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1 **Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea)**

2

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31

32 **Abstract**

33 We present a large-scale molecular phylogeny that includes 320 of the 761 recognized valid
34 species of the cone snails (*Conus*), one of the most diverse groups of marine molluscs, based
35 on three mitochondrial genes (COI, 16S rDNA and 12S rDNA). This is the first phylogeny of
36 the taxon to employ concatenated sequences of several genes, and it includes more than twice
37 as many species as the last published molecular phylogeny of the entire group nearly a decade
38 ago. Most of the numerous molecular phylogenies published during the last 15 years are
39 limited to rather small fractions of its species diversity. Bayesian and maximum likelihood
40 analyses are mostly congruent and confirm the presence of three previously reported highly
41 divergent lineages among cone snails, and one identified here using molecular data. About 85
42 % of the species cluster in the single Large Major Clade; the others are divided between the
43 Small Major Clade (~ 12%), the *Conus californicus* lineage (one species), and a newly
44 defined clade (~ 3%). We also define several subclades within the Large and Small major
45 clades, but most of their relationships remain poorly supported. To illustrate the usefulness of
46 molecular phylogenies in addressing specific evolutionary questions, we analyse the evolution
47 of the diet, the biogeography and the toxins of cone snails. All cone snails whose feeding
48 biology is known inject venom into large prey animals and swallow them whole. Predation on
49 polychaete worms is inferred as the ancestral state, and diet shifts to molluscs and fishes
50 occurred rarely. The ancestor of cone snails probably originated from the Indo-Pacific; rather
51 few colonisations of other biogeographic provinces have probably occurred. A new
52 classification of the Conidae, based on the molecular phylogeny, is published in an
53 accompanying paper.

54 **Keywords:** Ancestral state reconstruction, Conidae, *Conus*, COI, 16SrRNA, 12SrRNA.

55

56 **1. INTRODUCTION**

57

58 A molecular phylogeny of a taxon is a hypothesis of its evolutionary patterns and
59 processes, and a framework for clarifying its classification. A strongly supported molecular-
60 based phylogenetic tree can help to estimate diversification rates, divergence times, ancestral
61 distributions, and community compositions, and it can provide evidence relevant to taxonomic
62 hypotheses. However, many taxa of considerable evolutionary and practical importance have
63 very incomplete species-level molecular phylogenies, based on few species with appropriate
64 genes sequenced, not representative of the diversity of the group, or largely unresolved.

65 The gastropod family Conidae, commonly known as cone snails, includes the widely
66 distributed, mainly tropical *Conus*, a relatively young genus first appearing in the Early
67 Eocene. The family Conidae is one of the most diverse in the marine environment (Kohn,
68 1990), with 761 valid Recent species currently (21th January 2014) recognized in the World
69 Register of Marine Species ([WoRMS](#), 2013) and new species descriptions are published each
70 year. It is also the most rapidly diversifying marine molluscan genus (Kohn, 1990; Stanley,
71 2008, 1979) and is ecologically important especially in coral reef environments where up to
72 36 species, specialized predators on worms, other molluscs, and fishes, co-occur on a single
73 reef (Kohn, 2001).

74 These latter attributes all likely relate to the extremely diverse peptide venoms that cone
75 snails use to overcome and capture prey and that also make the Conidae a most promising
76 source for neurobiologic and therapeutic applications (Biass et al., 2009; Lluisma et al., 2012;
77 Olivera, 2006). Molecular geneticists, evolutionary biologists, pharmacologists, and
78 toxicologists thus all require a robust phylogeny and taxonomy for this group. New drug
79 discovery is particularly likely to benefit from a clear phylogenetic context that permits

80 targeting divergent lineages and thus potential novel toxins (Biggs et al., 2010; Olivera,
81 2006).

82 Since the first published molecular phylogenies for *Conus* (Duda and Palumbi, 1999a;
83 Monje et al., 1999), many others have appeared, either for the cone snails and their relatives
84 (Puillandre et al., 2011a, 2008), or subgroups (Bandyopadhyay et al., 2008; Biggs et al., 2010;
85 Cunha et al., 2008, 2005; Duda and Kohn, 2005; Duda and Palumbi, 2004, 1999b; Duda and
86 Rolán, 2005; Duda et al., 2008, 2001; Espino et al., 2008; Espiritu et al., 2001; Kaufenstein et
87 al., 2011, 2004; Kraus et al., 2012, 2011; Nam et al., 2009; Pereira et al., 2010; Puillandre et
88 al., 2010; Williams and Duda, 2008). The most comprehensive includes 138 species, ca. 20%
89 of the known diversity of cone snails (Duda and Kohn, 2005).

90 Ancestral states of morphological, ecological, and developmental traits have been inferred
91 from some of these phylogenetic studies (Cunha et al., 2005; Duda and Palumbi, 2004, 1999a;
92 Duda et al., 2001; Kohn, 2012) and lineages of toxins with unknown functions identified
93 (Puillandre et al., 2010). However, these authors generally agree that available phylogenies
94 are not complete enough to robustly test hypotheses about how natural history attributes relate
95 to factors that could explain the evolutionary history of the cone snails.

96 Cone snails experienced several episodes of enhanced diversification since their origin
97 (Duda and Kohn, 2005; Kohn, 1990; Williams and Duda, 2008) and exhibit the highest rate of
98 diversification of any marine gastropod or bivalve group (Stanley, 1979), a remarkable
99 radiation that was likely driven by ecological speciation (Stanley, 2008). Currently they occur
100 mostly throughout tropical regions of our world's oceans, although the overwhelming
101 majority of species, both fossil and recent, are restricted to single marine biogeographic
102 provinces (e.g., Indo-Pacific, East Pacific, West Atlantic, East Atlantic and South Africa)
103 (Duda and Kohn, 2005). Results from previous molecular phylogenetic analysis suggest that
104 three major lineages arose shortly after the origination of the group: one with extant species

105 mostly occurring in the present-day Indo-Pacific, another with most extant species found in
106 the present-day East Pacific and West Atlantic, and a third that today consists of a single
107 species that is restricted to the East Pacific (Duda and Kohn, 2005). Based on the geographic
108 distributions of species in these clades, there has apparently been very little interchange of
109 lineages among the major marine biogeographic provinces (Duda and Kohn, 2005; Duda and
110 Lessios, 2009). Nonetheless, this work included analyses of sequence data from only one-fifth
111 of the recognized cone snail species and the authors caution that their results are preliminary
112 and the patterns that they observed may change with more complete taxonomic coverage
113 (Duda and Kohn, 2005). Here we examine the biogeography of this group with a much more
114 exhaustive taxonomic and geographic coverage than available previously.

115 While most cone snail species are vermivorous (i.e., feed on a variety of worms, including
116 mostly polychaetes but also hemichordates), others are either piscivorous or molluscivorous,
117 with few species exhibiting more than one feeding mode. In addition, diets tend to be species-
118 specific, especially in areas where multiple species co-occur (Kohn and Nybakken, 1975;
119 Kohn, 1968, 1959). A previous investigation of the evolution of diets of cone snails reports
120 that major shifts in diet were relatively rare (Duda et al., 2001), although piscivory originated
121 at least twice (Duda and Palumbi, 2004). However, as with all past molecular phylogenetic
122 studies of this group, these studies relied on limited taxonomic coverage. Analyses of a much
123 larger dataset may provide additional insights of the evolution of diet that were not available
124 previously.

125 We propose here a molecular phylogeny of the Conidae sensu Bouchet *et al.* (2011),
126 based on three mitochondrial genes (COI, 12S, 16S) sequenced for 320 species (>40% of the
127 known species diversity), and including representatives from the main lineages defined in
128 previous DNA studies: *C. californicus*, the Small Major Clade and the Large Major Clade
129 (Duda and Kohn, 2005). Tucker and Tenorio (2009) classified the Small Major Clade as the

130 Family Conilithidae – it included *C. californicus* – and the Large Major Clade as the family
131 Conidae (see Table 1 for a comparison of the recent classifications of cone snails and related
132 species). We then analyse the evolution of three character sets: diet category, biogeographic
133 province and toxin diversity. Previous molecular phylogenetic studies analysed the main
134 evolutionary diet shifts (from worms to fishes or molluscs) (Duda and Kohn, 2005; Duda and
135 Palumbi, 2004; Duda et al., 2001), but never on such a large dataset. Disentangling the
136 evolution of these traits throughout this hyperdiverse taxon should help to generate and
137 critically examine hypotheses of the factors that promoted its exceptional ecological and
138 evolutionary diversification.

139

140 **2. MATERIAL AND METHODS**

141

142 *2.1. Sampling*

143 The analysed dataset is the result of a joint effort from several museums and laboratories.
144 The Museum National d'Histoire Naturelle (MNHN), Paris provided 493 specimens collected
145 during several recent expeditions in the Indo-Pacific (details are provided in the
146 Supplementary data 1); 88 specimens were collected during the CONCO project in New
147 Caledonia and South Africa, and processed in the University of Frankfurt; 319 specimens
148 were collected and processed by CPM, TFD and BMO or their lab groups. Additionally,
149 sequences from 1207 vouchers were downloaded from GenBank and added to the datasets.
150 Specimens were morphologically identified by the authors and by Eric Monnier, Loïc
151 Limpalaër and Manuel Tenorio; for the GenBank sequences, we followed the identifications
152 provided by the respective authors.

153 Nine vouchers from GenBank were only identified at the genus level (as “*Conus* sp.”). For
154 various reasons, the voucher specimens were not available for all the non-GenBank

155 specimens, but in some cases digital images of shells were available (unpublished data) for
156 confirmation of identifications. In most cases, the morphological identification was double- or
157 triple-checked by several taxonomic specialists of the group. We followed the cone snail
158 taxonomy provided in the World Register of Marine Species (WoRMS, version of 14th May
159 2013) in applying species names to the vouchers: only species names considered valid in
160 WoRMS were applied. All other species-level names that could have been attributed to the
161 specimens were considered subspecies, form or variety names, or as synonyms. In total, the
162 2107 specimens were attributed to 320 species names, representing >40% of the total number
163 of cone snail species considered valid in WoRMS (Supplementary data 2). Additionally, we
164 recognize nine morphospecies as potentially corresponding to undescribed species (numbered
165 from a to i). In total, 1740 COI, 928 16S and 599 12S sequences were analyzed, of which
166 1523 are newly published (Supplementary data 1).

167 Outgroups were chosen according to Puillandre *et al.* (2011a). To test the monophyly of
168 the Conidae, representatives from related groups within the superfamily Conoidea were
169 included: *Benthofascis lozoueti* (Conorbidae), *Bathytoma neocaledonica*, *Borsonia* sp.,
170 *Genota mitriformis* and *Microdrillia* cf. *optima* (Borsoniidae), *Clathurella nigrotincta* and
171 *Etrema* cf. *tenera* (Clathurellidae), *Mitromorpha metula* and *Lovellona atramentosa*
172 (Mitromorphidae), *Anticlinura* sp. and *Benthomangelia* cf. *trophonoidea* (Mangeliidae) and
173 *Eucyclotoma cymatodes* and *Thatcheria mirabilis* (Raphitomidae). Less closely related genera
174 were used as more distant outgroups: *Turris babylonica* (Turridae), and *Terebra textilis*
175 (Terebridae). The non-conoidean *Harpa kajiyamai* (Harpidae) is the most distant outgroup.

176

177 2.2. DNA Extraction and Sequencing

178 Although all laboratories mentioned above utilized the same primer pairs [12S1/12S3
179 (Simon *et al.*, 1991), 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer *et al.*,

180 1994)] and all amplification products were sequenced in both directions, our laboratories used
181 a variety of DNA extraction protocols, amplification conditions and sequencing approaches to
182 obtain sequences of regions of the mitochondrial 12S, 16S and COI genes. For brevity, only
183 methodologies employed at the MNHN are described here. DNA was extracted using 6100
184 Nucleic Acid Prepstation system (Applied Biosystems), the Epmotion 5075 robot (Eppendorf)
185 or DNeasy_96 Tissue kit (Qiagen) for smaller specimens, following the manufacturers'
186 recommendations. All PCR reactions were performed in 25 µl, containing 3 ng of DNA, 1X
187 reaction buffer, 2.5 mM MgCl₂, 0.26 mM dNTP, 0.3 mM each primer, 5% DMSO, and 1.5
188 units of Qbiogene Q-Bio Taq. Amplification consisted of an initial denaturation step at 94°C
189 for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 12S
190 gene, 52°C for 16S and 50°C for COI, followed by extension at 72°C for 1 min. The final
191 extension was at 72°C for 5 min. PCR products were purified and sequenced by sequencing
192 facilities (Genoscope and Eurofins). Specimens and sequences were deposited in GenBank
193 (Supplementary data 1 & 2).

194

195 2.3. Phylogenetic Analyses

196 Sequences were manually (COI gene) or automatically aligned using Muscle 3.8.31
197 (Edgar, 2004) (16S and 12S genes). Preliminary analyses were performed for each gene
198 separately using the Neighbor-Joining algorithm (with a K2P model) implemented in MEGA
199 4 (Tamura et al., 2007) to remove obviously misidentified or contaminated sequences from
200 the dataset. One voucher (GU227112.1 and GU226998.1) identified as *Conus* sp. in GenBank
201 actually corresponded to a member of the Raphitomidae, and eight others were obviously
202 misidentified or contaminated (the sequence clustered with a non-phylogenetically related
203 species: AF126172.1 was identified as *C. monachus* but clustered with *C. radiatus*;
204 AF174157.1 was identified as *C. circumactus* but clustered with *C. parius*; AF036532.1 was

205 identified as *C. distans* but clustered with *C. bandanus*; AF174169.1 was identified as *C.*
206 *frigidus* but clustered with *C. sanguinolentus*; AJ717598.1 was identified as *C. magus* but
207 clustered with *C. furvus*; AF174184.1 was identified as *C. muriculatus* but clustered with *C.*
208 *striatellus*; AB044276.1 was identified as *C. praecellens* but clustered with *C. boholensis* and
209 AY726487.1 was identified as *C. ventricosus* but clustered with *C. venulatus*). Additionally,
210 the unique sequence labelled as *C. centurio* (AY382002.1) was also removed from the
211 dataset, as it also corresponded to a misidentified specimen (M. Tenorio, pers. com.). Finally,
212 28 short COI sequences from GenBank (< 200bp) were also removed from the dataset; all
213 corresponded to species represented by several other specimens in the final dataset. Because
214 COI is generally more variable than 16S and 12S gene regions, COI is usually more valuable
215 for specimen identification and distinction of closely related species. It was thus used to
216 assign unidentified specimens from GenBank and to point at species-level issues. We
217 analysed the COI dataset with ABGD (Puillandre et al., 2012b). This method relies on genetic
218 distances only and seeks to identify in the distribution of genetic distances a gap that would
219 correspond to a threshold between intra-specific and inter-specific distances. The defaults
220 parameters provided on the web version of ABGD (version of March, 2014) were applied.

221 Each gene was analysed independently to check for incongruency between trees. The best
222 model of evolution was selected for each gene and for each codon position of the COI gene
223 using Modelgenerator V.85 (Keane et al., 2006) under the Hierarchical Likelihood Ratio Tests
224 (with four discrete gamma categories): GTR+I+G was always identified as the best model,
225 with $I = 0.98, 0.85, 0.66, 0.58$ and 0.49 and $\alpha = 0.66, 0.25, 0.24, 0.34$ and 0.16 for the COI
226 (first, second and third position of the codon), 16S and 12S genes respectively. Maximum
227 Likelihood analyses (ML) were performed using RAxML 7.0.4 (Stamatakis, 2006), with a
228 GAMMAI model for each gene. Three partitions were defined for the COI gene,
229 corresponding to each position of the codon. RaxML analyses were performed on the Cipres

230 Science Gateway (<http://www.phylo.org/portal2/>) using the RAxML-HPC2 on TG Tool.

231 Accuracy of the results was assessed by bootstrapping (1000 replicates).

232 After visual inspection of the absence of supported incongruencies between the
233 independent trees, a concatenated dataset was prepared by including only one representative
234 of each species name represented in the independent gene datasets. When several specimens
235 were available for a single named species, the preferred specimen had the highest number of
236 genes and with, if possible, an available voucher. Three unnamed morphospecies and 16
237 species were represented by specimens sequenced for only one gene: they were excluded from
238 the concatenated dataset. In several cases, specimens of a named species were found not to be
239 monophyletic (see section 3). In all of these cases, the different specimens remained closely
240 related and only one was included in the final dataset. Finally, 326 specimens (including 16
241 outgroups) were included in the concatenated dataset. ML analyses were performed as
242 described before, with five partitions (three codon positions of the COI gene, 12S and
243 16S). Bayesian Analyses (BA) were performed running two parallel analyses in MrBayes
244 (Huelsenbeck et al., 2001), consisting each of eight Markov chains of 200,000,000
245 generations each with a sampling frequency of one tree each thousand generations. The
246 number of swaps was set to five, and the chain temperature at 0.02. Similarly to the ML
247 approach, unlinked models (each with six substitution categories, a gamma-distributed rate
248 variation across sites approximated in four discrete categories and a proportion of invariable
249 sites) were applied for each partition. Convergence of each analysis was evaluated using
250 Tracer 1.4.1 (Rambaut and Drummond, 2007), and analyses were terminated when ESS
251 values were all superior to 200. A consensus tree was then calculated after omitting the first
252 25% trees as burn-in.

253 The COI gene is more variable than 16S or 12S, COI sequences were available for the
254 largest number of morphospecies, and many of these were represented by several individuals.

255 For these reasons, COI gene trees were used to explore the species-level α -taxonomy of cone
256 snails.

257

258 2.4. Character Evolution

259 The evolution of two characters was analysed by mapping their character states on the
260 Bayesian phylogenetic tree obtained with the concatenated dataset: geographic distribution
261 (five states: East Atlantic; East Pacific; Indo-Pacific; South Africa; West Atlantic) and prey
262 type (four states: worms; fishes; molluscs; worms, fishes, crustaceans and molluscs). The prey
263 type was based on direct observation for 100 species, was inferred from the radula type for
264 103 species and remains unknown for 107 species
265 (<http://biology.burke.washington.edu/conus/>). It should be noted that the vermivorous type
266 may refer to preys from different phyla. However, among the 53 species for which the
267 vermivorous diet was based on direct observation, only one species (*C. leopardus*) is known
268 to mainly feed on a non-polychaete (enteropneust *Ptychodera* - Kohn, 1959) The evolution of
269 the prey type was assessed with Mesquite V2.74 (Maddison and Maddison, 2009), using the
270 option ‘tracing character history’ and the likelihood ancestral reconstruction method. The
271 BBM (Bayesian Binary MCMC) method implemented in RASP (Yu et al., 2013, 2010) was
272 used to reconstruct ancestral ranges for each node. To account for uncertainties, the 10,000
273 last trees obtained with the Bayesian analyses were loaded. Analyses were run with default
274 parameters, except the number of cycles (set to 500,000) and the root distribution (set to
275 “wide”).

276

277 3. RESULTS

278

279 3.1. Species-Level Phylogeny

280 Final alignments included 658bp, 457bp and 553bp for the COI, 16S and 12S genes
281 respectively. Single-gene analyses produced poorly resolved trees (Supplementary data 3-5),
282 with only a few clades supported. Trees constructed with the concatenated dataset also
283 recovered these clades, albeit with higher support. However, single-gene trees are useful to
284 identify unknown specimens and for evaluation of species-level taxonomy of cone snails.

285 The eight remaining unidentified *Conus* from GenBank (after one was discarded from the
286 dataset because it was not a cone snail) were identified following a barcoding approach in
287 which an unknown specimen is identified based on the identity of its closest neighbour in the
288 tree (Austerlitz et al., 2009): one specimen which consisted of an egg capsule collected in the
289 Philippines (Puillandre et al., 2009) matched *C. australis*; five other specimens (Cunha et al.,
290 2008, 2005) belonged to the *C. venulatus* complex; another matched *C. capitaneus* (Dang et
291 al., unpublished); and the last corresponded to *C. tabidus* (Cunha et al., 2005).

292 In addition to the phylogenetic analyses, the ABGD method was also used to interpret the
293 species complexes. In the vicinity of the barcode gap, the ABGD method constantly returns a
294 partition in 343 primary species hypotheses (PSH). Because it is not the primary objective of
295 this article, and because most species are represented by one or a few specimens only, we will
296 not discuss in detail the ABGD results, but instead identify the problematic cases and suggest
297 that they deserve more in-depth analyses. In numerous cases several species names were
298 mixed in a single clade. For most of them (*C. aulicus*/*C. episcopatus*/*C. magnificus*, *C.*
299 *dalli*/*C. canonicus*, *C. frigidus*/*C. flavidus*, *C. jaspideus*/*C. mindanus*, *C. mucronatus*/*C.*
300 *sutanorcum*, *C. muriculatus*/*C. floridulus*, *C. sulcatus* complex, *C. striatellus*/*C. planorbis*/*C.*
301 *ferrugineus*, *C. ximenes*/*C. mahogani*, *C. loyaltiensis*/*C. kanakinus*/*C. vaubani*, *C.*
302 *pennaceus*/*C. crocatus*/*C. lohri*, *C. bandanus*/*C. marmoreus*, *C. pagodus*/*C. aff. eucoronatus*
303 and *C. tessulatus*/*C. eburneus*/*C. suturatus*/*C. sandwichensis*) correlating these preliminary
304 results with morphological, geographical or bathymetrical variation would require analyses of

305 additional specimens. Nonetheless, in some cases we can propose preliminary hypotheses to
306 interpret the results. *Conus arenatus* occurs in two clades, one corresponding to the form
307 *aequipunctatus* and the other being mixed with *C. pulicarius*; ABGD places these two
308 lineages in two different PSH. In the case of *C. lividus* and *C. sanguinolentus* (only two
309 specimens from GenBank, one for each name), specimens may have been incorrectly
310 identified as *C. lividus* or *C. sanguinolentus* or the morphological criteria used to delimit these
311 species are inappropriate. For members of the *C. teramachii/C. smirna/C. aff. profundorum/C.*
312 *n. sp. g* complex (Fig. 1A), four clades are recognized: two restricted to New Caledonia (one
313 including *C. n. sp. g* and the second containing specimens with *C. profundorum*-like shells),
314 another to Madagascar (it would correspond to the form *neotorquatus* of *C. teramachii*), and
315 one that occurs in the Philippines, Solomon Islands, Papua-New Guinea and New Caledonia
316 (with *C. smirna* and *C. teramachii*-like shells). In this complex ABGD recognizes only three
317 PSH, merging the *C. profundorum*-like shells and the Philippines/Solomon Islands/Papua-
318 New Guinea/New Caledonia clade in a single PSH. Also, several species complexes were
319 revealed that have been treated previously (*C. sponsalis* complex in Duda *et al.* (2008), *C.*
320 *orbigny* complex in Puillandre *et al.* (2011b), *C. ventricosus* complex in Cunha *et al.* (2005)
321 and Duda and Rolan (2005) and *C. venulatus* complex in Cunha *et al.* (2005), Cunha *et al.*
322 (2008) and Duda and Rolan (2005), but our results suggest that their taxonomy is not fully
323 resolved yet, and that numerous cryptic species still need formal description.

324 Sequences of specimens representing 11 species names were not monophyletic and
325 included two (*C. miliaris*, *C. glans*, *C. longurionis*, *C. mappa*, *C. quercinus*, *C. villepinii*, *C.*
326 *generalis*, *C. regius*) or three (*C. australis*, *C. daucus*, *C. imperialis*) lineages. All these
327 lineages correspond to different PSH as defined by ABGD, the high genetic distances thus
328 suggesting that they may belong to different species. In some cases, one of the lineages is
329 geographically (e.g., *C. longurionis*) or bathymetrically (e.g., two of the *C. imperialis* lineages

330 – Fig. 1B) distinct. In other cases, one is associated with a previously recognized subspecies
331 or forms (e.g., *granarius* for one lineage of *C. mappa*, *fulgetrum* for *C. miliaris* – Fig. 1C,
332 *maldivus* for *C. generalis*, *abbotii* for *C. regius*, *gabryae* for *C. australis*, *boui* for *C. daucus*,
333 and *fuscatus* for *C. imperialis*). The two lineages of *C. quercinus* (one being identified as “aff
334 *quercinus*”) were not found with the 12S and 16S genes. In several other cases, divergent
335 lineages within a single morphospecies were revealed, although the corresponding
336 morphospecies remained monophyletic, thus suggesting the presence of cryptic species (e.g.,
337 *C. consors*), some of which are associated with a previously described subspecies or form
338 (e.g. *archiepiscopus* for *C. textile*). ABGD defines two PSH associated with the name *C.*
339 *consors* and three with the name *C. textile*. Finally, in a few cases (e.g., *C. recurvus* and *C.*
340 *virgatus*), two species names shared identical or very similar sequences, suggesting
341 synonymy; ABGD places them in a single PSH. However, the low number of specimens
342 sequenced for each species name prevents adequate evaluation of this hypothesis.

343

344 3.2. Phylogeny Above the Species Level

345 Analyses of the concatenated dataset revealed four main highly divergent clades (Fig 2,
346 Supplementary data 6). Three of them correspond to previously reported lineages with
347 molecular data: one with only one species (*C. californicus*), a second corresponding to the
348 Small Major Clade (SMC – *sensu* (Duda and Kohn, 2005) and roughly to the Conilithinae
349 (*sensu* Tucker and Tenorio, 2009), and a third, the most species-rich, corresponding to the
350 Large Major Clade (LMC – *sensu* (Duda and Kohn, 2005) and roughly to the Conidae (*sensu*
351 Tucker and Tenorio, 2009). A fourth main clade was found here for the first time with DNA
352 characters. It roughly corresponds to *Profundiconus sensu* Tucker and Tenorio (2009) and
353 includes a number of deep-water species from the Indo-Pacific that were not examined in
354 previous molecular phylogenetic analyses. *Profundiconus* is sister-group to all the other

355 Conidae, but this relationship is not supported. The inclusion of *Profundiconus* in Conidae
356 thus remains doubtful, although the morphological characters would place it in cone snails.

357 Within the SMC and LMC, reconstructed phylogenies show several well-resolved
358 subclades that generally correspond to genus-level groups defined by Tucker and Tenorio
359 (2009). However, most of the relationships among the subclades of the SMC and LMC were
360 not resolved; this could be due to a lack of phylogenetic signal for the three mitochondrial
361 genes analysed here and/or to a radiation process that led to multiple lineages originating in a
362 short period of time. Nonetheless, some groupings can be noted, although in most cases only
363 supported by the Bayesian analysis (Fig. 2). Within the SMC, all the species except for *C.*
364 *arcuatus* and *C. mazei* clustered together (PP = 1, bootstrap = 34). *Conus distans* is the sister-
365 species of all other members of the LMC (PP = 1; this relationship was absent in the ML
366 analysis). Half of the members of the LMC (from *Puncticulis* to the bottom of Fig. 2) occur
367 within a well-supported clade (PP = 1; relationship not found with the ML analysis).

368 Similar to the results obtained by Puillandre *et al.* (2011a) with similar outgroups,
369 monophyly of the cone snails (= Conidae sensu Bouchet *et al.*, 2011 – see Table 1) is not
370 supported, suggesting that more taxa, in particular within the closely related families
371 (Borsoniidae, Clathurellidae, Conorbiidae), and additional genes, especially nuclear, with
372 lower rates of evolution, should be analysed to fully resolve the relationships of cone snails
373 and other Conoidea. The diversity pattern within Conidae remained unchanged from previous
374 studies (e.g. Duda and Kohn, 2005; Tucker and Tenorio, 2009), with very disparate numbers
375 of species between the main lineages. By far most cone snails species (~ 85%) are in the
376 LMC.

377

378 **4. DISCUSSION**

379

380 4.1. *Species diversity and phylogenetic relationships*

381 In most cases (213 of the 320 named species), DNA analyses were congruent with species
382 delimitation based on shell characters. For the remaining species, DNA analyses were not
383 found congruent with species delimitation based on morphological characters, and we
384 examined four hypotheses that could explain this high number of discrepancies: 1. Specimens
385 were not identified correctly. Although specimens with vouchers (or at least a picture) were
386 examined by several experts to verify identification, a large proportion of the sequences
387 (especially those from GenBank) lacked voucher material and could not be evaluated. 2. The
388 sequence obtained belongs to a contaminant. Several identical sequences independently
389 obtained by different laboratories reduce the likelihood of contamination, but checking for
390 contamination is more difficult when only a single specimen is available for a given named
391 species. 3. The three analysed genes all belong to the maternally transmitted mitochondrial
392 genome, and its evolutionary history is distinct from the species tree. In particular, the non-
393 monophyly of a given morphospecies may be linked to the fact that the analysed gene(s) have
394 not yet coalesced (Funk and Omland, 2003). 4. Lack of morphological variability (e.g. cryptic
395 species) or, conversely, high within species morphological variability (e.g. linked to
396 phenotypic plasticity) resulted in incorrectly delimited species, suggesting that the taxonomy
397 needs to be revised.

398 The recovery of a new clade, namely *Profundiconus*, illustrates the fact that more
399 complete taxon sampling can provide a much better view of the evolutionary history and
400 taxonomic diversity of groups. Although our current phylogenetic treatment more than
401 doubles the number of species examined, our analyses included less than 50% of the
402 recognized cone snail species; inclusion of additional species and analyses of additional gene
403 sequence regions will be instrumental in reconstructing the history of the Conidae and may
404 reveal additional previously unrecognized groups.

405 Most, if not all, previously published molecular phylogenies are congruent with the
406 phylogenetic results presented here; this does not come as a surprise as most of the specimens
407 and sequences analysed in these studies were combined in our dataset. However, phylogenetic
408 trees that were reconstructed with other gene regions (intron 9 CIS Kraus *et al.* (2011); and
409 calmodulin exon+intron gene sequences, Duda and Palumbi (1999a) are also consistent with
410 those produced here. All clades defined in these prior trees were recovered in our trees (taking
411 into account that not all the same species were included in all studies). The inclusion of many
412 more species compared to the previously published phylogenies, however, revealed many
413 clades that were previously unrecognized either because members of these clades were not
414 included in the previous analyses or because the inclusion of additional species and/or
415 sequences improved the resolution of the tree. The phylogenetic analysis of the 320 cone snail
416 species has been turned into a new classification for the family Conidae that now includes 4
417 genera and 71 subgenera (Puillandre *et al.*, in press).

418

419 4.2. *Evolution of Diet*

420 Most cone snails feed on polychaete worms, and reconstruction of the evolution of their
421 diets supports the hypothesis that the cone snail ancestor was vermivorous (Fig. 3). The form
422 of its radular tooth (Kohn *et al.*, 1999) and its position in the tree (Fig. 3) also support the
423 evolution of the unusual diet of *C. californicus*—this species is able to feed on molluscs,
424 worms, crustaceans and fishes (Biggs *et al.*, 2010)—from a worm-hunting ancestor. This is
425 also likely in the few clades whose members specialize on fishes (members of *Chelyconus*,
426 *Phasmoconus*, *Gastridium* and *Pionoconus*) and molluscs (most of the members of the
427 subgenera *Conus*, *Leptoconus*, *Calibanus*, *Darioconus*, *Cylinder*, and *Eugeniconus*). The
428 capacity to feed on molluscs likely appeared only once, with a probable reversion to worm-
429 hunting behaviour in *C. nobilis* (diet predicted from radular tooth characters).

430 Reconstruction of the evolution of the cone snail diet shows that the capacity to prey on
431 fishes probably appeared several times during the evolution of the group. If we rely only on
432 the species for which piscivory has been confirmed by direct observation, and not on the
433 species for which the diet has been inferred from the radula (marked “2” in Fig. 3), the
434 piscivorous diet evolved only twice, in *C. ermineus* and *C. purpurascens* within *Chelyconus*,
435 and in several species of the clade (*Asprella*, *Afonsoconus*, *Textilia*, *Pionoconus*, *Embrikena*,
436 *Gastridium*, *Phasmoconus*), as represented by the two grey boxes in the Figure 3. However,
437 the relationships between these two clades are not supported, and we thus cannot rule out that
438 piscivory evolved only once. Similarly, several previous phylogenetic investigations of cone
439 snails suggest that fish-eating arose multiple times during the evolution of this group, but
440 many of the resultant trees from these studies lacked rigorous support to reject the hypothesis
441 that fish-eating evolved only once (Duda et al., 2001, Fig 1-3, 5; Kraus et al., 2011, Fig. 2 and
442 3; but see Duda and Palumbi, 2004).

443

444 4.3. Clade Specificity of Venom Peptides.

445 In this section we relate an independent dataset – the major peptide toxins expressed in the
446 venom of each species in Conidae (see Supplementary data 7 for the GenBank accession
447 numbers) – to the phylogeny based on standard mitochondrial marker genes shown in Fig. 2.
448 At present, the range of species whose venom has been comprehensively analyzed is far more
449 phylogenetically restricted than the species for which the mitochondrial markers are available
450 (as shown by the asterisks in Fig. 3); consequently, it was thus not possible to directly map
451 the evolution of the toxins on the tree, as done with the diet and biogeography. Nevertheless,
452 it is clear even from the more limited dataset available that the major venom peptides
453 expressed in a given species tightly correlate with the clade to which that particular species is

454 assigned, based on the molecular data (Fig. 2). Consequently, venom peptides can be used as
455 an independent dataset to confirm or refute the clades defined using mitochondrial genes.

456 We specifically tested this hypothesis with the fish-hunting clades. As discussed above,
457 the phylogeny suggests that worm hunting was the ancestral state. One family of venom
458 peptides that are well understood at the mechanistic level are the α -conotoxins, targeted to the
459 nicotinic acetylcholine receptor, a molecular target that is key to prey capture. Blocking this
460 receptor at the synapse between nerve and muscle results in the paralysis of potential prey.
461 The major toxins in the venoms of cobra-related snake species, such as cobratoxin or α -
462 bungaratoxin, similarly target the nicotinic acetylcholine receptor of their prey. In the shift
463 from worm hunting to fish hunting, the α -conotoxins were clearly under selection to diverge
464 from the ancestral worm-hunting nicotinic antagonists, and to target the very distinctive
465 nicotinic acetylcholine receptor expressed in the skeletal muscle of vertebrates. Thus, the
466 members of the α -conotoxin family in worm-hunting cones mostly belong to a specific toxin
467 gene subfamily called the α 4/7 subfamily. These have the canonical sequence CCX₄CX₇C:
468 the peptides in the gene superfamily, as defined from the similarity in the signal sequence,
469 have 4 cysteine residues with diverse amino acids in between them. In the typical ancestral
470 peptide there are 4 and 7AA respectively in the two inter-cysteine intervals. Supplementary
471 data 7 shows examples of α 4/7 subfamily peptides from two different clades of worm-hunting
472 *Conus* snails, *Puncticulis* and *Dendroconus*; peptide sequences from two species in each clade
473 are shown.

474 As shown in the Supplementary data 7, in one specific clade of fish-hunting cone snails
475 (*Pionoconus*), the α -conotoxin family peptides that are highly expressed diverge
476 systematically from the ancestral canonical sequence, and belong to a different subclass of α -
477 conotoxins, the α 3/5 toxin gene subfamily (canonical sequence: CCX₃CX₅C). However, in a
478 different clade of fish-hunting cone snails (*Chelyconus*), the ancestral subfamily has also been

479 altered, but the change is entirely different: an extra disulfide bond has been added (leading to
480 peptides with 6 instead of 4 cysteines). Thus, all piscivorous species in *Pionoconus* express
481 the α 3/5 subfamily member as the major venom peptide for inhibiting the nicotinic
482 acetylcholine receptor at the neuromuscular junction. However, in the piscivorous *Chelyconus*
483 clade, it is the longer peptides with an extra disulfide linkage (known as α A-conotoxins) that
484 have this physiological role. Thus, although the Bayesian analysis in Fig 2 does not
485 statistically allow the unequivocal conclusion of independent origins of fish-hunting in the
486 *Pionoconus* and *Chelyconus* clades, this is strongly supported by the type of venom-peptide
487 expression data shown in the Supplementary data 7. The same divergence between venom
488 peptides in *Pionoconus* and *Chelyconus* is found if the peptides targeted to voltage-gated K
489 channels are examined.

490 Furthermore, the major nicotinic acetylcholine receptor antagonists in some highly
491 specialized worm-hunting lineages, such as *Stephanoconus* (specialized to prey on
492 amphinomid polychaetes), also diverge systematically from the canonical α 4/7 subfamily, to
493 peptides in the α 4/3 subfamily (CCX₄CX₃C). In this case, the most highly expressed nicotinic
494 antagonist targets a different nicotinic receptor subtype, presumably similar to the isoform
495 expressed at the neuromuscular synapse of the amphinomid prey of species in the
496 *Stephanoconus* clade.

497

498 4.4. Biogeography

499 Mapping geographic distributions of species onto the reconstructed phylogeny requires
500 more transition events than the evolution of the diet (Fig. 4). Based on the tree, most species
501 occur in the Indo-Pacific (IP), which may be the ancestral source of the Conidae (frequency of
502 occurrence of Indo-Pacific region at the node 1 – Fig. 4: 90.5%) and of *Conus* (node 2:
503 99.2%). However, the fossil record supports the view that the center of diversity of Conidae in

504 the Eocene was the former Tethys region (Kohn, 1985), also the region of its oldest known
505 fossils (Kohn, 1990). In total, 22 events of dispersals and 27 events of vicariance are inferred.
506 Several of these events are relatively recent and involve species from the IP and EP, e.g., the
507 clades containing the EP species *C. nux*, *C. dalli* and *C. diadema*, that suggest recent
508 migration events across the East Pacific Barrier to establish these species in the EP. Several
509 other clades included sets of species from both the EP and WA, e.g., the clade containing the
510 piscivores *C. purpurascens* and *C. ermineus*, suggesting recent allopatric speciation events
511 linked to vicariance of lineages associated with the emergence of Isthmus of Panama. In
512 addition, in one case it is possible to reconstruct a scenario of consecutive speciation (and
513 possible dispersion and/or vicariance) events to explain the origins of current IP, EP, WA and
514 EA distributions of members of a clade: a first dispersion or vicariance event between the IP
515 and EP led to the origin of *C. fergusonii* and *C. gladiator* in the EP, followed by another
516 dispersion or vicariance event that gave rise to *C. mus* in the WA (possibly associated with the
517 emergence of the Isthmus of Panama), which was then followed by separation of lineages in
518 (or a migration event between) the WA and EA and ultimate origin of *C. tabidus* in the EA.
519 *Conus tabidus* is the only EA cone snail species on the tree that is restricted to the EA and
520 does not occur in a clade with other EA species.

521 Overall, the number of suspected migration and vicariance events is low relative to the
522 number of species included in the analysis. Indeed, few cone snail species occur in more than
523 one of the main marine biogeographic provinces (e.g., *C. ermineus* occurs in the WA and EA
524 and as stated above *C. chaldaeus*, *C. ebraeus* and *C. tessulatus* occur in both the IP and EP).
525 The low levels of connectivity between these provinces is probably linked to large-scale
526 historical-geological events, such as the existence of the East Pacific Barrier between the
527 islands of the central Pacific and the offshore islands and coast of the Americas and the Mid-
528 Atlantic Barrier that separates the Atlantic Ocean into western and eastern regions (Duda and

529 Kohn, 2005) as well as physiological barriers that prevent migration through cold water
530 barriers at higher latitudes.

531 The only previous analysis of the biogeographic history of cone snails (Duda and Kohn,
532 2005) inferred that the group contains two main groups, the SMC and LMC, that were largely
533 restricted to the EP+WA and IP respectively and that this geographic separation likely
534 promoted the divergence of the lineages that gave rise to these clades. That study was able to
535 include only nine SMC species, and with increased taxonomic coverage, this pattern is no
536 longer apparent. Most (70%) SMC species occur in the IP, while the others are evenly
537 distributed in the EP and WA (Fig. 4). The IP SMC members are deep-water species, while
538 most of the EP and WA members are not. Thus, bathymetric isolation, and not isolation in
539 separate biogeographic provinces as inferred by Duda and Kohn (2005), may account for the
540 separation of the SMC and LMC.

541

542 *4.5. Speciation Patterns in Cone Snails*

543 Allopatric patterns, either linked to a speciation event or to within-species differentiation
544 that has not led to speciation, occur throughout Conidae (e.g. Duda and Lee, 2009a; Duda and
545 Rolan, 2005; Puillandre et al., 2011b). The likely propensity of such populations to evolve
546 different venoms (Duda and Lee, 2009b; Duda et al., 2009) that may be linked to prey shifts,
547 make cone snails a promising model to also explore the effects of non-geographic factors on
548 the diversification of the group. Prey shifts after speciation could induce strong positive
549 selection on venom properties and the evolution of new toxins more adapted to new
550 prey (Duda et al., 2008), in agreement with the hypothesis proposed for snakes (Barlow et al.,
551 2009; Kordis and Gubensek, 2000; Lynch, 2007) and scorpions (Kozminsky-Atias et al.,
552 2008). Duda & Lee (2009b) also proposed that ecological release, occurring when an isolated
553 population is under relaxed selective pressure (e.g. from a predator-prey arms race), may lead

554 to the appearance of new toxins, even without prey shift, in *C. miliaris*. However, the
555 available data on conotoxins remain too scarce (species with an asterisks in Fig. 3) to
556 reconstruct the evolution of the conotoxins from the phylogenetic tree presented here and to
557 eventually identify shifts in venom composition between closely related species that could be
558 linked to prey shift or ecological release (but see pararagraph 3.4.). Only 71 species of cone
559 snails are represented by at least one nucleotide sequence of conotoxin in GenBank
560 (Puillandre et al., 2012a), and for most of them the conotoxin sampling is not saturated, as
561 revealed by recent next-gen sequencing (Terrat et al., 2011; Violette et al., 2012), precluding a
562 robust comparison of venom composition at a large-scale.

563 Because our analysis revealed only a few diet shifts, one could argue that this could
564 explain only few speciation events in cone snails. However, we limited prey categories to
565 only the three major types (molluscs, worms and fishes), and important shifts likely occur at
566 finer taxonomic levels of prey. Actually, closely related sympatric *Conus* species of cone
567 snails typically exhibit different feeding specializations, as shown before (e.g. (Kohn and
568 Nybakken, 1975; Kohn, 2001, 1959), and additional comparative analyses may provide
569 stronger evidence linking prey shift to speciation events in some cases.

570

571 4.6. Conclusion

572 Molecular phylogenetic analysis has confirmed that cone snails constitute a largely
573 heterogeneous group in spite of overall morphological homogeneity that justified their
574 inclusion in a single genus until recently. Speciation in cone snails results from different
575 evolutionary processes, since several models of speciation, either linked to geography or
576 ecology, may apply to the group. This propensity to speciate following several evolutionary
577 processes is likely one of the key factors to explain why cone snails are one of the most
578 diverse groups of marine invertebrates. We also argue that the pharmacological diversity of

579 the peptides found in the venom gland of the cone snails have been underestimated, since
580 most of the studies of the last three decades focused on species that belong to only a few
581 lineages (Puillandre et al., 2012a), and several lineages remain largely understudied (or even
582 not studied at all – e.g. *Profundiconus*). The newly defined, highly divergent lineages of cone
583 snails may represent novel biological strategies not found in the limited set of cone snail
584 lineages analyzed so far. One indication of this is the high diversity of conotoxins found in *C.*
585 *californicus* (only half of the subfamilies found in *C. californicus* are also found in *Conus*
586 species – Biggs et al., 2010), this would imply that conotoxin study is only in its infancy,
587 suggesting a promising future for the discovery of new conotoxins and new therapeutic
588 applications.

589

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616

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790

791 **FIGURE CAPTION**

792

793 Figure 1: Three sub-parts of the COI Bayesian tree that illustrate discrepancies between COI
794 diversity and morphological diversity. a) *C. teramachii* complex. b) Putative cryptic species in
795 *C. imperialis*. c) *C. miliaris* complex (black arrows).

796

797 Figure 2: Bayesian tree based on a concatenation of the COI, 16S and 12S genes for the
798 reduced dataset of 326 specimens. Posterior probabilities (> 0.95) are shown for each node.
799 Genus and subgenus names follow the classification based on the phylogenetic tree and
800 published in Puillandre *et al.* (in press).

801

802 Figure 3: Mapping of the type of prey on the Bayesian tree based on a concatenation of the
803 COI, 16S and 12S genes for the reduced dataset of 326 specimens. *: species for which at
804 least one nucleotide sequence of conotoxin is registered in GenBank. 1: species for which the
805 diet is known from direct observations. 2: species for which the diet has been inferred from the
806 radula. ?: species for which the diet is unknown. When species for which the diet has been
807 inferred from the radula are not taken into account for the ancestral state reconstruction, the
808 clade delimited by the light grey box is inferred to include only mollusc-hunting species and
809 the two clades delimited by the dark grey boxes are inferred to include only fish-hunting
810 species.

811

812 Figure 4: Mapping of the geographic distribution (EA = East Atlantic; EP = East Pacific; IP =
813 Indo-Pacific; SA = South Africa; WA = West Atlantic) on the Bayesian tree based on a
814 concatenation of the COI, 16S and 12S genes for the reduced dataset of 326 specimens.

815 **SUPPLEMENTARY DATA**

816

817 Supplementary data 1

818 List of 2123 specimens analysed in the independent gene analyses. Sequences of different
819 genes published by the same author and identified with the same species name were
820 considered to correspond to the same specimen (only when only one sequence per species was
821 in GenBank).

822

823 Supplementary data 2

824 List of 345 species analysed in the concatenated dataset, with 19 species not included because
825 only one gene over three was available (grey lines) and 16 outgroups (at the end of the list).
826 Voucher numbers in the first column refer to the Supplementary data 1. Type of prey (M =
827 Mollusc, F = Fish, W = Worm, C = Crustaceans), geographic province (EA = East Atlantic;
828 EP = East Pacific; IP = Indo-Pacific; SA = South Africa; WA = West Atlantic) and GenBank
829 accession numbers for the three genes are indicated. *: type of prey inferred from the radula.

830

831 Supplementary data 3

832 Maximum likelihood tree based on COI sequences. Bootstraps values > 80 are shown for each
833 node.

834

835 Supplementary data 4

836 Maximum likelihood tree based on 16S sequences. Bootstraps values > 80 are shown for each
837 node.

838

839 Supplementary data 5

840 Maximum likelihood tree based on 12S sequences. Bootstraps values > 80 are shown for each
841 node.

842

843 Supplementary data 6

844 Statistical support (Bayesian and Maximum likelihood analyses) for the clades associated to a
845 genus or subgenus name in the new classification (Puillandre et al., in press)

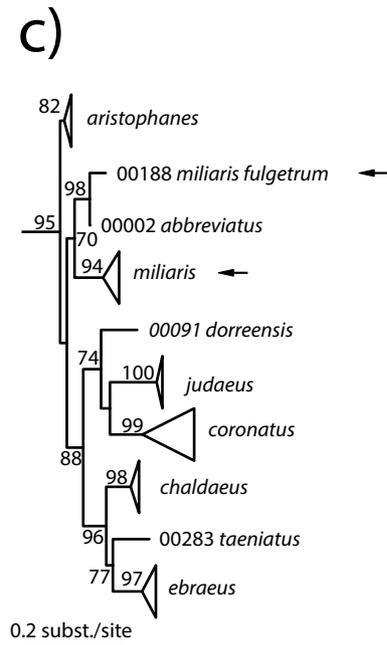
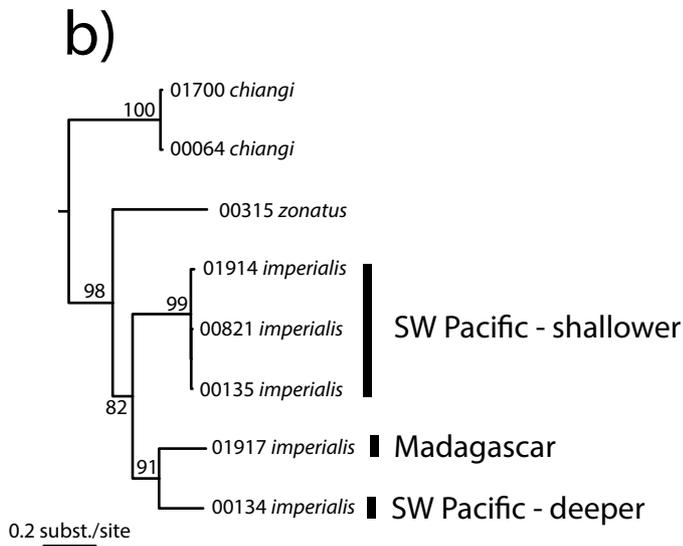
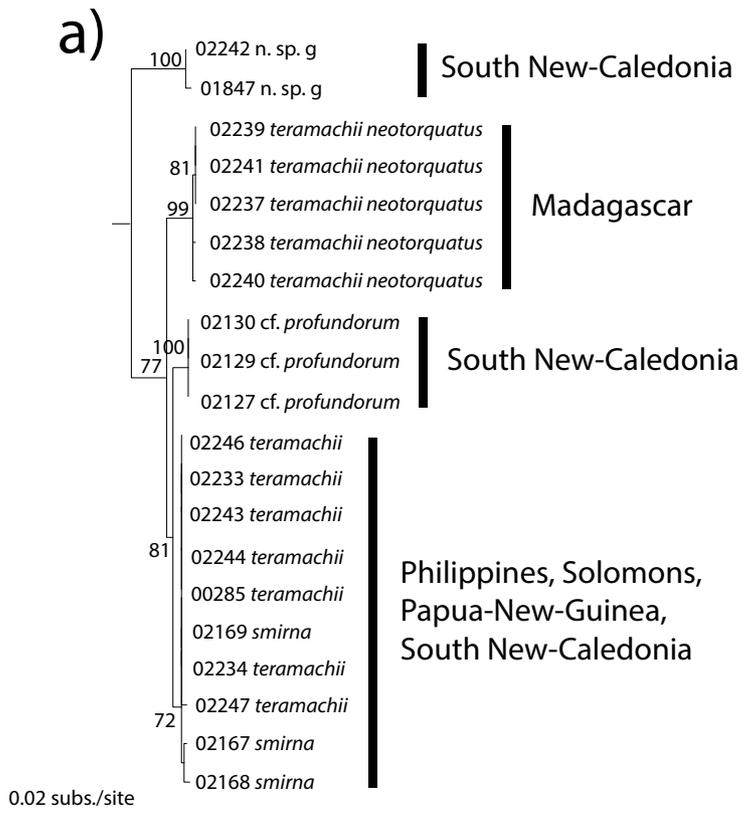
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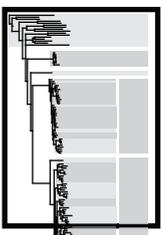
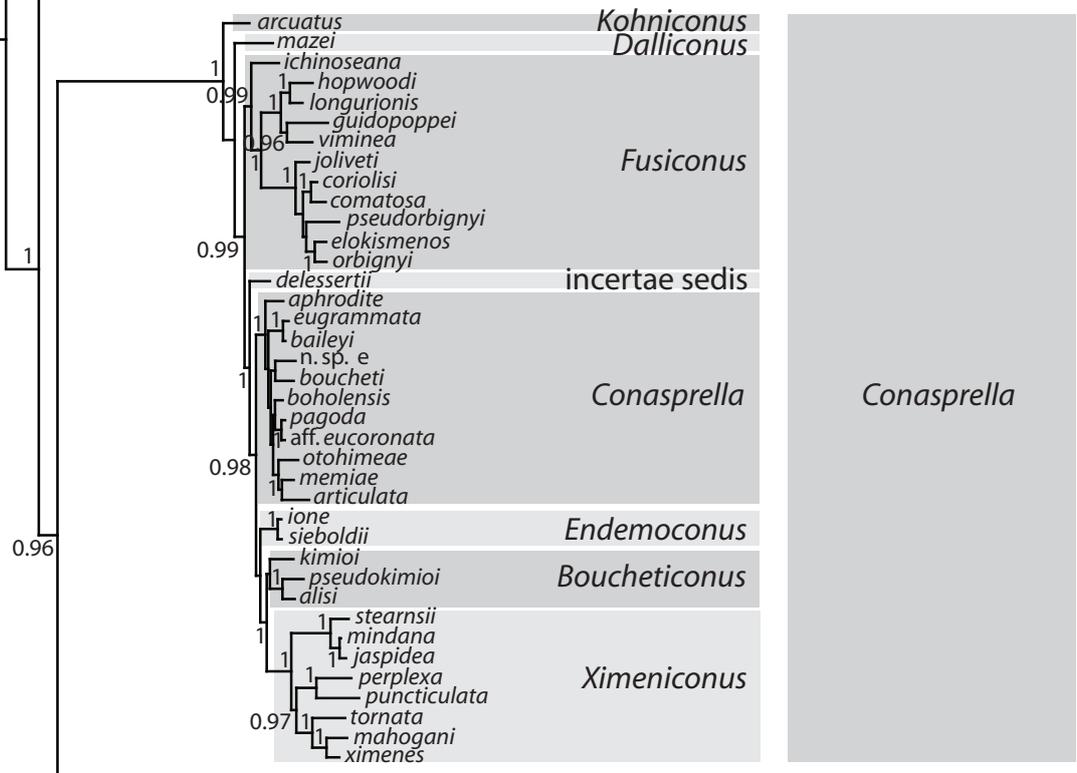
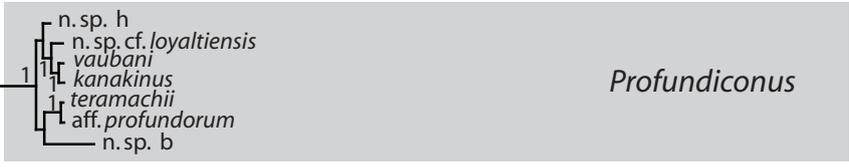
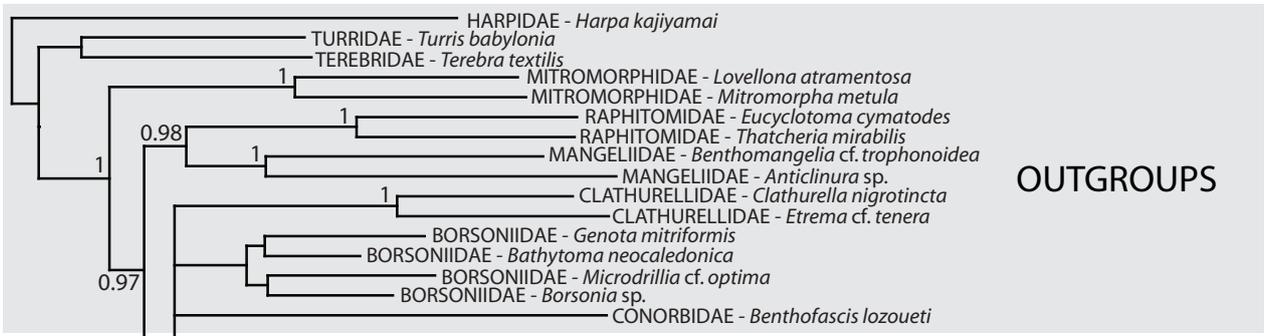
847 Supplementary data 7

848 Venom peptides in the a-conotoxin family in five clades.

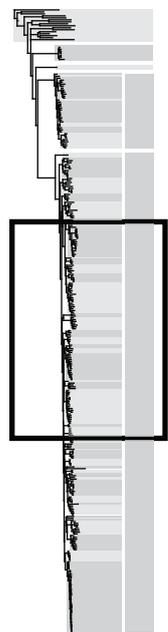
TABLE 1: Comparison of recent classifications of cone snails and related species. In this article, the cone snails are restricted to the Conidae sensu Bouchet et al. (2011). †: fossil taxa.

| Taylor et al., 1993 | Bouchet and Rocroi, 2005 | Duda and Kohn, 2005 | Tucker and Tenorio, 2009 | | (Bouchet et al., 2011) | New classification | | | |
|---------------------|--------------------------|---------------------|--------------------------|----------------------|------------------------|--------------------|---------|--------------|-----------------------|
| Coninae | Coninae | Large Major Clade | Taranteconidae | | Conidae | Conidae | Conidae | <i>Conus</i> | |
| | | | Conidae | Coninae | | | | | |
| | | | | Puncticulinae | | | | | |
| | | Small Major Clade | Conilithidae | Californiconinae | | | | | <i>Californiconus</i> |
| | | | | Conilithinae | | | | | <i>Conasprella</i> |
| | | | | Hemiconidae† | | | | | <i>Profundiconus</i> |
| Conorbinae | Conorbinae | | Conorbidae | <i>Conorbis</i> † | Conorbidae | | | | |
| | | | | <i>Artemidiconus</i> | | | | | |
| | | | | <i>Benthofascis</i> | | | | | |
| | | | Cryptoconidae | <i>Cryptoconus</i> † | | | | | |
| Clathurellinae | | | | <i>Genota</i> | Borsoniidae | | | | |
| | | <i>Genotina</i> | Mangeliidae | | | | | | |

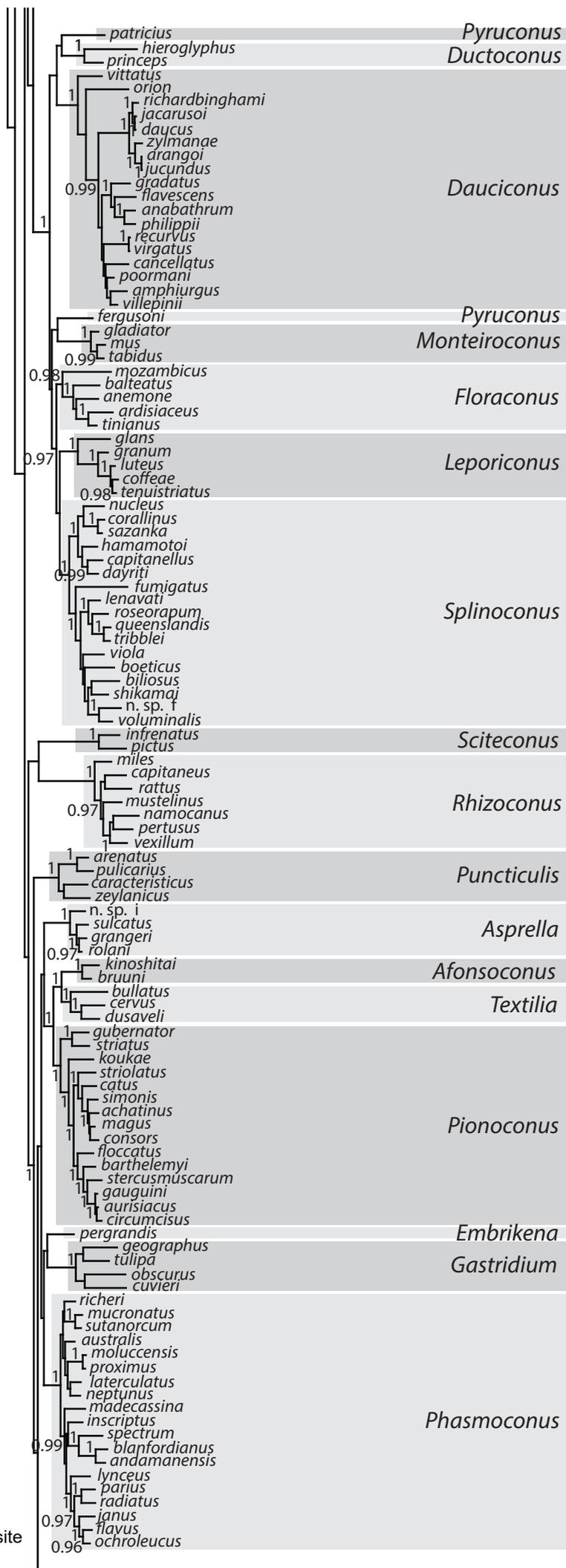


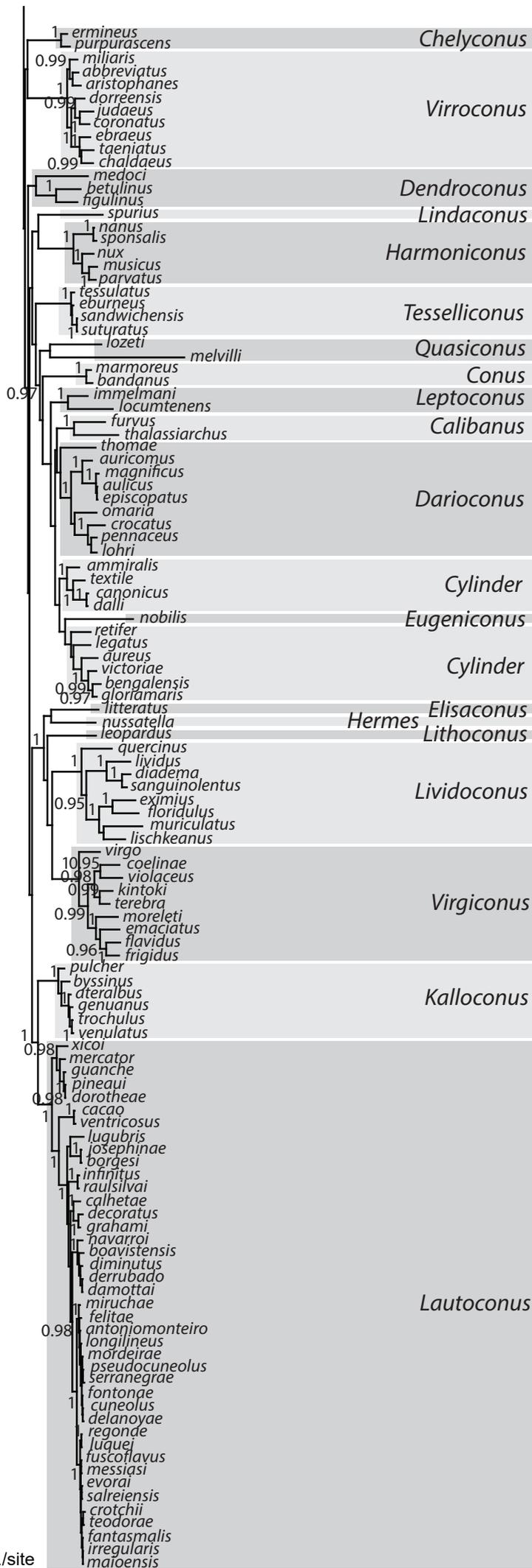


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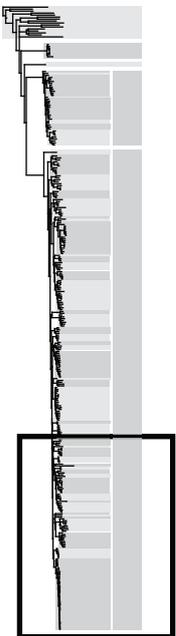
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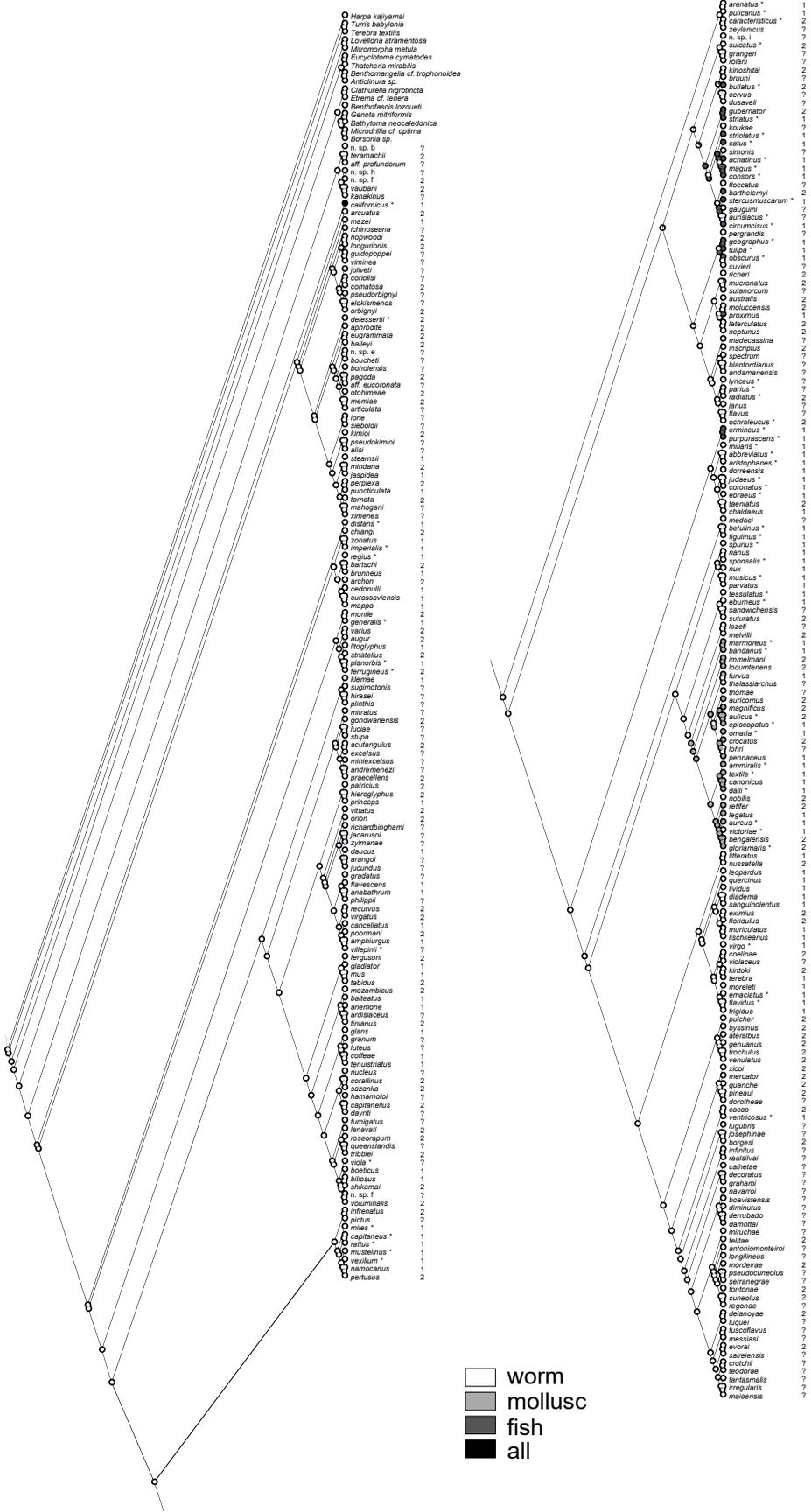




Conus

0.1 subs./site





Harpa kajiyamai
Turris babyfonia
Terebra lextilis
Loveilona atramentosa
Mitromorpha metala
Eucyclidoma cymatodes
Thalassia minimalis
Benthonangella cf. trophonoides
Anticlinura sp.
Clathurella nigroincta
Etrema cf. tenera
Benthofascia locuetti
Genota miniiformis
Eathlytoma neocaledonia
Microtrilia cf. optima
Borsonia sp.
n. sp. b
feramachii
aff. procladurum
n. sp. h
n. sp. f
vulstani
kanakinus
californicus *
arcuatus
mazii
chrysozona
hopwoodi
longunus
guidopoppeli
viminea
olivieri
conrolli
comata
pseudodrigbryi
elokimenes
orbigny
delesseri *
saprodia
eugrammata
bellii
n. sp. e
boucheti
bolivensis
pagode
aff. mucronata
olohimense
mentiae
articulata
ione
saboddi
kimoi
pseudokimoi
aitai
stearnsi
miriciana
aspidea
perplexa
purcillucata
formata
malhogeri
ximenes
dasens
changii
conatus
imperiata *
regius *
bartuzi
brunneus
arcton
cedronuli
curassavensis
maspoe
monile
generata *
varius
nagui
illoglyphus
striatellus
clancorba *
ferrugineus *
hemiae
sugimotois
hirasei
olreth
mitratus
pondiceniensis
luciae
stuppe
leucangulus
excellent
moisecelstus
andremenezi
graeckelens
patricius
hieroglyphus
ornocaps
vittatus
orion
richardbinghami
dearuso
tyrimnase
daucus
anagor
lucundus
gradatus
flavescens
anabathrum
ohivooi
recurvus
virgatus
cancellatus
poomamii
amphurges
villipini *
fergusoni
gladiator
mus
babius
mozanbicus
balbatus
anemone
ardillaceus
sinianus
glans
granum
luteus
coffiae
tenuistratus
nucleus
coreallius
sazanka
hansamotzi
capitaneus
dayrilli
tumigatus
tenavalli
rooseoprum
queenslandis
hibbii
viole *
boeticus
oliticus
shikamai
n. sp. f
voluminalis
niffrenatus
ocius
miles *
capitaneus *
rattus *
musellinus *
exellum *
namocatus
perustus

arenatus * 1
 pulchellus * 1
 caelestis * 2
 zeylanicus * 2
 n. sp. i 1
 sulcatus * 2
 grangeri 1
 roleri 1
 kinoshitai 2
 kruii 2
 bullatus * 2
 lenius 2
 dusaveii 1
 gubernator 1
 sinus * 2
 thymus 1
 koukai 2
 sinolatus * 2
 catus 1
 amoni 1
 echnatus * 1
 magus * 1
 conors * 1
 foccatus * 2
 barthelemyi 2
 stercorarius * 2
 gausumii 1
 aurissius * 2
 circumcissus * 1
 bergandii 1
 geographus * 1
 tulpa * 1
 obscurus * 2
 cuxieri 1
 richeri 1
 mucronatus 2
 autanorum 2
 autreus 2
 mucocensius 2
 promus 1
 laterculatus 2
 resilius 2
 marsecassina 2
 inscriptus 2
 spectrum 2
 bianfordianus 2
 andamanensis 2
 lynceus * 1
 parvus 2
 rielatus * 2
 jarus 2
 flavus 2
 ochroleucus * 2
 ermineus * 1
 purpuraceus * 1
 milars 1
 abbreviatus * 1
 aristophanes * 1
 domentis 1
 judeus * 1
 coronatus * 1
 ebraeus * 1
 taenatus 2
 chaddeus 2
 medoci 2
 betulinus * 1
 figurinus * 1
 apurus * 1
 lenius 1
 sponsalis * 1
 rus 1
 musicus * 1
 parvulus * 1
 leucosus * 1
 eburnus * 1
 sarcochichensis 1
 saturatus 2
 lozeli 2
 mevilli 2
 marmoratus * 1
 bardensis * 1
 imneimani 2
 lucumtensis 2
 furus 2
 thassasarchus 2
 thomae 2
 auricomus 2
 magnificus 2
 aulicus 2
 episcopatius * 1
 oniae 1
 crocatus 2
 bini 1
 pennaceus 1
 armialis * 1
 legie 1
 canonicus 1
 dalli * 1
 nodalis 2
 reifer 2
 legatus * 1
 aureus * 1
 victorae * 1
 bengalensis 2
 gauriamis * 2
 litleratus 2
 rusastella 1
 leogardus 1
 quercinus 1
 lividus 1
 diadema 1
 sanguinolentus 1
 eximus 2
 floridus 2
 muricatus 1
 ischkeanus 1
 virgo * 2
 coelinae 2
 violaceus 2
 kinosi 2
 herbra 1
 moniei 1
 emaciatus * 1
 flavus * 1
 figus 2
 pulcher 2
 bysianus 2
 ateralbus 2
 genuanus 2
 trochilus 2
 verulatus 2
 xopi 2
 mercator 2
 guiriche 2
 pinus 2
 dorothaeae 2
 caeso 2
 ventricosus * 1
 lugubris 2
 josephiinae 2
 borgesi 2
 infusus 2
 rausilvai 2
 cathetae 2
 decoratus 2
 grahmi 2
 nasarovi 2
 boavistensis 2
 dimutus 2
 derubado 2
 demotai 2
 minochae 2
 fellae 2
 antonomontevici 2
 longilneus 2
 mordaxae 2
 pseudocuneoidus 2
 serranegrae 2
 fontoriae 2
 cureolus 2
 regoniae 2
 delanyae 2
 lugae 2
 fuscoflavus 2
 messiasi 2
 evorei 2
 salirensis 2
 cratchi 2
 leodoreae 2
 fantasmalis 2
 frequens 2
 maioensis 2

◻ worm
 ◻ mollusc
 ◻ fish
 ◻ all

