*Stephanoconus*) (Watkins et al., 2010). The other study (Puillandre et al., 2014a) was based on probabilistic analyses of three partial mitochondrial (mt) genes and included 330 species belonging to Conidae, Conilithidae and Taranteconidae *sensu* Tucker and Tenorio (2009). The presence of several deep lineages within the analyzed taxa prompted for a new taxonomic classification (that we follow here naming the subgenus only the first time) with a single family Conidae,

which included four genera, namely *Californiconus*, *Profundiconus*, *Conasprella*, and *Conus* (Puillandre et al., 2014b). The latter two genera were further subdivided into 11 and 60 subgenera, respectively (Puillandre et al., 2014b). The reconstructed phylogeny showed that *Profundiconus* was the sister group of the remaining Conidae, although without support (and thus questioning the limits of the family; Puillandre et al., 2014a). Within the remaining taxa, *Californiconus* was the sister group of *Conasprella* and *Conus* (Puillandre et al., 2014a). Therefore, the genera *Conus* and *Conasprella sensu* Puillandre et al. (2014b) more or less corresponded to the families Conidae and Conilithidae *sensu* Tucker and Tenorio (2009), respectively. However, the genera *Profundiconus* and *Californiconus* were excluded from other Conilithidae (*Conasprella*) (Puillandre et al., 2014b). Besides that, major lineages within *Conasprella* and *Conus* were highly congruent between both studies, only differing in their subgeneric (Puillandre et al., 2014b) or generic (Tucker and Tenorio, 2009) status, and on the placement of some species for which there was no radula and/or DNA data available and that were ascribed based on shell characters only (Tucker and Tenorio, 2009). Moreover, the new molecular phylogeny was confirming previous ones (Duda and Kohn, 2005; Biggs et al., 2010) that had already distinguished *Conus californicus*, a "Small Major Clade" (*Conasprella*) and a "Large Major Clade (*Conus*). In addition, different lineages within *Conasprella* (Kraus et al., 2012) and *Conus* (Espiritu et al., 2001; Nam et al., 2009; Kraus et al., 2011) were also recovered in several previous molecular phylogenies.

Here, we aimed to confirm the main deep lineages reported within Conidae (*sensu* Puillandre et al., 2014b) and in particular to define the phylogenetic relationships between these main deep lineages, which were mostly unresolved in published phylogenies (e.g., Puillandre et al., 2014a). To achieve these goals, we used complete or almost complete (without control region) mt genome sequence data, which have proven useful in recovering internal nodes with high support at this level of divergence or higher in other gastropods (Grande et al., 2008; White et al., 2011; Uribe et al., 2016).

Thus far, the only complete mt genomes available for Conidae are those of *Conus* (*Cylinder*) *textile* (Bandyopadhyay et al., 2008); *Conus* (*Gastridium*) *tulipa* (Chen et al., 2015); *Conus* (*Lautoconus*) *borgesi* (Cunha et al., 2009); *Conus* (*Splinoconus*) *tribblei* (Barghi et al., 2015); and *Conus* (*Pionoconus*) *consors* (Brauer et al., 2012). No complete mt genomes are available for other cone snails genera and for related families within Conoidea, and the closest conoideans available are *Xenuroturris cerithiformis* (Turridae; (Bandyopadhyay et al., 2006), *Fusiturris similis* (Clavatulidae; (Cunha et al., 2009), and *Oxymeris dimidiata* (Terebridae; (Cunha et al., 2009), which some authors place in a different superfamily, Turroidea (Tucker and Tenorio, 2009). Therefore, we sequenced mtDNAs of several species representing the main lineages of Conidae (*Profundiconus*, *Californiconus*, *Conasprella*, and *Conus*), as well as closely related conoidean families (Mangeliidae, Clathurellidae, and Borsoniidae). In addition, we sequenced the mt genomes of two highly divergent species of cones that may represent additional genera (*Lilliconus* and *Pseudolilliconus*). Our aims were: (1) to confirm the previously identified main lineages within cone snails and eventually identify new ones; (2) to reconstruct a robust phylogeny of Conidae that could be used as framework for further evolutionary studies; (3) to assess whether there have been major rearrangements of the mtDNA genome organization among the analyzed conoidean families, and (4) to date main cladogenetic events within Conidae.

2. Materials and methods

2.1. Samples and DNA extraction

The complete list of species analyzed in this study corresponding to families Conidae, Borsoniidae, Mangeliidae and Clathurellidae, is shown in Table 1, along with their respective sampling localities and museum vouchers. Specimens from the MNHN were either found in old collections or newly collected during several recent expeditions (Atimo Vatae in Madagascar, Papua Niugini and Kavieng in Papua New-Guinea). All

samples were stored in ethanol 100% and total genomic DNA was isolated from up to 30-50 mg of foot tissue following a standard phenol-chloroform extraction.

2.2. PCR amplification and sequencing

Complete or nearly complete (without the control region; see results and discussion) mt genomes were amplified through long PCR using different combinations of conserved primers newly designed in mt *cox1, cox3, rrnL* and *trnF* genes (Supplementary material 1). The long PCR reactions contained 2.5 µl of 10 × LA Buffer II (Mg^{+2} plus), 3 µl of dNTPs (2.5 mM each), 0.5 µl of each primer (10 mM), 0.5-1 µl (10-40 ng) of template DNA, 0.2 µl TaKaRa LA Taq DNA polymerase (5 units/µl), and sterilized distilled water up to 25 µl. The following PCR conditions were used: initial denaturing step at 94°C for 60 s; 45 cycles of denaturing at 98°C for 10 s, annealing at 53°C for 30 s and extending at 68°C for 60 s per kb; final extending step at 68°C for 12 min. In addition, two standard PCR reactions were performed (Supplementary material 1). One used the *rrnL* gene universal primers (Palumbi et al., 1991) to close the gap between long PCR *rrnL* primers, and the other used *cox1*gene universal primers (Folmer et al., 1994) to amplify a fragment, which after Sanger sequencing at the MNHN was used to check that final assemblies corresponded to the correct species. The standard PCR reactions contained 2.5 µl of 10x buffer, 1.5 µl of $MgCL_2$ (25 mM), 0.5 µl of dNTPs (2.5 mM each), 0.5 µl of each primer (10mM), 0.5-1 µl (10-40 ng/µl) of template DNA, 0.2 µl of Taq DNA polymerase 5PRIME (Hamburg, Germany), and sterilized distilled water up to 25 µl. The following program was applied: initial denaturing step at 94°C for 60 s; 45 cycles of denaturalization at 94°C for 30 s, annealing at 44°C for 60 s and extending at 72°C for 90 s; final extending step at 72°C for 5 m.

Long-PCR products were purified by ethanol precipitation. Amplified fragments

from the same mt genome were pooled together in equimolar concentrations and subjected to massive parallel sequencing. For each conoidean mt genome a separate indexed library was constructed using the NEXTERA XT DNA library prep Kit (Illumina, San Diego, CA, USA) at AllGenetics (A Coruña, Spain). Each of the libraries contained in addition mt genomes of unrelated animals (e.g., snakes, spiders) from different projects. The indexed libraries were run in a single lane in an Illumina HiSeq2000 (100 Pair-ended) at Macrogen (Seoul, Korea).

2.3. Genome assembly and annotation

Reads were sorted according to their indexes, and the assembly of the different mt genomes was performed in the TRUFA webserver (Kornobis et al., 2015). Briefly, adapters were removed using SeqPrep (StJohn, 2011), quality of the reads was checked using FastQC v.0.10.1 (Andrews, 2010), and raw sequences were trimmed and filtered out according to their quality scores using PRINSEQ v.0.20.3 (Schmieder and Edwards, 2011). Filtered reads were used for *de novo* assembly of each mt genome using TRUFA default settings (minimum contig length; 200; sequence identity threshold: 0.95) only retaining contigs with a minimum length of 3kb. These contigs were finally overlapped in Sequencher 5.0.1 to render the different complete or nearly complete mt genomes included within each index (the one belonging to a conoidean species and those belonging to a snake or a spider). In order to estimate mean coverage, each assembled conoidean mt genome was used as reference to map the original (raw) reads with a minimum identity of 100% using Geneious® 8.0.3.

The newly determined mt genomes were annotated using Geneious® 8.0.3 by setting a limit of nucleotide identity of 75% to previously reported conoidean mt genomes (i.e., *C. textile*, *C. borgesi*, *C. consors*, *F. similis*, *X. cerithiformis*, and *O.*

dimidiata). Annotations of the 13 mt protein-coding genes were corroborated manually identifying the corresponding open reading frames using the invertebrate mitochondrial code. The transfer RNA (tRNA) genes were further identified with tRNAscan-SE 1.21 (Schattner et al., 2005), which infer cloverleaf secondary structures (with a few exceptions that were determined manually). The ribosomal RNA (rRNA) genes were identified by sequence comparison with previously reported conoidean mt genomes, and assumed to extend to the boundaries of adjacent genes (Boore et al., 2005). GenBank accession numbers of each mt genome are provided in Table 1.

2.4. Sequence alignment

The newly sequenced complete or nearly complete mt genomes were aligned with all orthologous conoidean mt genomes available in NCBI (Table 1). Two sequence data sets were constructed and analyzed: the first data set (hereafter referred to as the Conidae data set) was aimed to test the monophyly of Conidae and included main lineages within the family as well as closely related conoidean families. Three species of less related conoideans were selected as outgroup taxa following Puillandre et al., (2011): *F. similaris* (Clavatulidae); *X. cerithiformis* (Turridae), and *O. dimidiata* (Terebridae). This data set included the deduced amino acid sequences of the 13 mt protein coding genes and the nucleotide sequences of the two rRNA genes. The second data set (hereafter referred to as the *Conus* data set) was aimed to test the internal phylogenetic relationships of *Conus*. This data set included newly determined and previously published *Conus* species and it was rooted with *Conasprella wakayamaensis* and *Californiconus californicus*. The data set included 13 mt protein-coding genes and two rRNA genes, both analyzed at the nucleotide level. Phylogenetic analyses of the protein-coding genes at the amino acid and nucleotide levels in the Conidae and *Conus*

data sets, respectively, was aimed at maximizing phylogenetic information (by selecting the appropriate levels of sequence variation) as each data set was addressing different taxonomic questions (see discussion). In order to construct these two data sets, the deduced amino acid sequences of the 13 mt protein-coding genes were aligned separately and used to guide the alignment of the corresponding nucleotide sequences with Translator X (Abascal et al., 2010). Nucleotide sequences of the mt rRNA genes were aligned separately using MAFFT v7 (Katoh and Standley, 2013) with default parameters. Ambiguously aligned positions were removed using Gblocks, v.0.91b (Castresana, 2000) with the following settings: minimum sequence for flanking positions: 85%; maximum contiguous non-conserved positions: 8; minimum block length: 10; gaps in final blocks: no. Finally, the different single alignments were concatenated into the two data matrices using Geneious® 8.0.3.

2.5. Phylogenetic analyses

Phylogenetic relationships of family Conidae and genus *Conus* were inferred using maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Huelsenbeck and Ronquist, 2001). ML analyses were conducted with RAxML v7.3.1 (Stamatakis, 2006) using the rapid hill-climbing algorithm and 10,000 bootstrap pseudoreplicates (BP). BI analyses were conducted using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), running four simultaneous Markov chains for 10 million generations, sampling every 1000 generations, and discarding the first 25% generations as burn-in (as judged by plots of ML scores and low SD of split frequencies) to prevent sampling before reaching stationarity. Two independent Bayesian inference runs were performed to increase the chance of adequate mixing of the Markov chains and to increase the chance of detecting failure to converge, as determined using Tracer v1.6 (Rambaut and

Drummond, 2007). Node support was assessed based on Bayesian Posterior Probabilities (BPP).

The best partition schemes and best-fit models of substitution for the two data sets were identified using Partition Finder and Partition Finder Protein (Lanfear et al., 2012) with the Bayesian Information Criterion (BIC; Schwarz, 1978). For the protein-coding genes of the Conidae data set (analyzed at the amino acid level) the partitions tested were: all genes grouped; all genes separated (except nad4/ 4L and atp6/8); genes grouped by enzymatic complexes (*nad*, *cox*, *atp*, *cob*; see Supplementary Material 2 for selected best fit partitions and models). For the protein-coding genes of the *Conus* data set, which were analyzed at the nucleotide level, the partitions tested were: all genes grouped; all genes separated (except nad4/ 4L and atp6/8); genes grouped by subunits (see Supplementary Material 2). In addition, these three partitions schemes were tested taking into account separately the three codon positions). The rRNA genes (analyzed at the nucleotide level) in both data sets were tested separately with two different schemes, as genes separated or combined.

2.6. *Estimation of divergence times*

The program BEAST v.1.7 (Drummond and Rambaut, 2007) was used to perform a Bayesian estimation of divergence times among major conoidean lineages based on the mt amino acid data set. An uncorrelated relaxed molecular clock was used to infer branch lengths and nodal ages. The tree topology was set based on a combination of the Conidae and *Conus* trees. For the clock model, the lognormal relaxed-clock model was selected, which allows rates to vary among branches without any a priori assumption of autocorrelation between adjacent branches. For the tree prior, a Yule process of speciation was employed. The partitions selected by Partition Finder Protein (see above) were applied. The final Markov chain was run twice for 100 million generations,

sampling every 10,000 generations and the first 10 million was discarded as part of the burn-in process, according to the convergence of chains checked with Tracer v.1.5. (Rambaut and Drummond, 2007). The effective sample size of all the parameters was above 200.

The posterior distribution of the estimated divergence times was obtained by specifying two calibration points as priors for divergence times of the corresponding splits. Fossils provided hard minimum bounds (offset) and mean and standard deviations were chosen so that the 95% probability limit corresponds to a soft maximum bound. For the divergence of Conidae, a calibration point was set at a minimum of 55 million years ago (Mya) with a 95% upper limit of 58.1 MYA (lognormal distribution, offset: 55; mean: 1; standard deviation: 1) based on the oldest known fossils of *Hemiconus rouaulti* (France) and *Hemiconus concinnus* (England) that likely belong to the stem group of the family Conidae (Tucker and Tenorio, 2009) and were documented from the Lower Eocene (Kohn, 1990). A second calibration point was set at the divergence time between *C. ventricosus* and *C. borgesi*. Fossils of *C. (Lautoconus) ventricosus* become recognized in the Middle-Lower Miocene (16.4 to 20.5 Mya) of Cuenca de Piemonte (Italy) (Sacco, 1893). This interval coincides with the inferred origin of Cape Verde cone snails and the age of the archipelago (Cunha et al., 2005). Therefore, a normal distribution (recommended for inferred a secondary calibrations and biogeographical datings; Ho and Phillips, 2009) was applied. The 95% upper and lower limits were set to 21 and 16 MYA, respectively (mean: 18.5; standard deviation: 1.5).

3. Results

3.1. Sequencing and assembly

Within Conidae, the mt genomes of *C. californicus*, *Conus* (*Kalloconus*) *venulatus*, and C. *ventricosus* were determined complete whereas those of *Conus* (*Lautoconus*) *hybridus*, *Conus* (*Eugeniconus*) *nobilis* (subspecies *victor*), *C. wakayamaensis*, *Lilliconus sagei*, *Profundiconus teramachii*, and *Pseudolilliconus traillii* lacked the control region because it could not be amplified. In addition, the nearly complete (without control region) mt genomes of *Benthomangelia* sp. (Mangeliidae), *Tomopleura* sp. (Borsoniidae), and *Glyphostoma* sp. (Clathurellidae) were also amplified and sequenced. The number of reads, mean coverage, and length of each mt genome are provided in Table 1. The mt genomes of *C. californicus* and *C. ventricosus* received the minimum (15,542) and maximum (249,121) reads, respectively. The minimum (119x) and maximum (1,619x) coverage corresponded to *Benthomangelia* sp. and *C. ventricosus*, respectively.

3.2. Structural features and mitochondrial organization

The newly determined mt genomes had the usual 13 protein coding, 2 rRNA, and 22 tRNA genes reported in other animal mt genomes (see annotation and main features of each of these mt genomes in Supplementary Material 3). In few instances, the control region between *trnF* and *cox3* genes was also amplified allowing the completion of the mt genome. All but two of the analyzed conoidean mt genomes conformed to the consensus genome organization described for Caenogastropoda (Osca et al., 2015) with most genes encoded by the major strand and only a cluster of tRNA genes (MYCWQGE) and the *trnT* gene encoded by the minor strand (Fig. 1). The only exceptions were the mt genome of *L. sagei*, which showed the translocation of the *trnL*(uag) and *trnL* (uaa) genes and the inversion and translocation of the *trnT* gene, as well as the mt genomes of *P. traillii* and *Tomopleura* sp., which showed the

translocation of the *trnT* gene (Fig.1). In addition, we were not able to find the *trnR* gene of the mt genome of *L. sagei* in its usual position (within the cluster KARNI), but we cannot discard that it might have been translocated near to the control region, which could not be amplified in this mt genome (Fig. 1).

3.3. Phylogenetic relationships of Conidae

The molecular phylogeny of Conidae was reconstructed based on the Conidae data set using probabilistic methods (Fig. 2). The final matrix was 5870 positions long. Both, ML (-lnL = 57997.47) and BI (-lnL = 59051.42 for run1; -lnL = 59051.06 for run2) arrived at almost identical topologies (Fig. 2) only differing in the internal relationships within *Conus.* The reconstructed phylogeny recovered Borsoniidae + Clathurellidae as sister group to Mangeliidae + Conidae, although both groupings received moderate and low statistical support, respectively (Fig. 2). The monophyly of Conidae received strong statistical support (1 BPP, 90% BP; Fig. 2). Within Conidae, *Profundiconus* was recovered as sister group of the remaining members of the family. Within the latter, a clade including *Californiconus* and *Lilliconus* + *Pseudolilliconus* was the sister group of *Conasprella* to the exclusion of *Conus* (Fig. 2). All recovered phylogenetic relationships within Conidae received strong support (Fig. 2) except those within *Conus*.

3.4. Phylogenetic relationships of **Conus**

In order to further determine phylogenetic relationships within the genus *Conus*, a second alignment named Conus data set was analyzed with probabilistic methods (Fig. 3). The final matrix was 13473 positions long. Both ML (-lnL = 69568.03) and BI (-lnL = 69594.80 for run1; -lnL = 69592.68 for run2) arrived at fully resolved phylogenetic trees with all nodes strongly supported (above 70% BP and 0.99 BPP; Fig. 3). Among

Conus studied species, *C. tribblei* was recovered as sister group of the remaining, which were organized into two sister clades. One clade included *C. consors* + *C. tulipa* as sister group of *C. textile* + *C. nobilis*. The other clade included *C. venulatus* as sister group of a clade including *C. hybridus* and *C. ventricosus* + *C. borgesi* (Fig. 3).

3.5. Divergence times

Major cladogenetic events within Conoidea were dated using an uncorrelated relaxed molecular clock model, which was calibrated with several European fossils belonging to the stem and crown groups of Conidae. The origin of the conoidean families closely related to Conidae is dated at a mean of 67 (84-57, credible intervals) Mya, quite close in geological times to the origin of the family Conidae itself about 59 (73-55) Mya (Fig. 4). The branching of *Profundiconus* is estimated to have occurred around 56 (70-49) Mya and the split between the lineage leading to extant *Conus* and the clade containing *Californicus*, *Conasprella*, *Lilliconus*, and *Pseudolilliconus* was dated at 51 (64-44) Mya (Fig. 4). Divergence among these latter four genera occurred successively between 46-30 (59-22) Mya. The radiation of the analyzed *Conus* species was estimated to have occurred between 24-15 (30-12) Mya (Fig. 4).

4. Discussion

Thanks to the combination of long PCR and massive sequencing techniques, we were able to add in the present work up to12 new mt genomes to the catalogue of conoidean mt genomes. Not only we more than double the number of available mt genomes for this superfamily of Caenogastropoda, but also we provide a better representation of the diversity of the superfamily by adding the first representatives of five genera within Conidae and three closely related families. Both, number of reads per mt genome and

final coverage were high, with a direct relationship between both parameters except in the case of the mt genomes of *Californiconus* and *Pseudolilliconus*, which showed higher coverage than expected. The presence of reads with the same index and in the same lane corresponding to distantly related animal species did not interfere in the correct assembly of each conoidean mt genome as assessed by empirical PCR amplification and sequencing of the *cox1* gene of each analyzed species. We were able to complete only three out of the 12 mt genomes. Completed mt genomes showed short control regions and interestingly, the coverage in these regions was much lower than average (despite being part of a longer PCR fragment in equimolar concentration). It is likely that longer and more complex (with secondary structures) control regions in the remaining mt genomes prevented *Taq* polymerase for completing the PCR reactions in some species. In those cases, outward primers were designed in the *trnF* and *cox3* genes at the boundaries of the control region (see Supplementary Material 1).

4.1. Gene order evolution

The mt genomes of mollusks, and of gastropods in particular, are known for having relatively high rates of gene rearrangement (Grande et al., 2008; Stöger and Schrödl, 2013). Major changes in mt genome organization including translocations and inversions of protein coding and/or rRNA genes normally occur between main lineages of gastropods (e.g., Patellogastropoda or Heterobranchia versus other gastropod lineages; Grande et al., 2008) or in particular groups within main lineages (e.g., superfamily Vermetoidea witihin Caenogastropoda; Rawlings et al., 2010). Interestingly, these high rates of rearrangement are normally associated with high mutational rates, leading to long branches in phylogenetic trees (Stöger and Schrödl, 2013; Osca et al., 2015; Uribe et al., 2016). Nevertheless, for the majority of groups and species within a

main gastropod lineage, gene order is relatively stable and rearrangements are restricted to tRNA genes, if any (Grande et al., 2008). Hence, it is possible to reconstruct a consensus gene order for the hypothetical ancestor of the different main gastropod lineages (Grande et al., 2008; Stöger and Schrödl, 2013; Osca et al., 2014; Osca et al., 2015; Uribe et al., 2016). The gene order of the 12 mt genomes here sequenced generally conforms to the consensus genome organization for Caenogastropoda and is identical to the one inferred for Neogastropoda (Cunha et al., 2009; Osca et al., 2015). Among previously published conoidean mt genomes, it was reported the translocation of the *trnV* and *trnS* in *O. dimidiata* and *F. similis*, respectively (Cunha et al., 2009). Here, the *Tomopleura* sp. mt genome shows a translocation of the *trnT*, which is normally found next to the *trnS* (uga) gene and encoded by the minor strand, to a location between the *cox1* and *cox2* genes. The rearrangement of this tRNA gene is relatively frequent among caenogastropods (Osca et al., 2015) and occupies the same position in *P. traillii*. In addition, the mt genome of *L. sagei* presents a translocation and inversion of the *trnT* gene, which is found next to the *rrnL* gene and encoded by the major strand, in a position were normally the *trnL* (uag) and *trnL* (uaa) genes are found. In this mt genome, however, the two *trnL* genes have moved next to the *cox2* gene. Interestingly, both events seem to be connected because at the same position where the *trnT* is found, the minor strand could putatively encode for a *trnL* (uaa) gene (see Supplementary Material 4), indicating that the *trnT* and the reverse complementary *trnL (uaa)* gene sequences are very similar. Moreover, between the two *trnL* genes there is space for the coding of a *trnT* gene in the major strand (see Supplementary Material 4), which could be the remnant of an ancient duplication. In addition, we were not able to detect the *trnR* gene, in the otherwise highly conserved KARNI cluster. This missing gene might have moved next to the control region, which could not be sequenced.

Finally, it is worth mentioning that in many gastropod mt genomes high rates of rearrangement and of substitution rates are normally correlated (Rawlings et al., 2010; Stöger and Schrödl, 2013; Osca et al., 2014; Osca et al., 2015). However, here this correlation does not hold. The mt genomes of *Tomopleura* and *Pseudolliliconus* have the same gene order, but only the latter genus has a very long branch in the phylogenetic tree, much longer than that of *Lilliconus*, whose mt genome has more rearrangements than any other (and even in this case only associated to minor tRNA gene rearrangements).

4.2. Phylogenetic relationships of Conidae

The hyperdiverse superfamily Conoidea has been the subject of recent molecular phylogenetic studies (Puillandre et al., 2008; Puillandre et al., 2011; Puillandre et al., 2014a) that supported some morphology-based classifications (Taylor et al., 1993) and allowed discerning the closest families to Conidae, i.e., Conorbidae, Raphitomidae, Mangeliidae, Borsoniidae, Clathurellidae, and Mitromorphidae. These molecular phylogenies were based on the concatenation of partial mt genes and were unable to resolve phylogenetic relationships among these families, and thus determining the sister group of Conidae. A clade including Conorbidae and Borsoniidae was tentatively recovered as sister group of Conidae but without statistical support (Puillandre et al., 2011). In the phylogeny here reconstructed based on complete mt genomes, the Mangeliidae were recovered as sister group of Conidae but this relationship showed low statistical support impeding the resolution of this long-standing question. Here, we added a considerable amount of sequence data (mt genomes) in trying to gain further resolution in this part of the Conoidea tree but without success. However, our data set was biased towards representatives of the family Conidae. Hence, in future studies, it

would be important to increase taxon representation within closely related families, as well as include missing important families such as Conorbidae, Raphitomidae, and Mitromorphidae. Moreover, the possibility nowadays of obtaining a considerable number of nuclear loci using next-generation sequencing techniques opens a potent approach to increase phylogenetic resolution. In any case, it is clear from the reconstructed phylogenetic trees that the lengths of internal nodes connecting these families are rather short, which may indicate and ancient radiation, and therefore that achieving high statistical support and final resolution of these phylogenetic relationships will be challenging.

The monophyly of the family Conidae was highly supported in the reconstructed phylogeny, as were relationships among its main deep lineages. In this case, we had a complete representation of main lineages and even new ones, allowing us to reach stronger conclusions. The genus *Profundiconus* was recovered as sister group to the remaining members of Conidae in agreement with previous molecular phylogenies (Puillandre et al., 2011; Puillandre et al., 2014a) but here showing high statistical support. Phylogenetic relationships among the remaining Conidae differed with respect to previous studies. Here, *Conus* was recovered as the sister group of a clade containing *Conasprella* as sister group of *Californiconus* and *Lilliconus + Pseudolilliconus*. In previous molecular phylogenies (Puillandre et al., 2011; Puillandre et al., 2014a), *Californiconus* was recovered as sister group of *Conasprella + Conus*, with low BP support (50-63 %) in ML and relatively high BPP support in BI (0.96-0.98). The differences between the present study and previous ones are the increased number of analyzed positions, the use of amino acids, which show a better phylogenetic information/ noise ratio at deeper nodes due to lower saturation levels, and the inclusion of new lineages of Conidae that proved to be highly divergent. Interestingly, a close

relationship between *Californiconus* and *Conasprella* (*Lilliconus* and *Pseudolilliconus* were not included in the study) was already suggested by Tucker and Tenorio (2009). The reconstructed phylogeny is statistically robust within Conidae and serves as a framework for studying evolutionary processes associated with the diversification of the family. All members of Conidae are presumed vermivorous except *C. californicus*, which has a wide diet based on worms, mollusks, crustaceans and fishes (Biggs et al., 2010), and certain derived groups of *Conus* that feed on fishes or mollusks. The strongly supported phylogenetic position of *C. californicus* deeply nested within the Conidae tree reinforces the hypothesis that the ancestor of Conidae was a hunter of polychaete worms (Puillandre et al., 2014a). The 16 extant species belonging to *Profundiconus* live in the deep sea in the Indo-Pacific region (Tenorio and Castelin, 2016). The relative phylogenetic position of this genus within the family Conidae suggests that the group represents an early offshoot that has survived since the middle Eocene.

4.3. Phylogenetic relationships of Conus

The reconstructed phylogeny based on the Conidae data set lacked resolution within *Conus*. This was likely due to low levels of variation in the amino acids at this hierarchical taxonomic level. In order to maximize phylogenetic information, a second data set was constructed with protein coding genes analyzed at the nucleotide level. The *Conus* data set rendered a fully resolved phylogeny with high statistical support in all nodes. The reconstructed phylogenetic relationships are fully congruent with those recovered in a previous molecular phylogeny with an extended taxon sampling (Puillandre et al., 2014a). The presumed vermivorous species *C. tribblei* from the West Pacific and Indian Oceans was recovered as sister group of the remaining taxa. Within this, two main groups were recovered, corresponding to Indo-Pacific and Western

Atlantic- Mediterranean species, respectively. Within the Indo-Pacific clade, two species, *C. consors* and *C. tulipa*, feeding on fish clustered together as sister group of two species, *C. textile* and *C. nobilis*, feeding on mollusks (Tucker and Tenorio, 2009; Puillandre et al., 2014a). All species in the Western Atlantic- Mediterranean clade are worm hunters. Obviously, the present phylogenetic tree has only a minor representation of the species diversity of the genus (nine out of 800 species) and is biased in terms of taxonomy (three of the species belong to the same subgenus), distribution (half of the species are from the Atlantic Ocean), and life history traits (about half of the species are fish- or mollusk-hunters) when compared with the genus as a whole. Therefore, we limited our interpretation of the results to character states at the tips of the tree and refrained from performing proper ancestral character-state reconstructions, which would be meaningless at this moment. Nevertheless, the present work emphasizes that complete mt genomes are a very promising tool for achieving important levels of resolution within *Conus*, and that a more complete data set will certainly help a better understanding of the evolutionary processes (diet and conotoxin evolution, biogeography) that led to the extraordinary diversity encompassed by the genus.

4.4. Divergence times and taxonomic levels within family Conidae

The reconstructed time tree using a relaxed molecular clock model dated the origin of the family Conidae in the Paleocene, shortly after the Cretaceous-Tertiary boundary (about 59 Mya, at the Danian/ Selanian transition), which is right before the earliest fossils of cone snails are documented. According to the chronogram, the first burst of cladogenetic events within the family Conidae occurred successively during the Paleocene and Eocene and corresponded to the origin of the major lineages (genera) in parallel to the appearance of closely related conoidean families (Fig. 4; Kohn, 1990).

The fossil record would suggest that some of these conoidean families may have appeared before (Powell, 1942), but this would need to be confirmed with a full revision of the different reported fossils and confirmation of their ascriptions to the different families. The diversity of Conidae increased steadily during the Oligocene until a major radiation in the Indo-Pacific region occurred in the Miocene corresponding to the appearance of subgenera within *Conus* (Fig. 4; Kohn, 1990). However, the analyzed *Conus* species correspond to clades that appeared relatively late during the evolution of the genus (Puillandre et al., 2014a): the inclusion of species belonging to the subgenera *Fraterconus, Stephanoconus, Strategoconus, Klemaeconus*, and *Turriconus*, which supposedly diverged before (Puillandre et al., 2014a), will likely push back our estimates for the original radiation of *Conus*. Given our taxon sampling we could not date the most recent radiation in the family corresponding to the appearance of extant species in the Pleistocene (Kohn, 1990; the magnitude of this radiation, >800 living species versus 100-150 fossils species at the maximum diversity 10 Mya, directly depends on how complete and unbiased is the fossil record). Another important radiation of *Conus* occurred locally during the middle-lower Miocene in the Cape Verde archipelago shortly after the emergence of these volcanic islands (Cunha et al., 2005).

In an ultrametric tree, the distances from the root to every branch tip are equal, the length of the branches is proportional to the time of divergence, and hence, branch length of the different lineages can be roughly compared in order to provide a criterion for taxonomic level delimitation above species (Johns and Avise, 1998). The hierarchical level of the main clades within Conidae has been an important source of conflict between morphological- (Tucker and Tenorio, 2009) and molecular-based (Puillandre et al., 2014b) classifications. By comparing the branch lengths of the different accepted families within Conoidea (Puillandre et al., 2011), it seems that

earlier lineages within Conidae could have appeared before some other related families of Conoidea. However, the fossil record suggests that some families of "turrids" (i.e., Conoidea except Conidae and Terebridae) likely appeared before the cone snails (Powell, 1942). Furthermore, while cone snails are represented by multiple lineages, and several species within *Conus*, in our phylogenetic analyses, the closely related families are represented by only one species each, and even some families, also suggested as closely related to cone snails (Puillandre et al. 2011), e.g., Clathurellidae, Mitromorphidae, Raphitomidae, are absent. Therefore, more data, and in particular a better coverage of the Conoidea diversity, together with calibration points for non-cone-snails Conoidea, are needed to provide a time-calibrated phylogeny that could be used to discuss taxonomic ranks.

5. Conclusions

The ancient radiation at the origin of conoidean families combined with the extraordinary species diversity within Conidae have hindered past attempts of resolving the phylogeny of the family based on concatenated partial mt genes. Here, up to 12 complete or nearly complete (without control region) mt genomes of all main lineages of Conidae and certain selected closely related conoidean families were sequenced and used for phylogenetic analyses. The monophyly of the family including genus *Profundiconus* was recovered with high support, and high resolution of phylogenetic relationships was achieved not only among all genera, but also among an abridged representation of species within the most diverse genus (*Conus*). Our results indicate that complete mt genomes are a very promising phylogenetic tool to reconstruct a statistically robust phylogeny of the family. This approach could be complemented with the development (using next generation sequencing techniques) of nuclear markers,

which could be particularly useful for resolving deeper phylogenetic relationships i.e., those among conoidean families. Altogether, these robust molecular phylogenies would allow setting the needed framework to further our understanding of the evolutionary processes that generated and maintain the remarkable taxonomic and ecological diversity of cone snails.

6. Acknowledgements

The MNHN Indo-Pacific material originates from a series of expeditions and workshops, conducted in the context of the "Our Planet Reviewed" programme with Pro-Natura International (ATIMO VATAE to South Madagascar in partnership with Université de Tuléar; PAPUA NIUGINI 2012 and KAVIENG 2014 to Papua New Guinea, in partnership with University of Papua New Guinea and the National Fisheries College). The organizers thank the Total Foundation, Prince Albert II of Monaco Foundation, Stavros Niarchos Foundation, Fondation EDF, Vinci Entrepose Contracting and Richard Lounsbery Foundation for their support to these expeditions. We are indebted to José Utge, Agnès Dettaï and Laetitia Aznar (MNHN, Paris), and Ana Echeverry (MNCN) for technical assistance in the laboratory. Several specimens of *C. californicus* were kindly donated by Rick Negus. We are grateful to Jesús Marco and Luis Cabellos who provided access to the supercomputer Altamira at the Institute of Physics of Cantabria (IFCA-CSIC), member of the Spanish Supercomputing Network, for performing phylogenetic analyses. This work was supported by the Spanish Ministry of Science and Innovation (CGL2010-18216 and CGL2013-45211-C2-2-P to RZ; BES-2011-051469 to JEU), by the “Service de Systématique Moléculaire” (UMS 2700 CNRS-MNHN), by the ATM (“Action transversal du Muséum”) Emergences of the MNHN and by the project CONOTAX, funded by the French Agence Nationale de la

Recherche (ANR-13-JSV7-0013-01 to NP). Access of JEU to the MNHN collections and facilities was funded by the EU Synthesys Project.

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Legends to Figures

Figure 1. Mitochondrial gene orders of conoidean mitochondrial genomes. The consensus genome organization is shown as well as known exceptions. The genes encoded in the major and minor strands are shown in the top and bottom lines, respectively. Gene rearrangements (restricted to tRNA genes) are indicated by arrows. Translocated genes are in green. A gene both translocated and inverted is in blue. Striped boxes indicate regions not sequenced (note that the *trnR* gene is missing in *L. sagei*).

Figure 2. Phylogenetic relationships of Conoidea based on complete mt genomes. The reconstructed ML phylogram using Terebridae, Turridae and Clavtulidae as outgroup is shown. The family Conidae is indicated in blue. Numbers at nodes are statistical support values for ML (bootstrap proportions in percentage)/ BI (posterior probabilities). Drawings are taken from (Puillandre et al., 2014a).

Figure 3. Phylogenetic relationships within *Conus* based on complete mt genomes. The reconstructed ML phylogram using *Californiconus* and *Conasprella* as outgroup is shown. Numbers at nodes are statistical support values for ML (bootstrap proportions in percentage)/ BI (posterior probabilities). The distributions of the taxa (Indo-Pacific region in blue; Western Atlantic Ocean and Mediterranean in orange) and their diet are indicated.

Figure 4. Chronogram with age estimates of major divergence events among conoideans, based on the Conidae data set, and using Bayesian relaxed dating methods

(BEAST). Horizontal bars represent 95% credibility intervals of relevant nodes, and calibration constraints are indicated with an asterisk on the corresponding nodes. Dates (and credibility intervals) are in millions of years. A geological table with periods is shown as well as species diversity of cone snails in the fossil record (modified from (Kohn, 1990)

Figure 1

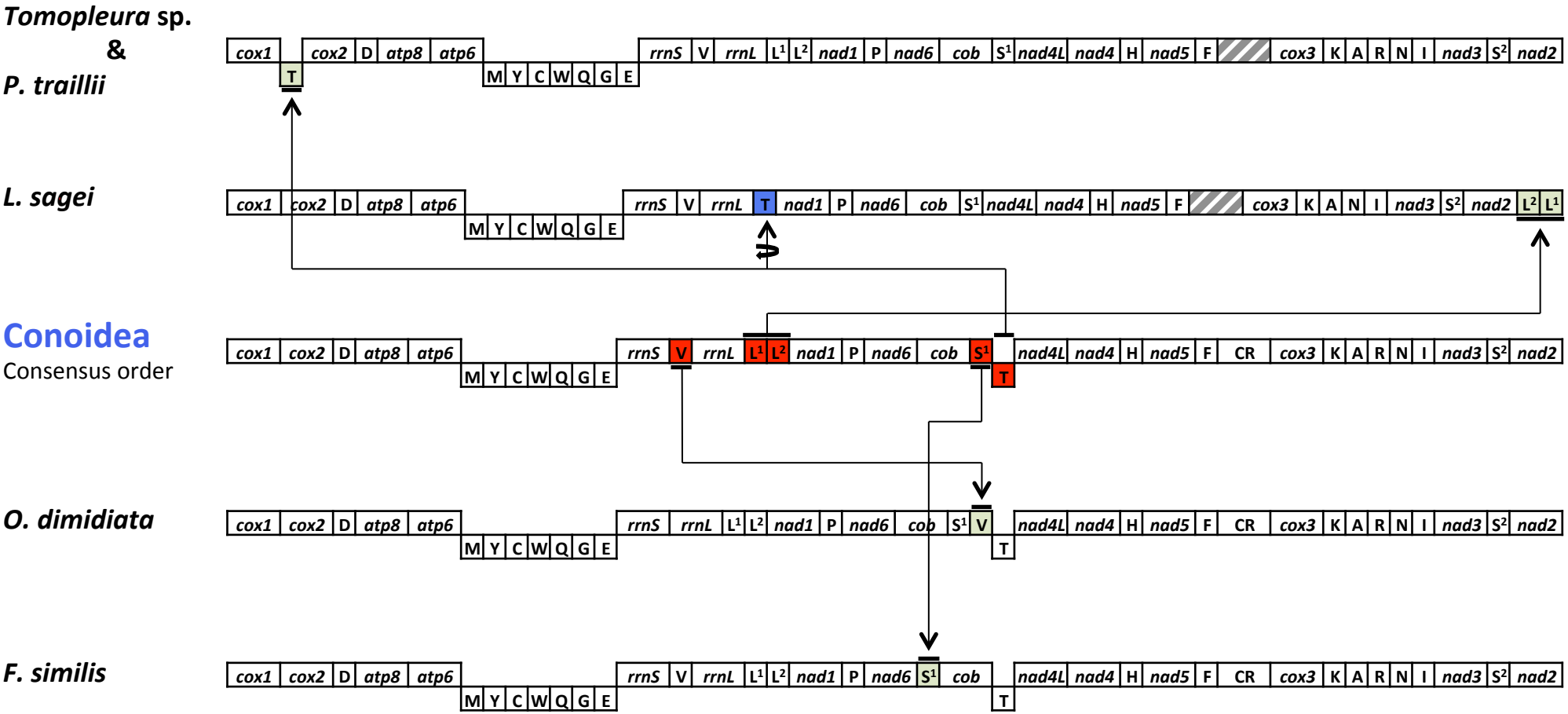

Tomopleura sp.
&
P. traillii
L. sagei
Conoidea
Consensus order
O. dimidiata
F. similis
cox1 cox2 D atp8 atp6 M Y C W Q G E rrnS V rrnL L1 L2 nad1 P nad6 cob S1 nad4L nad4 H nad5 F cox3 K A R N I nad3 S2 nad2 L2 L1
T
CR

Figure 2

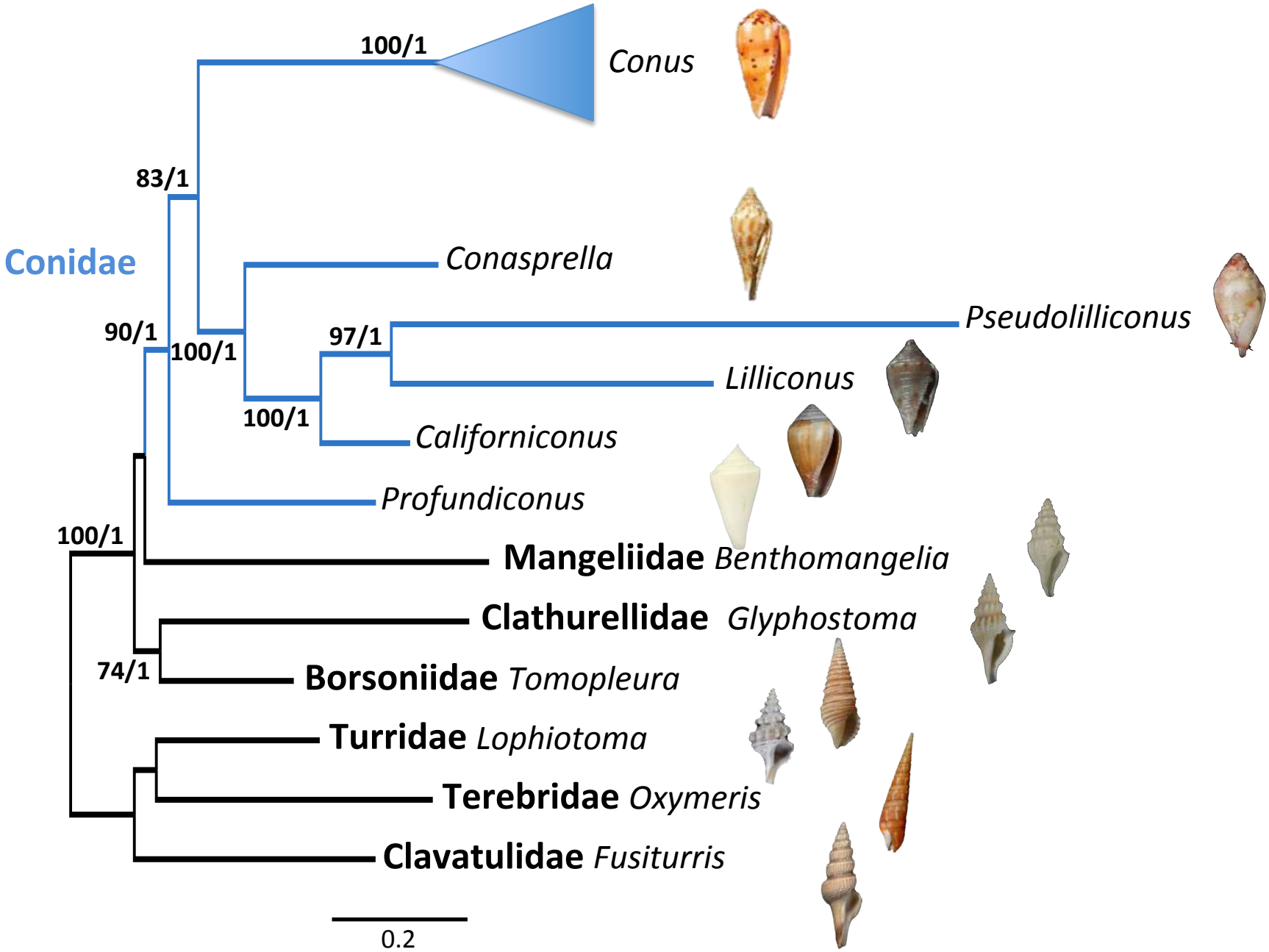

100/1
Conus
83/1
Conidae
Conasprella
90/1
97/1
Pseudolilliconus
100/1
Lilliconus
100/1
Californiconus
Profundiconus
100/1
Mangeliidae Benthomangelia
Clathurellidae Glyphostoma
74/1
Borsoniidae Tomopleura
Turridae Lophiotoma
Terebridae Oxymeris
Clavatulidae Fusiturris
0.2

Figure 3

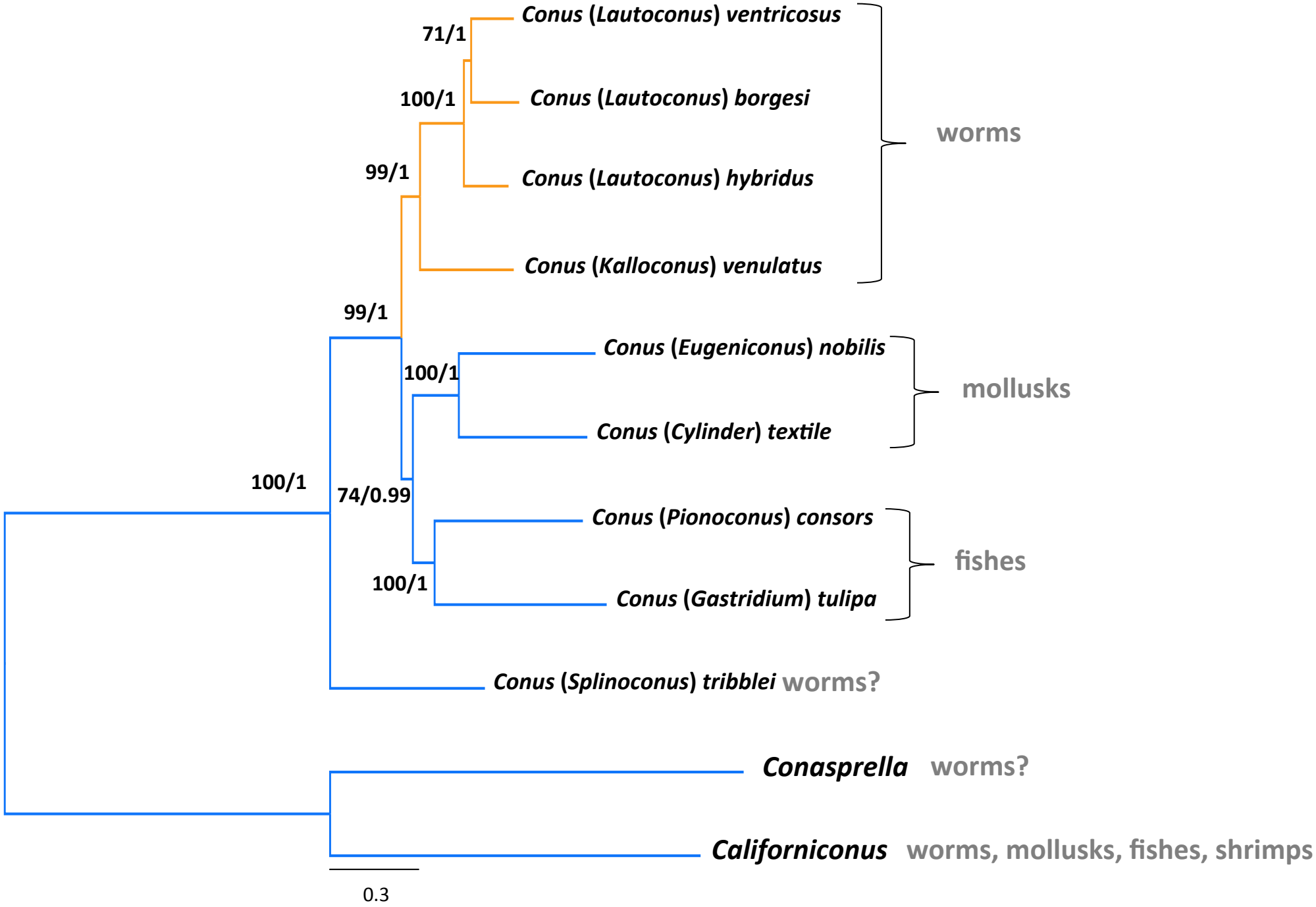

Conus (Lautoconus) ventricosus
71/1
Conus (Lautoconus) borgesi
100/1
Conus (Lautoconus) hybridus
99/1
Conus (Kalloconus) venulatus
worms
99/1
Conus (Eugeniconus) nobilis
100/1
Conus (Cylinder) textile
mollusks
100/1
74/0.99
Conus (Pionoconus) consors
100/1
Conus (Gastridium) tulipa
fishes
Conus (Splinoconus) tribblei worms?
Conasprella worms?
Californiconus worms, mollusks, fishes, shrimps
0.3

Figure 4

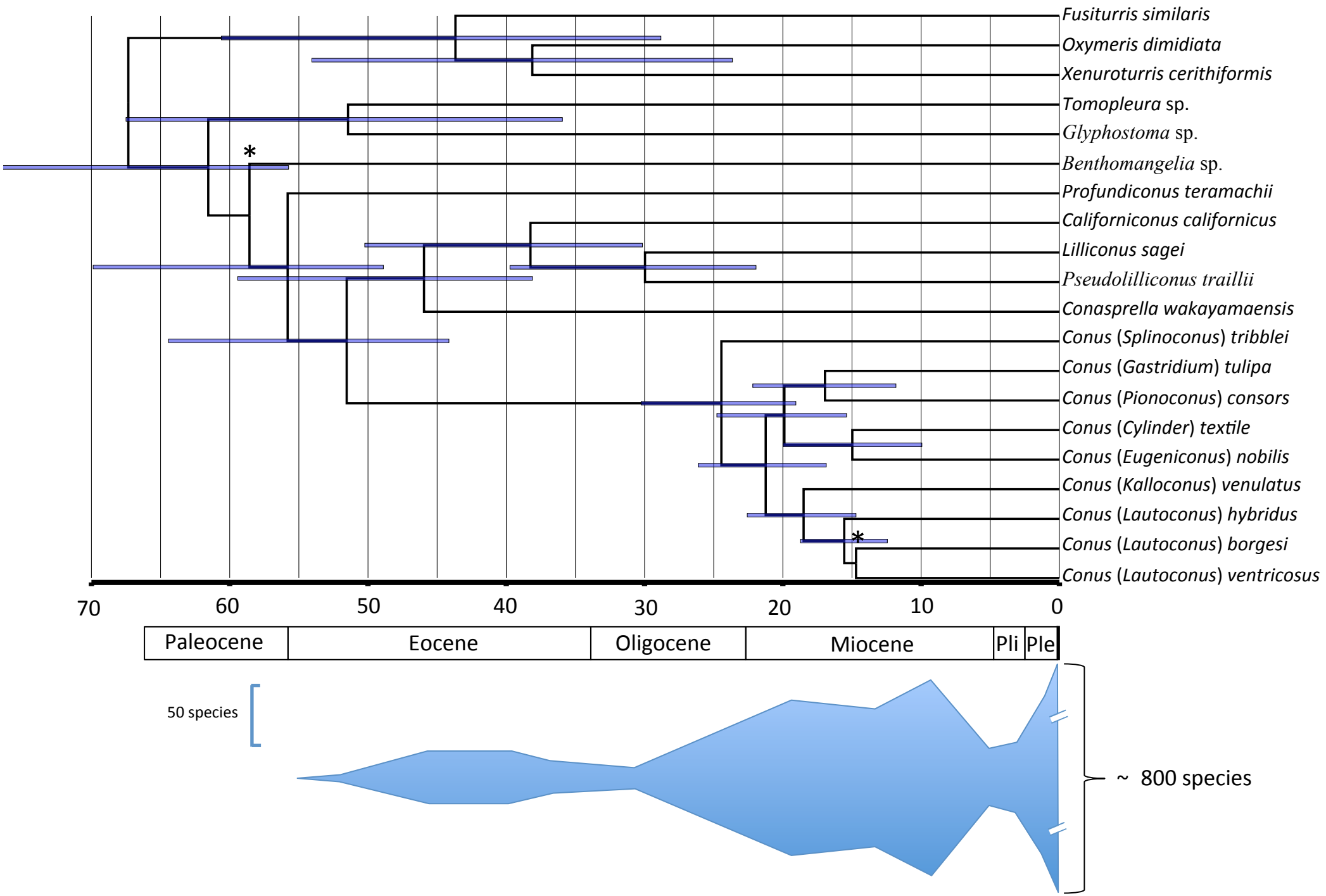

Fusiturris similaris
Oxymeris dimidiata
Xenuroturris cerithiformis
Tomopleura sp.
Glyphostoma sp.
Benthomangelia sp.
Profundiconus teramachii
Californiconus californicus
Lilliconus sagei
Pseudolilliconus traillii
Conasprella wakayamaensis
Conus (Splinoconus) tribblei
Conus (Gastridium) tulipa
Conus (Pionoconus) consors
Conus (Cylinder) textile
Conus (Eugeniconus) nobilis
Conus (Kalloconus) venulatus
Conus (Lautoconus) hybridus
Conus (Lautoconus) borgesi
Conus (Lautoconus) ventricosus
*
*
70
60
50
40
30
20
10
0
Paleocene
Eocene
Oligocene
Miocene
Pli
Ple
50 species
~ 800 species

Table 1

Table 1. Mitochondrial (mt) genomes analyzed in this study. Numero (#) of reads and mean (M) coverage of each specie.

New mt genomes

Species	Family	Length (bp)	GenBank Acc. No.	Location	Voucher Museum	# reads	M. Coverage
*Californiconus californicus**	Conidae	15444	KX263249	Aqua Hedionda lagoon, Carlsbad, California, USA	MNCN:ADN:86740	15542	693
Conus (*Kalloconus*) *venulatus**	Conidae	15524	KX263250	Boavista Island, Ilheu Sal Rei, Cape Verde	MNCN:ADN:86741	155050	1009
Conus (*Lautoconus*) *ventricosus**	Conidae	15534	KX263251	Faro, Portugal	MNCN:ADN:86742	249121	1619
Conus (*Lautoconus*) *hybridus*	Conidae	15276	KX263252	Dakar, 0-2 m, 14° 45 ' N; 17°32' W	MNHN-IM-2009-18301	54697	355
Conus (*Eugeniconus*) *nobilis*	Conidae	15379	KX263253	Indonesia, NE Flores	MNHN-IM-2009-29800	80526	521
Conasprella wakayamaensis	Conidae	15927	KX263254	Papua Niugini expedition (Papua New-Guinea), st. CP4059, 335 m, 02°38'S; 141°18'E	MNHN-IM-2013-19091	128645	801
Lilliconus sagei	Conidae	15485	KX263255	Atimo Vatae expedition (Madagascar), BS03, 14-18 m, 25°26.4'S; 44°56.1'E	MNHN-IM-2009-31328	27906	177
Profundiconus teramachii	Conidae	15279	KX263256	Papua Niugini expedition (Papua New-Guinea), st. CP3979, 540-580 m, 04°44'S; 146°11'E	MNHN-IM-2013-19686	99314	645
Pseudolilliconus traillii	Conidae	14963	KX263257	Kavieng expedition (Papua New-Guinea), st. KB8, 13m, 02°33,2'S; 150°48,2'E	MNHN-IM-2013-47771	34311	636
Benthomangelia sp.	Mangeliidae	15034	KX263258	Papua Niugini expedition (Papua New-Guinea), st. CP4024, 420-490 m, 05°22'S; 145°48'E	MNHN-IM-2013-09858	18107	119
Tomopleura sp.	Borsoniidae	15182	KX263259	Papua Niugini expedition (Papua New-Guinea), st. CP4023, 340-385 m, 05°22'S; 145°48'E	MNHN-IM-2013-09849	30684	271
Glyphostoma sp.	Clathurellidae	13370	KX263260	Papua Niugini expedition (Papua New-Guinea), st. CP4065, 380, 03°19'S; 143°01'E	MNHN-IM-2013-19173	37382	277

GenBank mt genomes

Species	Family	Length (bp)	GenBank Acc. No.	Reference
Conus (*Lautoconus*) *borgesi*	Conidae	15536	NC_013243	Cunha et al., 2009
Conus (*Pionoconus*) *consors*	Conidae	16112	NC_023460	Brauer et al., 2012
Conus (*Cylinder*) *textile*	Conidae	15562	NC_008797	Bandyopadhyay et al., 2008
Conus (*Splinoconus*) *tribblei*	Conidae	15570	KT199301	Barghi et al., 2015
Conus (*Gastridium*) *tulipa*	Conidae	16599	NC_027518	Chen et al., 2015
Fusiturris similis	Clavatulidae	15595	NC_013242	Cunha et al., 2009
Xenuroturris cerithiformis	Turridae	15380	NC_008098	Bandyopadhyay et al., 2006
Oxymeris dimidiata	Terebridae	16513	NC_013239	Cunha et al., 2009

**Complete genomes*

Complementary Data 1. Amplification strategy. Long PCR and primer walking primers

Conus californicus

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Forcox1F	AGCTTTTGACTTTTACCCCCTGCTTTG	*cox1-rrnL* (5346)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Forcox1R	GTCTACCGAACCTCCTGCATGAGCTAGG	*rrnL-cox3* (10219)
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (425)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Conus (*Lautoconus*) *venulatus*

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Conus_cox1_F	AGTTTYTGRCTTCTTCCTCCTGCGCTT	*cox1-rrnL* (5371)
Conus_16S_R	GATTATGCTACCTTTGCACGGTCAGAG	
Conus_12S_F	GAGATAAGTCGTAACAYAGTAGGGGTAATG	*rrnL-cox1* (6024)
Conus_nd4_R	GAATTTAGGACTACCTCCGTGATGAATAG	
Conus_nd4_F	GTTTATTAAGCGTACTCGTCTTTGCAGCAT	*rrnL-cox1* (5926)
Conus_cox1_R	CCTAAAATAGAAGAHACMCCAGCAAGATG	

Conus (*Lautoconus*) *ventricosus*

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Conus_cox1_F	AGTTTYTGRCTTCTTCCTCCTGCGCTT	*cox1-rrnL* (5358)
Conus_16S_R	GATTATGCTACCTTTGCACGGTCAGAG	
Conus_12S_F	GAGATAAGTCGTAACAYAGTAGGGGTAATG	*rrnL-cox1* (6037)
Conus_nd4_R	GAATTTAGGACTACCTCCGTGATGAATAG	
Conus_nd4_F	GTTTATTAAGCGTACTCGTCTTTGCAGCAT	*rrnL-cox1* (5935)
Conus_cox1_R	CCTAAAATAGAAGAHACMCCAGCAAGATG	

Conus (*Lautoconus*) *hybridus*

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3339)
COIbfol_R	TATAAAATDGGATCHCCACCTCCTGC	
COIfol_F	TATTTTCTACHAATCATAAAGATATTGG	*cox1-rrnL* (5618)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (7011)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (426)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Conus (*Eugeniconus*) *nobilis victor*

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3397)
COIbfol_R	TATAAAATDGGATCHCCACCTCCTGC	
COIfol_F	TATTTTCTACHAATCATAAAGATATTGG	*cox1-rrnL* (5694)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (7005)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (427)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Conasprella wakayamaensis

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3324)
COIbfol_R	TATAAAATDGGATCHCCACCTCCTGC	
COIfol_F	TATTTTCTACHAATCATAAAGATATTGG	*cox1-rrnL* (6282)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (7037)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (445)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Liliconus sagei

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3863)
Lilicox1R	CTGCACCTAAAATTGATGAAGCACCAGC	
Lilicox1F	TTAAGTCAACCTGGGGCTCTGTTAGG	*cox1-rrnL* (5553)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (6964)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (427)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Profundiconus terimachi

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-rrnL* (8325)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (700)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (426)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Pseudolilliconus traillii

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-rrnS* (8124)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Pseu12sF	AATCTGTGAAAGTTTTGAGGGAAACCGGG	*rrnS-trnF* (8175)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Benthomangelia sp.

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)

Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3065)
Mangcox1R	ACAGCHCCTAAAATAGAAGAAACACC	
Mangcox1F	GGAGCTCCHGATATAGTWTTTCCTCG	*cox1-rrnL* (5261)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-trnF* (7019)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (422)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Tomopleura sp.

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3299)
Borcox1R	GATATAARATAGGATCWCCRCCTCCTGC	
Borcox1F	ATTGGAGGATTTGGRAATTGRTTRGTTCC	*cox1-rrnL* (5380)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-cob* (3249)
BorcobR	GGGCACCTTCCAATCCAAGTTAAAAC	
Borcob4F	GAAGTCCTATTCGAAAAGTTCATCCGG	*cobtrnF* (4737)
CdeaPheR	TACYTTAGCATCTTCAGCGCTAYGCTCT	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (423)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Glyphostoma sp.

Long PCR

Primer	Sequence 5'-3'	Fragment (bp)
Cdeacox3F	ATGGCACGAAATCCATTTCATTTRGTTGA	*cox3-cox1* (3145)
Clathcox1R	AGCACCTAAAATAGAAGAAACACCNGCAAG	
Clathcox1F	GGGGCTCCYGATATGGTYTTTCCTCG	*cox1-rrnL* (5351)
Cdea16sR	CTACCTTTGCACGGTCAGAGTACC	
Cdea16sF	GCCTTATAATTGAAGGCTRGWATGAATGG	*rrnL-nad4* (5147)
Conus_nd4_R	GAATTTAGGACTACCTCCGTGATGAATAG	

Primer Link *rrnL*

Primer	Sequence 5'-3'	
16sinicioF2	TTCTGCCTGTTTAKCAAAAACATGGCTTC	*rrnL-rrnL* (422)
16sfinR	AAAGATAATGCTGTTATCCCTRCGG	

Supplementary Material 2. Best fit partitions and evolutionary substitution models as selected by Partition Finder

Conidae matrix

	Set Partition	Best Model	Alpha	Pinvar	A<->C	A<->G	A<->T	C<->G	C<->T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition to CDS genes (BIC =72340.71)	1 *atp6-8*	MtMam+I+G+F	0.34	0.09									
	2 *cob*	MtArt+I+G	0.98	0.54									
	3 *cox1-2-3*	MtMam+I+G+F	0.61	0.62									
	4 *nad1-2-3-4-4L-5-6*	MtMam+I+G+F	0.37	0.10									
Best Partition to rARNs genes (BIC =46628.80)	5 *rrnL-S*	GTR+I+G	0.61	0.27	0.29	5.14	1.04	0.08	5.80	0.36	0.12	0.18	0.33

Conus matrix

	Set Partition	Best Model	Alpha	Pinvar	A<->C	A<->G	A<->T	C<->G	C<->T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition CDS genes (BIC =117487.53)	1 *atp6-8* 1th	HKY+G	1.57	0.56	1.79	35.88	3.42	1.57	43.41	0.28	0.15	0.20	0.34
	2 *atp6-8* 2th	GTR+I+G	1.12	0.70	0.73	10.38	0.97	8.95	6.46	0.17	0.22	0.13	0.47
	3 *atp6-8* 3th	HKY+I+G	1.11	0.02	27.02	226.17	4.16	12.15	367.55	0.28	0.08	0.13	0.49
	4 *cob* 1th	GTR+G	1.45	0.68	2.09	13.92	1.22	0.00	47.87	0.22	0.20	0.26	0.30
	5 *cob* 2th	GTR+I+G	0.77	0.81	0.00	2.88	0.69	4.58	6.03	0.21	0.21	0.15	0.41
	6 *cob* 3th	HKY+I+G	0.57	0.03	15.09	1745.51	4.62	54.55	1796.65	0.32	0.10	0.09	0.47
	7 *cox1-2-3* 1th	GTR+I+G	0.68	0.65	0.96	5.93	0.00	0.00	33.81	0.23	0.16	0.31	0.28
	8 *cox1-2-3* 2th	GTR+I+G	1.20	0.89	1.90	6.12	0.46	9.23	3.83	0.19	0.21	0.18	0.39
	9 *cox1-2-3* 3th	HKY+G	0.96	0.00	4.13	295.70	6.55	32.23	342.50	0.30	0.06	0.16	0.47
	10 *nad1-2-3-4-4L-5-6* 1th	GTR+I+G	0.47	0.27	3.16	15.82	1.71	2.01	40.21	0.28	0.15	0.21	0.33
	11 *nad1-2-3-4-4L-5-6* 2th	GTR+I+G	0.69	0.62	1.09	12.35	1.11	9.74	8.34	0.16	0.20	0.16	0.46
	12 *nad1-2-3-4-4L-5-6* 3th	GTR+I+G	1.65	0.02	13.47	256.02	6.36	25.46	197.03	0.33	0.10	0.12	0.42
Best Partition to rARNs genes (BIC =25260.64)	13 *rrnL-S*	GTR+I+G	0.28	0.06	0.98	22.03	3.82	1.24	23.43	0.35	0.13	0.19	0.32

Supplementary Material 3. Mitochondrial genome features

Californiconus californicus

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	1	1548	1548	ATG	TAA	119	forward
cox2	CDS	1668	2354	687	ATG	TAA	3	forward
trnD	tRNA	2358	2427	70			0	forward
atp8	CDS	2428	2586	159	ATG	TAA	2	forward
atp6	CDS	2589	3284	696	ATG	TAA	38	forward
trnM	tRNA	3323	3389	67			10	reverse
trnY	tRNA	3400	3467	68			0	reverse
trnC	tRNA	3468	3534	67			0	reverse
trnW	tRNA	3535	3600	66			-3	reverse
trnQ	tRNA	3598	3664	67			20	reverse
trnG	tRNA	3685	3750	66			-1	reverse
trnE	tRNA	3750	3817	68			1	reverse
rrnS	rRNA	3819	4770	952			1	forward
trnV	tRNA	4772	4838	67			0	forward
rrnL	rRNA	4839	6198	1360			1	forward
trnL (tag)	tRNA	6200	6268	69			8	forward
trnL (taa)	tRNA	6277	6345	69			0	forward
nad1	CDS	6346	7287	942	ATG	TAA	6	forward
trnP	tRNA	7294	7361	68			0	forward
nad6	CDS	7362	7859	498	ATG	TAA	18	forward
cob	CDS	7878	9017	1140	ATG	TAA	13	forward
trnS (tga)	tRNA	9031	9095	65			0	forward
trnT	tRNA	9096	9161	66			20	reverse
nad4L	CDS	9182	9478	297	ATG	TAG	-7	forward
nad4	CDS	9472	10843	1372	ATG	T--	0	forward
trnH	tRNA	10844	10907	64			0	forward
nad5	CDS	10908	12626	1719	ATG	TAA	0	forward
trnF	tRNA	12627	12694	68			97	forward
cox3	CDS	12792	13571	780	ATG	TAA	24	forward
trnK	tRNA	13596	13662	67			8	forward
trnA	tRNA	13671	13737	67			4	forward
trnR	tRNA	13742	13811	70			7	forward
trnN	tRNA	13819	13886	68			8	forward
trnI	tRNA	13895	13964	70			0	forward
nad3	CDS	13965	14318	354	ATG	TAA	1	forward
trnS (cgt)	tRNA	14320	14387	68			0	forward
nad2	CDS	14388	15443	1056	ATG	TAG	1	forward

Conus (*Lautoconus*) *venulatus*

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	1	1548	1548	ATG	TAA	130	forward
cox2	CDS	1679	2365	687	ATG	TAA	0	forward
trnD	tRNA	2366	2432	67			0	forward
atp8	CDS	2433	2594	162	ATG	TAG	6	forward
atp6	CDS	2601	3296	696	ATG	TAA	40	forward
trnM	tRNA	3337	3404	68			13	reverse
trnY	tRNA	3418	3483	66			4	reverse
trnC	tRNA	3488	3552	65			0	reverse
trnW	tRNA	3553	3618	66			1	reverse
trnQ	tRNA	3620	3677	58			14	reverse
trnG	tRNA	3692	3757	66			1	reverse
trnE	tRNA	3759	3826	68			0	reverse
rrnS	rRNA	3827	4781	955			0	forward
trnV	tRNA	4782	4848	67			0	forward
rrnL	rRNA	4849	6212	1364			0	forward
trnL (tag)	tRNA	6213	6282	70			6	forward
trnL (taa)	tRNA	6289	6357	69			0	forward
nad1	CDS	6358	7299	942	ATG	TAA	3	forward
trnP	tRNA	7303	7369	67			0	forward
nad6	CDS	7370	7870	501	ATG	TAA	11	forward
cob	CDS	7882	9021	1140	ATG	TAA	12	forward
trnS (tga)	tRNA	9034	9098	65			9	forward
trnT	tRNA	9108	9174	67			21	reverse
nad4L	CDS	9196	9492	297	ATG	TAG	-7	forward
nad4	CDS	9486	10867	1382	ATG	TA-	0	forward
trnH	tRNA	10868	10934	67			0	forward
nad5	CDS	10935	12649	1715	ATG	TA-	0	forward
trnF	tRNA	12650	12715	66			126	forward
cox3	CDS	12842	13621	780	ATG	TAA	25	forward
trnK	tRNA	13647	13716	70			4	forward
trnA	tRNA	13721	13787	67			16	forward
trnR	tRNA	13804	13872	69			10	forward
trnN	tRNA	13883	13951	69			14	forward
trnI	tRNA	13966	14035	70			5	forward
nad3	CDS	14041	14394	354	ATG	TAA	8	forward
trnS (cgt)	tRNA	14403	14470	68			0	forward
nad2	CDS	14471	15524	1054	ATG	T--	0	forward

Conus (*Lautoconus*) *ventricosus*

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	1	1548	1548	ATG	TAA	130	forward
cox2	CDS	1679	2365	687	ATG	TAA	0	forward
trnD	tRNA	2366	2432	67			0	forward
atp8	CDS	2433	2594	162	ATG	TAA	6	forward
atp6	CDS	2601	3296	696	ATG	TAA	35	forward
trnM	tRNA	3332	3399	68			12	reverse
trnY	tRNA	3412	3477	66			1	reverse
trnC	tRNA	3479	3543	65			0	reverse
trnW	tRNA	3544	3610	67			1	reverse
trnQ	tRNA	3612	3669	58			15	reverse
trnG	tRNA	3685	3750	66			2	reverse
trnE	tRNA	3753	3818	66			0	reverse
rrnS	rRNA	3819	4769	951			0	forward
trnV	tRNA	4770	4837	68			0	forward
rrnL	rRNA	4838	6201	1364			0	forward
trnL (tag)	tRNA	6202	6271	70			18	forward
trnL (taa)	tRNA	6290	6358	69			0	forward
nad1	CDS	6359	7300	942	ATG	TAG	4	forward
trnP	tRNA	7305	7372	68			0	forward
nad6	CDS	7373	7873	501	ATG	TAG	11	forward
cob	CDS	7885	9024	1140	ATG	TAG	11	forward
trnS (tga)	tRNA	9036	9100	65			9	forward
trnT	tRNA	9110	9176	67			20	reverse
nad4L	CDS	9197	9493	297	ATG	TAG	-7	forward
nad4	CDS	9487	10868	1382	ATG	TA-	0	forward
trnH	tRNA	10869	10935	67			0	forward
nad5	CDS	10936	12651	1716	ATG	TAA	10	forward
trnF	tRNA	12662	12726	65			126	forward
cox3	CDS	12853	13632	780	ATG	TAA	23	forward
trnK	tRNA	13656	13725	70			6	forward
trnA	tRNA	13732	13797	66			18	forward
trnR	tRNA	13816	13884	69			10	forward
trnN	tRNA	13895	13964	70			9	forward
trnI	tRNA	13974	14043	70			5	forward
nad3	CDS	14049	14402	354	ATG	TAA	8	forward
trnS (cgt)	tRNA	14411	14478	68			0	forward
nad2	CDS	14479	15534	1056	ATG	TAA	0	forward

Conus (*Lautoconus*) *hybridus*

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2655	4202	1548	ATG	TAA	120	forward
cox2	CDS	4323	5009	687	ATG	TAG	0	forward
trnD	tRNA	5010	5076	67			0	forward
atp8	CDS	5077	5238	162	ATG	TAA	6	forward
atp6	CDS	5245	5940	696	ATG	TAG	34	forward
trnM	tRNA	5975	6042	68			12	reverse
trnY	tRNA	6055	6121	67			1	reverse
trnC	tRNA	6123	6187	65			0	reverse
trnW	tRNA	6188	6253	66			-3	reverse
trnQ	tRNA	6251	6317	67			10	reverse
trnG	tRNA	6328	6393	66			2	reverse
trnE	tRNA	6396	6461	66			0	reverse
rrnS	rRNA	6462	7409	948			0	forward
trnV	tRNA	7410	7476	67			0	forward
rrnL	rRNA	7477	8840	1364			0	forward
trnL (tag)	tRNA	8841	8910	70			2	forward
trnL (taa)	tRNA	8913	8981	69			0	forward
nad1	CDS	8982	9923	942	ATG	TAA	4	forward
trnP	tRNA	9928	9994	67			0	forward
nad6	CDS	9995	10495	501	ATG	TAA	12	forward
cob	CDS	10508	11647	1140	ATG	TAG	12	forward
trnS (tga)	tRNA	11660	11724	65			10	forward
trnT	tRNA	11735	11801	67			20	reverse
nad4L	CDS	11822	12118	297	ATG	TAG	-7	forward
nad4	CDS	12112	13493	1382	ATG	TA-	0	forward
trnH	tRNA	13494	13560	67			0	forward
nad5	CDS	13561	15276	1716	ATG	TAA	0	forward
cox3	CDS	1	753	753	---	TAA	22	forward
trnK	tRNA	776	847	72			5	forward
trnA	tRNA	853	919	67			18	forward
trnR	tRNA	938	1006	69			10	forward
trnN	tRNA	1017	1085	69			9	forward
trnI	tRNA	1095	1164	70			5	forward
nad3	CDS	1170	1523	354	ATG	TAA	8	forward
trnS (cgt)	tRNA	1532	1599	68			0	forward
nad2	CDS	1600	2654	1055	ATG	TA-	0	forward

Conus (*Eugeniconus*) *nobilis victor*

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2701	4248	1548	ATG	TAA	159	forward
cox2	CDS	4408	5094	687	ATG	TAA	0	forward
trnD	tRNA	5095	5161	67			0	forward
atp8	CDS	5162	5323	162	ATG	TAG	6	forward
atp6	CDS	5330	6025	696	ATG	TAG	36	forward
trnM	tRNA	6062	6129	68			8	reverse
trnY	tRNA	6138	6206	69			0	reverse
trnC	tRNA	6207	6270	64			0	reverse
trnW	tRNA	6271	6336	66			-3	reverse
trnQ	tRNA	6334	6412	79			-4	reverse
trnG	tRNA	6409	6474	66			1	reverse
trnE	tRNA	6476	6540	65			0	reverse
rrnS	rRNA	6541	7502	962			0	forward
trnV	tRNA	7503	7570	68			0	forward
rrnL	rRNA	7571	8942	1372			0	forward
trnL (tag)	tRNA	8943	9012	70			6	forward
trnL (taa)	tRNA	9019	9087	69			0	forward
nad1	CDS	9088	10029	942	ATG	TAA	4	forward
trnP	tRNA	10034	10100	67			0	forward
nad6	CDS	10101	10601	501	ATG	TAA	9	forward
cob	CDS	10611	11750	1140	ATG	TAA	12	forward
trnS (tga)	tRNA	11763	11827	65			9	forward
trnT	tRNA	11837	11903	67			20	reverse
nad4L	CDS	11924	12220	297	ATG	TAG	-7	forward
nad4	CDS	12214	13595	1382	ATG	TA-	0	forward
trnH	tRNA	13596	13663	68			0	forward
nad5	CDS	13664	15379	1716	ATG	TAA	0	forward
cox3	CDS	1	780	780	---	TAG	31	forward
trnK	tRNA	812	881	70			20	forward
trnA	tRNA	902	968	67			15	forward
trnR	tRNA	984	1054	71			9	forward
trnN	tRNA	1064	1131	68			7	forward
trnI	tRNA	1139	1211	73			4	forward
nad3	CDS	1216	1569	354	ATG	TAG	8	forward
trnS (cgt)	tRNA	1578	1645	68			0	forward
nad2	CDS	1646	2700	1055	ATG	TA-	0	forward

Conasprella wakayamaensis

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2639	4210	1572	ATG	TAA	734	forward
cox2	CDS	4945	5630	686	ATG	TA-	0	forward
trnD	tRNA	5631	5699	69			0	forward
atp8	CDS	5700	5858	159	ATG	TAA	3	forward
atp6	CDS	5862	6557	696	ATG	TAA	46	forward
trnM	tRNA	6604	6670	67			1	reverse
trnY	tRNA	6672	6738	67			0	reverse
trnC	tRNA	6739	6804	66			0	reverse
trnW	tRNA	6805	6870	66			-2	reverse
trnQ	tRNA	6869	6945	77			-2	reverse
trnG	tRNA	6944	7010	67			0	reverse
trnE	tRNA	7011	7077	67			0	reverse
rrnS	rRNA	7078	8029	952			0	forward
trnV	tRNA	8030	8097	68			0	forward
rrnL	rRNA	8098	9479	1382			0	forward
trnL (tag)	tRNA	9480	9548	69			5	forward
trnL (taa)	tRNA	9554	9621	68			0	forward
nad1	CDS	9622	10563	942	ATG	TAA	5	forward
trnP	tRNA	10569	10638	70			0	forward
nad6	CDS	10639	11139	501	ATG	TAG	21	forward
cob	CDS	11161	12300	1140	ATG	TAA	11	forward
trnS (tga)	tRNA	12312	12376	65			1	forward
trnT	tRNA	12378	12445	68			36	reverse
nad4L	CDS	12482	12778	297	ATG	TAG	-7	forward
nad4	CDS	12772	14143	1372	ATG	T--	0	forward
trnH	tRNA	14144	14208	65			0	forward
nad5	CDS	14209	15927	1719	ATG	TAG	0	forward
cox3	CDS	1	759	759	---	TAA	24	forward
trnK	tRNA	784	852	69			6	forward
trnA	tRNA	859	925	67			0	forward
trnR	tRNA	926	995	70			11	forward
trnN	tRNA	1007	1077	71			10	forward
trnI	tRNA	1088	1158	71			2	forward
nad3	CDS	1161	1512	352	ATG	T--	0	forward
trnS (cgt)	tRNA	1513	1580	68			0	forward
nad2	CDS	1581	2636	1056	ATG	TAA	0	forward

Lilliconus sagei

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2964	4511	1548	ATG	TAA		forward
cox2	CDS	4584	5270	687	ATG	TAA		forward
trnD	tRNA	5277	5346	70				forward
atp8	CDS	5347	5508	162	ATG	TAA		forward
atp6	CDS	5518	6213	696	ATG	TAA		forward
trnM	tRNA	6249	6317	69				reverse
trnY	tRNA	6318	6387	70				reverse
trnC	tRNA	6386	6451	66				reverse
trnW	tRNA	6454	6520	67				reverse
trnQ	tRNA	6518	6584	67				reverse
trnG	tRNA	6592	6658	67				reverse
trnE	tRNA	6657	6724	68				reverse
rrnS	rRNA	6799	7762	964				forward
trnV	tRNA	7763	7831	69				forward
rrnL	rRNA	7832	9174	1343				forward
trnT	tRNA	9181	9250	70				forward
nad1	CDS	9317	10258	942	ATG	TAG		forward
trnP	tRNA	10262	10328	67				forward
nad6	CDS	10329	10826	498	ATG	TAA		forward
cob	CDS	10837	11976	114	ATG	TAA		forward
trnS (tga)	tRNA	11977	12041	65				reverse
nad4L	CDS	12043	12339	297	ATG	TAG		forward
nad4	CDS	12333	13701	1369	GTG	T--		forward
trnH	tRNA	13702	13766	65				forward
nad5	CDS	13767	15485	1719	ATG	TAA		forward
cox3	CDS	1	756	756	---	TAA		forward
trnK	tRNA	767	833	67				forward
trnA	tRNA	844	912	69				forward
trnN	tRNA	951	1017	67				forward
trnI	tRNA	1021	1090	70				forward
nad3	CDS	1094	1447	354	ATG	TAA		forward
trnS (cgt)	tRNA	1449	1516	68				forward
nad2	CDS	1517	2590	1074	ATG	TAA		forward
trnL (taa)	tRNA	2619	2691	73				forward
trnL (tag)	tRNA	2836	2903	68				forward

Profundiconus terimachi

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2636	4183	1548	ATG	TAA	160	forward
cox2	CDS	4344	5028	685	ATG	T--	0	forward
trnD	tRNA	5029	5097	69			0	forward
atp8	CDS	5098	5256	159	ATG	TAA	4	forward
atp6	CDS	5261	5956	696	ATG	TAA	38	forward
trnM	tRNA	5995	6062	68			4	reverse
trnY	tRNA	6067	6133	67			0	reverse
trnC	tRNA	6134	6199	66			0	reverse
trnW	tRNA	6200	6265	66			-3	reverse
trnQ	tRNA	6263	6341	79			-10	reverse
trnG	tRNA	6332	6398	67			0	reverse
trnE	tRNA	6399	6466	68			0	reverse
rrnS	rRNA	6467	7422	956			0	forward
trnV	tRNA	7423	7490	68			0	forward
rrnL	rRNA	7491	8877	1387			0	forward
trnL (tag)	tRNA	8878	8946	69			2	forward
trnL (taa)	tRNA	8949	9017	69			0	forward
nad1	CDS	9018	9959	942	ATG	TAG	9	forward
trnP	tRNA	9969	10035	67			0	forward
nad6	CDS	10036	10536	501	ATG	TAG	6	forward
cob	CDS	10543	11682	114	ATG	TAA	8	forward
trnS (tga)	tRNA	11691	11755	65			2	forward
trnT	tRNA	11758	11824	67			15	reverse
nad4L	CDS	11840	12136	297	ATG	TAG	-7	forward
nad4	CDS	12130	13498	1369	ATG	T--	0	forward
trnH	tRNA	13499	13563	65			0	forward
nad5	CDS	13564	15279	1716	ATG	TAA	0	forward
cox3	CDS	1	771	771	---	TAA	18	forward
trnK	tRNA	790	856	67			8	forward
trnA	tRNA	865	931	67			0	forward
trnR	tRNA	932	1001	70			14	forward
trnN	tRNA	1016	1081	66			-5	forward
trnI	tRNA	1077	1153	77			2	forward
nad3	CDS	1156	1509	354	ATG	TAA	0	forward
trnS (cgt)	tRNA	1510	1577	68			0	forward
nad2	CDS	1578	2635	1058	ATG	TA-	0	forward

Pseudolilliconus traillii

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2611	4157	1547	ATG	TAA	-12	forward
trnT	tRNA	4146	4207	62			10	reverse
cox2	CDS	4218	4904	687	ATG	TAG	10	forward
trnD	tRNA	4915	4983	69			5	forward
atp8	CDS	4989	5144	156	ATG	TAA	16	forward
atp6	CDS	5161	5888	728	ATG	TA-	0	forward
trnM	tRNA	5889	5954	66			8	reverse
trnY	tRNA	5963	6029	67			1	reverse
trnC	tRNA	6031	6093	63			2	reverse
trnW	tRNA	6096	6159	64			-3	reverse
trnQ	tRNA	6157	6229	73			-4	reverse
trnG	tRNA	6226	6288	63			-1	reverse
trnE	tRNA	6288	6350	63			0	reverse
rrnS	rRNA	6351	7278	928			0	forward
trnV	tRNA	7279	7349	71			0	forward
rrnL	rRNA	7350	8657	1308			0	forward
trnL (tag)	tRNA	8658	8724	67			3	forward
trnL (taa)	tRNA	8728	8792	65			0	forward
nad1	CDS	8793	9737	945	GTG	TAA	2	forward
trnP	tRNA	9740	9806	67			0	forward
nad6	CDS	9807	10298	492	GTG	TAA	2	forward
cob	CDS	10301	11439	1139	ATG	TAG	15	forward
trnS (cgt)	tRNA	11455	11519	65			8	forward
nad4L	CDS	11528	11824	297	ATG	TAG	-7	forward
nad4	CDS	11818	13183	1366	ATG	T--	0	forward
trnH	tRNA	13184	13250	67			0	forward
nad5	CDS	13251	14963	1713	ATA	TAA	0	forward
cox3	CDS	1	771	771	---	TAA	23	forward
trnK	tRNA	794	860	67			3	forward
trnA	tRNA	863	929	67			7	forward
trnR	tRNA	936	999	64			5	forward
trnN	tRNA	1004	1074	71			2	forward
trnI	tRNA	1076	1144	69			1	forward
nad3	CDS	1145	1497	353	ATG	TA-	1	forward
trnS (cgt)	tRNA	1498	1565	68			1	forward
nad2	CDS	1566	2610	1045	ATG	T--	0	forward

Benthomangelia sp.

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2585	4135	1551	ATG	TAA	63	forward
cox2	CDS	4199	4883	685	ATG	T--	0	forward
trnD	tRNA	4884	4949	66			0	forward
atp8	CDS	4950	5108	159	ATG	TAA	2	forward
atp6	CDS	5111	5806	696	ATG	TAG	36	forward
trnM	tRNA	5843	5909	67			-1	reverse
trnY	tRNA	5909	5974	66			1	reverse
trnC	tRNA	5976	6038	63			-6	reverse
trnW	tRNA	6033	6104	72			-3	reverse
trnQ	tRNA	6102	6169	68			3	reverse
trnG	tRNA	6173	6237	65			-1	reverse
trnE	tRNA	6237	6301	65			0	reverse
rrnS	rRNA	6302	7252	951			0	forward
trnV	tRNA	7253	7315	63			0	forward
rrnL	rRNA	7316	8649	1334			0	forward
trnL (tag)	tRNA	8650	8715	66			0	forward
trnL (taa)	tRNA	8716	8784	69			0	forward
nad1	CDS	8785	9726	942	ATG	TAA	8	forward
trnP	tRNA	9735	9802	68			0	forward
nad6	CDS	9803	10304	502	ATG	T--	0	forward
cob	CDS	10305	11444	114	ATG	TAA	11	forward
trnS (tga)	tRNA	11456	11520	65			0	forward
trnT	tRNA	11521	11584	64			6	reverse
nad4L	CDS	11591	11887	297	ATG	TAG	-7	forward
nad4	CDS	11881	13252	1372	ATG	T--	0	forward
trnH	tRNA	13253	13317	65			1	forward
nad5	CDS	13319	15034	1716	ATG	TAG	0	forward
cox3	CDS	1	756	756	---	TAA	11	forward
trnK	tRNA	768	832	65			0	forward
trnA	tRNA	833	899	67			1	forward
trnR	tRNA	901	967	67			5	forward
trnN	tRNA	973	1041	69			4	forward
trnI	tRNA	1046	1112	67			1	forward
nad3	CDS	1114	1465	352	ATG	T--	0	forward
trnS (cgt)	tRNA	1466	1533	68			0	forward
nad2	CDS	1534	2584	1051	ATG	T--	0	forward

Tomopleura sp.

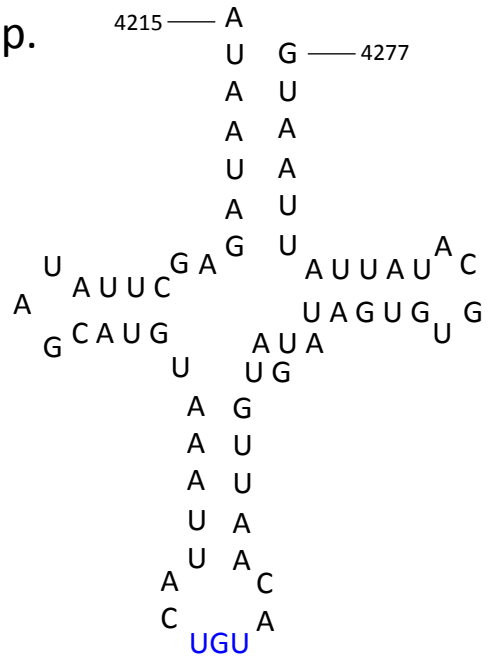
		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2615	4162	1548	ATG	TAG	44	forward
trnT	tRNA	4207	4276	70			-2	reverse
cox2	CDS	4275	4959	685	ATG	T--	0	forward
trnD	tRNA	4960	5028	69			0	forward
atp8	CDS	5029	5187	159	ATG	TAA	7	forward
atp6	CDS	5195	5890	696	ATG	TAA	36	forward
trnM	tRNA	5927	5993	67			3	reverse
trnY	tRNA	5997	6064	68			0	reverse
trnC	tRNA	6065	6129	65			0	reverse
trnW	tRNA	6130	6195	66			-3	reverse
trnQ	tRNA	6193	6263	71			-2	reverse
trnG	tRNA	6262	6326	65			0	reverse
trnE	tRNA	6327	6396	70			0	reverse
rrnS	rRNA	6397	7347	951			0	forward
renV	tRNA	7348	7414	67			0	forward
rrnL	rRNA	7415	8772	1358			0	forward
trnL (tag)	tRNA	8773	8841	69			5	forward
trnL (taa)	tRNA	8847	8915	69			0	forward
nad1	CDS	8916	9857	942	ATG	TAA	3	forward
trnP	tRNA	9861	9929	69			0	forward
nad6	CDS	9930	10430	501	ATG	TAA	11	forward
cob	CDS	10442	11581	114	ATG	TAG	6	forward
trnS (tga)	tRNA	11588	11652	65			84	forward
nad4L	CDS	11737	12033	297	ATG	TAG	-7	forward
nad4	CDS	12027	13365	1339	ATG	TAA	33	forward
trnH	tRNA	13399	13462	64			1	forward
nad5	CDS	13464	15182	1719	ATG	TAA	0	forward
cox3	CDS	1	756	756	---	TAG	29	forward
trnK	tRNA	786	855	70			5	forward
trnA	tRNA	861	928	68			0	forward
trnR	tRNA	929	994	66			0	forward
trnN	tRNA	995	1063	69			6	forward
trnI	tRNA	1070	1139	70			0	forward
nad3	CDS	1140	1493	354	ATG	TAA	0	forward
trnS (cgt)	tRNA	1494	1561	68			0	forward
nad2	CDS	1562	2614	1053	ATG	TAA	0	forward

Glyphostoma sp.

		Gene			Codon			
Gene	**Type**	**Start**	**Stop**	**Length**	**Start**	**Stop**	**Intergenic**	**Strand**
cox1	CDS	2672	4219	1548	ATG	TAG	93	forward
cox2	CDS	4313	4997	685	ATG	T--	0	forward
trnD	tRNA	4998	5066	69			0	forward
atp8	CDS	5067	5225	159	ATG	TAG	20	forward
atp6	CDS	5246	5941	696	ATG	TAA	32	forward
trnM	tRNA	5974	6040	67			1	reverse
trnY	tRNA	6042	6109	68			12	reverse
trnC	tRNA	6122	6186	65			-6	reverse
trnW	tRNA	6181	6254	74			-3	reverse
trnQ	tRNA	6252	6318	67			3	reverse
trnG	tRNA	6322	6388	67			0	reverse
trnE	tRNA	6389	6454	66			0	reverse
rrnS	rRNA	6455	7399	945			0	forward
trnV	tRNA	7400	7467	68			0	forward
rrnL	rRNA	7468	8845	1378			0	forward
trnL (tag)	tRNA	8846	8914	69			10	forward
trnL (taa)	tRNA	8925	8993	69			0	forward
nad1	CDS	8994	9935	942	ATG	TAA	5	forward
trnP	tRNA	9941	10009	69			0	forward
nad6	CDS	10010	10519	510	ATG	TAA	19	forward
cob	CDS	10539	11678	114	ATG	TAG	5	forward
trnS (cgt)	tRNA	11684	11748	65			0	forward
trnT	tRNA	11749	11815	67			9	reverse
nad4L	CDS	11825	12121	297	ATG	TAG	-7	forward
nad4	CDS	12115	13370	132	ATG	---	0	forward
cox3	CDS	1	741	741	---	TAA	14	forward
trnK	tRNA	756	820	65			34	forward
trnA	tRNA	855	921	67			1	forward
trnR	tRNA	923	988	66			17	forward
trnN	tRNA	1006	1071	66			24	forward
trnI	tRNA	1096	1163	68			4	forward
nad3	CDS	1168	1521	354	ATG	TAG	0	forward
trnS (cgt)	tRNA	1522	1589	68			0	forward
nad2	CDS	1590	2642	1053	ATG	TAA	29	forward

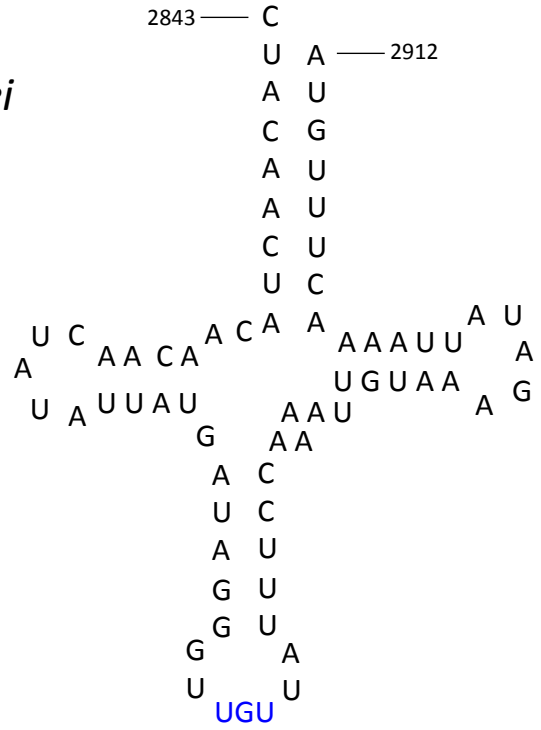
Tomopleura sp.

tRNA^{Thr} *reverce*



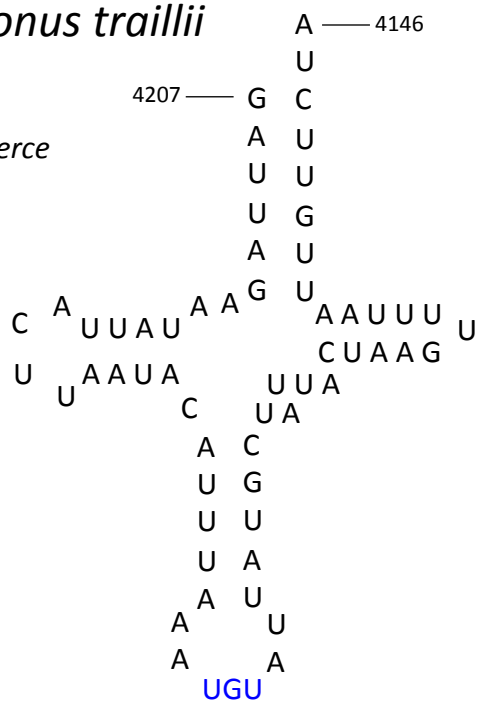
Liciconus sagei

tRNA^{Thr}



Pseudolilliconus traillii

tRNA^{Thr} *reverce*



Liciconus sagei

tRNA^{Leu} *reverce*