



HAL
open science

When everything converges: Integrative taxonomy with shell, DNA and venom data reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea)

Nicolas Puillandre, Reto Stöcklin, Philippe Favreau, Estelle Bianchi, Frédéric Perret, Audrey Rivasseau, Loïc Limpalaër, Eric Monnier, Philippe Bouchet

► To cite this version:

Nicolas Puillandre, Reto Stöcklin, Philippe Favreau, Estelle Bianchi, Frédéric Perret, et al.. When everything converges: Integrative taxonomy with shell, DNA and venom data reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea). *Molecular Phylogenetics and Evolution*, 2014, 80, pp.186-192. 10.1016/j.ympev.2014.06.024 . hal-02458200

HAL Id: hal-02458200

<https://hal.science/hal-02458200v1>

Submitted on 28 Jan 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **When everything converges: integrative taxonomy with shell, DNA and venomic data**
2 **reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea)**

3
4 Puillandre Nicolas^{a,b,*}, Stöcklin Reto^b, Favreau Philippe^b, Bianchi Estelle^b, Perret Frédéric^b,
5 Rivasseau Audrey^c, Limpalaër Loïc^d, Monnier Eric^e, Bouchet Philippe^f.

6
7 ^a Museum National d'Histoire Naturelle, Département Systematique et Evolution, ISyEB
8 Institut (UMR 7205 CNRS/UPMC/MNHN/EPHE), 43, Rue Cuvier, 75231 Paris, France

9
10 ^b Atheris Laboratories, Case postale 314, CH-1233 Bernex-Geneva, Switzerland

11
12 ^c UMS 2700, Museum National d'Histoire Naturelle, Département Systematique et Evolution,
13 43, Rue Cuvier, 75231 Paris, France

14
15 ^d 11, rue de la Poste 60510 Haudivillers

16
17 ^e Conservatoire National des Arts et Métiers, Département Chimie, Alimentation, Santé,
18 Environnement et Risque, 292, rue Saint-Martin, 75003 Paris, France

19
20 ^f Museum National d'Histoire Naturelle, Département Systematique et Evolution, ISyEB
21 Institut (UMR 7205 CNRS/UPMC/MNHN/EPHE), 55, Rue Buffon, 75231 Paris, France

22
23
24
25 * Corresponding author: Tel: +33 1 40 79 31 73; puillandre@mnhn.fr
26

27 **Abstract**

28 Cone snails have long been studied both by taxonomists for the diversity of their shells and by
29 biochemists for the potential therapeutic applications of their toxins. Phylogenetic approaches
30 have revealed that different lineages of *Conus* evolved divergent venoms, a property that is
31 exploited to enhance the discovery of new conotoxins, but is rarely used in taxonomy.
32 Specimens belonging to the Indo-West Pacific *Conus lividus* species complex were analyzed
33 using phenetic and phylogenetic methods based on shell morphology, COI and 28S rRNA
34 gene sequences and venom mRNA expression and protein composition. All methods
35 converged to reveal a new species, *C. conco* n. sp. (described in the supplementary data),
36 restricted to the Marquesas Islands, where it diverged recently (~3 mya) from *C. lividus*. The
37 geographical distribution of *C. conco* and *C. lividus* and their phylogenetic relationships
38 suggest that the two species diverged in allopatry. Furthermore, the diversity of the transcript
39 sequences and toxin molecular masses suggest that *C. conco* evolved unique toxins,
40 presumably in response to new selective pressure, such as the availability of new preys and
41 ecological niches. Furthermore, this new species evolved new transcripts giving rise to
42 original toxin structures, probably each carrying specific biological activity.

43

44 **Keywords:** Conidae, Conopeptides, MALDI-TOF-MS, Mass spectrometry, RNA-Seq,
45 Species delimitation, Toxins, Transcriptomes, Venom gland.

46

47 **1 Introduction**

48 Far ahead of snakes and together with scorpions and spiders, cone snails are among the three
49 most promising groups of venomous animals for pharmaceutical studies given the diversity of
50 their toxins (King et al., 2008). Recent proteomic approaches reveal that each cone snail
51 species may be able to produce at least 200 unique conotoxins or other proteins (Violette et
52 al., 2012) and possibly even thousands if all variants and fragments are explored (Dutertre et
53 al., 2013). Furthermore, as many as 761 species are now considered valid
54 (www.marinespecies.org) and this number is expanding. This would lead to an estimate of at
55 least 150,000 toxins, given that thousands of marine molluscs of the Conoidea superfamily
56 remain to be investigated.

57 The diversity of the conotoxins, driven by either mechanisms such as recombination or
58 gene duplication and positive selection (Duda, 2008), has been invoked for to explain the
59 higher rates of diversification of cone snails compared to other predatory - but non venomous
60 - neogastropods (Olivera, 2006). It has been shown that (i) even closely related species of
61 cone snails may exhibit different feeding specializations (Kohn, 2001) and (ii) each cone
62 species possesses its own arsenal of toxins, weakly overlapping with the arsenal of other
63 species (Kaas et al., 2010; Olivera, 2006). Duda (2008) thus suggested that prey shifts after
64 speciation induces a strong positive selection on venom, and the emergence of new toxins
65 more adapted to the new prey, in line with the hypotheses proposed for snakes (Barlow et al.,
66 2009) and scorpions (Kozminsky-Atias et al., 2008).

67 We present here an integrative approach, including morphological analysis, DNA
68 sequencing and venom characterization that led to the recognition of a new species of cone
69 snails in the *Conus lividus* complex, which we name *Conus conco* new species (description is
70 provided in the Supplementary Data 1). *Conus conco* is restricted to the Marquesas
71 Archipelago, where it had earlier been mistaken for *C. sanguinolentus* or *C. lividus*
72 (Moolenbeek et al., 2008). We used phenetic approaches for morphological characters and
73 phylogenetic methods for DNA sequences (COI and 28S genes) to distinguish this new
74 species from other species that belong to the same complex (*C. diadema*, *C. sanguinolentus*
75 and *C. lividus*). A new approach is also proposed: the presence/absence of the different toxins
76 detected by MALDI-TOF-MS analysis of the venom of multiple specimens from *C.*
77 *sanguinolentus*, *C. lividus* and *C. conco* n. sp. can be coded in a matrix, similar to traditional
78 analyses of morphological characters. Chemical signals, including toxins, have previously
79 been used as characters to confirm an existing phylogeny, using classic phylogenetic approach
80 or clustering methods (Nascimento et al., 2006), but rarely to help recognizing a new
81 species. These data, together with venom gland transcriptome investigation, revealed that *C.*
82 *conco* evolved specific conotoxins not found in its sister-species. The patterns of phylogenetic
83 relationships, divergence estimation, geographical distribution and venom diversity, within
84 and between species, are discussed to suggest a speciation scenario that led to the emergence
85 of this new species of cone snail.

86

87 **2 Material and methods**

88 2.1 Sampling

89 Most specimens were collected during three expeditions in 2007 to Tahiti and the Marquesas
90 and Chesterfield Islands. Specimens were photographed, the shell was broken and the animal
91 was then directly dissected on site. A piece of foot tissue was preserved in ethanol, radular
92 sacs were stored dry at -80°C. Extracts of the venom duct were dissolved in 10% acetic acid
93 in water, dried under vacuum and stored at -80°C. The remaining venom gland was preserved
94 in RNALater (Ambion) for further transcriptomic studies. Whenever possible, shells were
95 reconstructed for use as vouchers. Additional comparative material originates from
96 expeditions in the Philippines (Panglao 2004), Vanuatu (Santo 2006) and Madagascar (Atimo
97 Vatae 2011). A piece of foot tissue was cut from the body and placed in 95% ethanol. All

98 specimens were collected in shallow water (between 0 and 10 meters), and were identified in
99 the field as *C. lividus* or *C. sanguinolentus* (following the taxonomy of Röckel et al., 1995,
100 and referred to as *Lividoconus* in Tucker and Tenorio, 2013).

101 Outgroups for phylogenetic analyses were selected according to the last available
102 classification of cone snails (Tucker and Tenorio, 2013) and on the available phylogenetic
103 analyses for the group. *Conus muriculatus* was used as closely related outgroup. Other species
104 of *Conus* (*C. virgo*, *C. consors* and *C. marmoreus*) were also used as outgroups. *Conasprella*
105 *pagoda* (Conidae) and *Bathytoma carnicolor* (Conoidea, Borsoniidae) were used as distant
106 outgroups. Finally, in the COI gene dataset, *Conus quercinus* was also added to date the
107 species divergences (see 2.2).

108 2.2 DNA sequencing and phylogenetic analyses

109 DNA was extracted using the Epmotion 5075 robot (Eppendorf). Fragments of the COI and
110 rDNA28S genes of 658 and 750 bp, respectively, were amplified using the protocol described
111 in Kantor et al. (2013). PCR products were purified and sequenced by Eurofins in both
112 directions. Available COI and 28S sequences for *C. lividus*, *C. diadema* and *C.*
113 *sanguinolentus* in GenBank were downloaded and added to the dataset. GenBank accession
114 numbers are provided in the Supplementary Data 2.

115 Sequences were manually (COI gene) or automatically (28S gene) aligned using
116 ClustalW multiple alignment (BioEdit 7.0.5.3, Hall, 1999). The best model of evolution was
117 selected for each gene using Modelgenerator V.85 (Keane et al., 2006), following the
118 Hierarchical Likelihood Ratio Tests (with four discrete gamma categories). The best
119 substitution models are TrN+G for both COI and 28S genes. Bayesian Analyses (BA) were
120 performed including only one sequence per haplotype and running two parallel analyses in
121 MrBayes (Huelsenbeck et al., 2001), consisting each of eight Markov chains of 10,000,000
122 generations. A GTR+G model was applied to each gene. Tracer 1.4.1 (Rambaut and
123 Drummond, 2007) was used to check that ESS values were all greater than to 200. A
124 consensus tree was then calculated after omitting the first 25% trees as burn-in.

125 The divergences of the genus *Conus* from the other conoideans, and of *C. quercinus*
126 from *C. lividus*, respectively estimated at 55 mya and 11 mya based on fossil records (Duda
127 and Kohn, 2005), were used as calibration points to date the divergences between the different
128 species included in the *C. lividus* complex. BEAST 1.8.0 (Drummond and Rambaut, 2007)
129 was used to reconstruct a phylogenetic tree based on a concatenation of the COI and 28S
130 sequences including one specimen per species. The priors of the t_{MRC} of the cone snails and
131 of the clade including *C. quercinus*, *C. lividus*, *C. sanguinolentus* and *C. cono* were set to
132 follow a lognormal distribution. An uncorrelated lognormal relaxed clock with a “birth-death
133 incomplete sampling” speciation model was applied and independent models of substitutions
134 (TN93+G) were defined for each gene. Two MCMC chains were run for 100,000,000
135 generations. Convergence of the runs was tested as described previously. Tree annotator 1.8.0
136 (<http://beast.bio.ed.ac.uk>) was used to summarize the tree data generated by BEAST.

137 2.3 Venom MALDI-TOF-MS fingerprinting

138 MALDI-TOF-MS analyses were carried out on an Ultraflex TOF-TOF mass spectrometer
139 operated in positive reflector mode (m/z 480-5,000) under the control of the FlexControl 2.2
140 software (Bruker, Bremen, Germany). Samples (listed in the Supplementary Data 2) were
141 deposited on a 384 AnchorChip 600 plate using an affinity method based on manufacturer's
142 guide to sample preparation. External calibration was carried out and checked in the 700–
143 3,500 Da mass range with an error 50 ppm.

144 The FlexAnalysis 2.2 software (Bruker) was used for data processing and analysis.
145 Only the best representative mass spectrum of each venom was selected for data
146
147

148 interpretation, i.e. the one with the highest number of signals and the best signal-to-noise
149 ratio.

150 Each ionized molecular species obtained in the 41 mass spectra was considered as a
151 different character with two different states, present (1) or absent (0). A total of 444 characters
152 were coded, and the matrix obtained was analyzed using MrBayes (Huelsenbeck et al., 2001).
153 Two parallel analyses were performed, each consisting of eight Markov chains of 10,000,000
154 generations. Convergence of the runs was tested as described previously. A consensus tree
155 was calculated after omitting the first 25% trees as burn-in. The absence of outgroup in this
156 analysis is due to the unavailability of venom for closely related species, and to the fact that
157 venom available for more distant species had almost no mass in common with the three
158 species of the *lividus* complex.

159

160 2.4 Venom gland mRNA transcriptomic sequencing

161 Six venom glands (two per species) were used for transcriptomic analyses (specimens listed in
162 the Supplementary Data 2). Total RNA was extracted from the glands using a TissueLyzer LT
163 (Qiagen) and the method developed by Chomczynski (1993). Messenger RNA was purified
164 using the Dynabeads mRNA purification kit (Invitrogen) and the quality of total and mRNA
165 was verified using a 2100 Bioanalyzer (Agilent technologies). The construction of the whole
166 transcriptome libraries, the sample preparation for RNA-Seq sequencing and the sequencing
167 were performed according to the Ion Torrent protocols using the Ion Torrent Personal
168 Genome Machine (PGM).

169 Reads obtained from the Torrent Suite Software (version 3.6) were converted to FastQ
170 format for subsequent data analysis. Sequencing quality was assessed by aligning the reads to
171 housekeeping genes of Gastropods (CEGMA database and NCBI BLAST version 2.2.26).
172 Data belonging to rRNA and identical sequences were removed by matching reads against
173 public rRNA sequences (Silva database and NCBI BLAST version 2.2.26) followed by
174 clustering together reads of 100% identity (CD-HIT-454 version 4.5.4). *De novo* assembly
175 was performed using MIRA (version 3.9.15) with default parameters for Ion Torrent data.
176 Contigs represented by less than 10 reads were discarded. Sequences coding for toxins were
177 detected using GeneWise (EMBL-EBI version 2.4.1 – (Birney et al., 2004)) and HMMER2
178 models based on public sequences of conotoxins (ConoServer and UniProt). Signal sequences
179 in toxins were identified using an in-house (Koua et al., 2013).

180

181 **3 Results**

182 3.1 Morphological analyses

183 The specimens collected in the Marquesas Islands, and preliminarily identified as *C.*
184 *sanguinolentus*, formed two morphologically separable clusters (Fig 1). One morphotype,
185 with an average total shell length of 48 mm, is characterized by a lavender to purple
186 background color; the other morphotype, with an average total shell length of 35 mm, is olive
187 or yellowish brown and lacks the lavender to purple background color (the Supplementary
188 Data 1). The larger morphotype was only found in the Marquesas Islands, where it seemed to
189 replace *C. lividus*, which is found throughout the Indo-Pacific except in the Marquesas. This
190 purple morphotype likely represent a new species (and this hypothesis is supported by other
191 evidence – see below), and it is described as *Conus conco* new species (the Supplementary
192 Data 1).

193

194 3.2 Phylogenetic analyses

195 Analyses of the COI and 28S genes resulted in phylogenetic trees with identical topology
196 (Fig. 2). Specimens identified as *C. lividus* all belonged to a single clade (Posterior
197 Probability PP = 0.88 for the COI, 0.98 for the 28S). Similarly, the two COI sequences of *C.*
198 *diadema* from GenBank clustered in a highly supported clade (PP = 1). However, specimens

199 identified as *C. sanguinolentus* clustered in two different clades (each with PP = 1). One of
200 them included only specimens now identified as *C. conco* n. sp., and was the sister-group of
201 *C. lividus*. These four clades were all grouped in a single clade (PP = 1 for COI and 28S),
202 sister-group of *C. quercinus*.

203 Using two calibration points, the ages of the clade including *C. sanguinolentus*, *C.*
204 *lividus* and *C. conco* (*C. diadema* was not included in this analysis, because no 28S sequence
205 was available for it) and the clade including *C. lividus* and *C. conco* were estimated at 7.84
206 mya (5.12-12.46) and 2.738 mya (1.11-4.73) respectively (Fig. 3)

207 208 3.3 Toxin MS profile and venom gland RNA-Seq transcriptomes

209 A total of 444 monoisotopic signals (consisting of a single isotope) could be detected from the
210 41 venoms of *C. lividus*, *C. sanguinolentus* and *C. conco* in the mass range m/z 480-5,000
211 corresponding to the molecular masses of most known conopeptides (Conoserver; Kaas et al.,
212 2010). Each signal was generally found in only a few specimens (mean = 3.27) reflecting a
213 high level of intra-specific venom variation, and only 30 were found in more than 10
214 specimens. Each specimen of *C. lividus*, *C. sanguinolentus* and *C. conco* included a mean of
215 35.6, 31.5 and 37.19 different conotoxin masses, respectively. Eleven signals (given in Da)
216 found in at least 50% of the *C. conco* specimens, but never in *C. lividus* or *C. sanguinolentus*
217 were identified: 1053 (found in 17 specimens of *C. conco*), 1077 (12), 1585 (17), 1615 (18),
218 1754 (17), 1772 (13), 1812 (12), 1932.8 (14), 1970 (14), 2299 (16), 2321 (12). The
219 phylogenetic analysis of the 41 mass spectra resulted in a tree with three main groups, each
220 including only specimens from the same species (Fig. 4).

221 The sequencing of these *Conus* venom glands libraries yielded around 2 million reads
222 that were then assembled into 100,000 contigs on average. Transcriptome analyses confirmed
223 the high diversity of toxins found in the three species. In total, 88 different toxin precursors
224 were found in the six specimens analyzed, attributable to 12 different superfamilies using the
225 signal sequences (following the classification in Puillandre et al., 2012), plus one set of 8
226 sequences (found in the three species) characterized by a signal sequence different from the
227 known superfamily of conotoxins (Supplementary Data 3). Most precursor amino-acid
228 sequences are unique to a single species, but three are shared by the three species, four by *C.*
229 *lividus* and *C. sanguinolentus* and nine by *C. conco* and *C. lividus*, in agreement with the fact
230 that *C. conco* and *C. lividus* are closely related and diverged from each other more recently
231 than from *C. sanguinolentus*. Correlating the MALDI-TOF-MS signals and the transcriptome
232 data to infer potential mature conotoxin sequences was judged to be too hypothetical due to
233 the elevated number of matches resulting from unknown precursor cleavages sites, undefined
234 post-translational modifications and the lack of any previously characterized toxins from these
235 species.

236 237 **4 Discussion**

238 Congruence of the phylogenetic trees resulting from the two unlinked markers (COI,
239 mitochondrial, and 28S, nuclear) is a strong, although indirect, evidence for reproductive
240 isolation among *C. sanguinolentus*, *C. lividus* and *C. conco*. Moreover, they are characterized
241 by highly divergent sets of toxins and different shell characters. All these evidences strongly
242 support that *C. lividus* and *C. conco* are isolated species - and not divergent populations
243 within a single species that would be still able to exchange genes - and this justifies the
244 description of *C. conco* as a new species.

245 To understand how *C. conco* diverged from *C. lividus*, several patterns must be taken
246 into account. First, the geographical distributions of the two species are clearly allopatric.
247 *Conus lividus* is present in all the Indo-West Pacific except in the Marquesas Islands, and our
248 analyses show that specimens from the two extremes of the range (Madagascar and Tahiti) are
249 genetically indistinguishable (Fig 2). Conversely, *C. conco* is known only from the Marquesas

250 Islands. Second, phylogenetic analyses show that *C. conco* and *C. lividus* are sister groups.
251 Third, *C. conco* and *C. lividus* diverged recently (~ 2.738 mya), i.e. between 2 and 6 my after
252 the elevation of the first island of the Marquesas above sea level (Eiao Island is estimated to
253 have emerged between 4.99 and 8.72 mya – Clouard and Bonneville, 2005). Fourth, *C. conco*
254 expressed specific precursor sequences and toxin molecular masses not found in *C. lividus*
255 and *C. sanguinolentus* (Fig. 4). In particular, 11 toxin masses are present in most *C. conco*
256 specimens and not in the other two species, suggesting that this is not just a sampling bias.
257 Finally, the protoconchs of all *C. conco* specimens were eroded, but that of its sister species
258 *C. lividus* is multispiral (four whorls – (Röckel et al., 1995)), suggesting a high dispersal
259 potential (Jablonski and Lutz, 1980).

260 Given the geographic distribution of *C. conco* and *C. lividus* and the fact that the
261 speciation event occurred after the emergence of the Marquesas archipelago, it is more likely
262 that the speciation process occurred in the Marquesas Islands. If the speciation process that
263 led to the emergence of *C. conco* and *C. lividus* has occurred in sympatry, e.g. as a result of a
264 prey shift, then the two species diverged after the colonization of the Marquesas Islands by *C.*
265 *lividus*. This hypothesis is supported by the differentiation of the toxin repertoire in *C. conco*
266 (thus linked to the prey shift). *Conus lividus* is known to prey on polychaetes (direct
267 observations, Kohn, 1959), a taxonomically and ecologically very diversified group, and it is
268 probable that the guild of polychaete species found in the Marquesas differs significantly from
269 that occurring elsewhere in French Polynesia or the Indo-West Pacific. However, the
270 sympatric hypothesis does not explain the current geographical distribution. If *C. conco* and
271 *C. lividus* are now adapted to different habitats (i.e. different preys), they are no longer
272 competitors (Quenouille et al., 2011) and there is no simple explanation why *C. lividus* is
273 absent from the Marquesas Islands. Alternatively, the divergence may also be the result of a
274 peripatric speciation event, where a small peripheral population of *C. lividus* colonized the
275 Marquesas Islands, in agreement with the geographic distribution of the two species.
276 However, and following the peripatric hypothesis, the differentiation of the toxin repertoire in
277 *C. conco* would be more difficult to explain. Duda & Lee (2009) suggested that ecological
278 release, occurring when an isolated population is under relaxed selective pressure (e.g.
279 predator-prey arm race relaxed), led to the appearance of new toxins, even without prey shift,
280 in *C. miliaris*. This hypothesis may apply to *C. conco*. The absence of *C. lividus* in the
281 Marquesas Islands, although present in close archipelagos, would be explained by competitive
282 exclusion.

283 Demonstrating ecological speciation and identifying the underlying mechanisms
284 require methods that are difficult to apply to cone snails. Characterizing the prey of *C. conco*
285 and *C. lividus* would be a first step forward, either by direct observation or by analysis of the
286 gut contents. Furthermore, several questions linked to ecological speciation in cone snails
287 remain unanswered. How can two sympatric populations diverge when larvae are highly
288 dispersive? Existing hypotheses relate to the partition in microhabitats that would prevent
289 adults from meeting and maladapted larvae to settle (Krug, 2011). Then, what is the exact role
290 of the toxins in the prey shift? Conticello et al. (2001) suggested that non expressed toxins
291 present in the genomes can serve as a reservoir in prevision of the appearance of a new prey
292 (“lazarotoxins”), but the new toxins may also appear after the prey shift to increase the
293 specificity of the venom. Clearly, a model-based approach is required in order to better
294 characterize the genetic diversity, biology and ecology of the two species.

295 **5 Acknowledgments**

297 We are grateful to Felix Lorenz (Buseck-Buern) who first noticed that the "*Conus lividus*"
298 from the Marquesas was "something different", and generously handed it over for us to name
299 it in the context of this integrative approach. This project is part of CONCO, the cone snail
300 genome project for health, funded by the European Commission: LIFESCIHEALTH-6

301 Integrated Project LSHB-CT-2007-037592 and we are grateful to Dr Torbjörn Ingemansson,
302 scientific officer. The CONPOL 2007 CONCO expedition to French Polynesia (Nuku Hiva in
303 the Marquesas, Tahiti and Takapoto) was co-organised by Atheris Laboratories (Geneva,
304 Switzerland) and the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER)
305 in partnership with the Institut de Recherche pour le Développement (IRD). The CONFIELD-
306 I 2007 CONCO expedition to the Chesterfield Islands (New Caledonia) was co-organised by
307 Atheris Laboratories (Geneva, Switzerland) and the IRD in partnership with the French Navy
308 (vaisseau P400 "La Glorieuse"). The PANGLAO 2004 Marine Biodiversity Project was
309 funded by the Total Foundation and the French Ministry of Foreign Affairs. The MNHN-
310 IRD-PNI Santo 2006 (Vanuatu) and Atimo Vatae 2010 (Madagascar) expeditions was made
311 possible by grants, among others, from the Total Foundation, the Stavros Niarchos
312 Foundation, and the Prince Albert II of Monaco Foundation. This project was partly supported
313 by the network "Bibliothèque du Vivant" funded by the CNRS, the Muséum National
314 d'Histoire Naturelle, the INRA and the CEA (Genoscope) and by the "Service de
315 Systématique Moléculaire" (UMS 2700 CNRS-MNHN) and by the project CONOTAX,
316 funded by the French "Agence Nationale de la Recherche" (grant number ANR-13-JSV7-
317 0013-01). We would like to thank Evelyne Bachère, Michel Balleton, Philippe Favreau,
318 Antoine Gilbert, Peva Levy, David Piquemal, Joël Orempuller, Piapao and Reto Stöcklin,
319 who collected the specimens of *C. conco* in the Marquesas Archipelago, Napoleon
320 Colombani, Alain Gerbault, Jean-Louis Menou, Robin Offord, and Reto Stöcklin who
321 collected samples in the Chesterfield Islands, Sébastien Dutertre and Oliver Cheneval for the
322 preparation of the samples, Roman Mylonas and Florence Bandelier (Atheris Laboratories)
323 for the transcriptomic analyses, and Manuel Tenorio who prepared and photographed the *C.*
324 *conco* n. sp. radula. Last but not least, we express our gratitude to Jacques Puset (Toxinomics
325 Foundation), Claude Payri (IRD) and the governments of French Polynesia and New
326 Caledonia.

327

328 **6 References**

329

- 330 Barlow, A., Pook, C.E., Harrison, R.A., Wüster, W., 2009. Coevolution of diet and prey-
331 specific venom activity supports the role of selection in snake venom evolution. *Proc.*
332 *R. Soc. B Biol. Sci.* 276, 2443–2449.
- 333 Birney, E., Clamp, M., Durbin, R., 2004. GeneWise and genomewise. *Genome Res.* 14.5,
334 988–995.
- 335 Chomczynski, P., 1993. A reagent for the single-step simultaneous isolation of RNA, DNA
336 and proteins from cell and tissue samples. *BioTechniques* 15, 532–537.
- 337 Clouard, V., Bonneville, A., 2005. Ages of seamounts, islands and plateaus on the Pacific
338 plate, in: Foulger, G.R., Natland, J.H., Presnall, D.C., Anderson, D.L. (Eds.), *Plates,*
339 *Plumes, and Paradigms.* Geological Society of America, Boulder, USA, pp. 71–90.
- 340 Conticello, S.G., Gilad, Y., Avidan, N., Ben-Asher, E., Levy, Z., Fainzilber, M., 2001.
341 Mechanisms for evolving hypervariability: the case of conopeptides. *Mol. Biol. Evol.*
342 18, 120–131.
- 343 Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling
344 trees. *BMC Evol. Biol.* 7, 214.
- 345 Duda, T.F.J., 2008. Differentiation of venoms of predatory marine gastropods: divergence of
346 orthologous toxin genes of closely related *Conus* species with different dietary
347 specializations. *J. Mol. Evol.* 67, 315–321.
- 348 Duda, T.F.J., Kohn, A.J., 2005. Species-level phylogeography and evolutionary history of the
349 hyperdiverse marine gastropod genus *Conus*. *Mol. Phylogenet. Evol.* 34, 257–272.
- 350 Duda, T.F.J., Lee, T., 2009. Ecological release and venom evolution of a predatory marine
351 Snail at Easter Island. *PLoS ONE* 4, e5558.

352 Dutertre, S., Jin, A.H., Kaas, Q., Jones, A., Alewood, P.F., Lewis, R.J., 2013. Deep venomics
353 reveals the mechanism for expanded peptide diversity in cone snail venom. *Mol. Cell.*
354 *Proteomics* 12, 312–329.

355 Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis
356 program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.

357 Huelsenbeck, J.P., Ronquist, F., Hall, B., 2001. MrBayes: Bayesian inference of phylogeny.
358 *Bioinformatics* 17, 754–755.

359 Jablonski, D., Lutz, R.A., 1980. Molluscan larval shell morphology - ecological and
360 paleontological applications, in: Rhoads, D.C., Lutz, R.A. (Eds.), *Skeletal Growth of*
361 *Aquatic Organisms*. Plenum Press, New York, pp. 323–377.

362 Kaas, Q., Westermann, J.C., Craik, D.J., 2010. Conopeptide characterization and
363 classifications: An analysis using ConoServer. *Toxicon* 55, 1491–1509.

364 Kantor, Y.I., Puillandre, N., Fraussen, K., Fedosov, A.E., Bouchet, P., 2013. Deep-water
365 Buccinidae (Gastropoda: Neogastropoda) from sunken wood, vents and seeps:
366 Molecular phylogeny and taxonomy. *J. Mar. Biol. Assoc. U. K.* 93, 2177–2195.

367 Keane, T.M., Creevey, C.J., Pentony, M.M., Naughton, T.J., McInerney, J.O., 2006.
368 Assessment of methods for amino acid matrix selection and their use on empirical data
369 shows that ad hoc assumptions for choice of matrix are not justified. *BMC Evol. Biol.*
370 6, 1–17.

371 King, G.F., Gentz, M.C., Escoubas, P., Nicholson, G.M., 2008. A rational nomenclature for
372 naming peptide toxins from spiders and other venomous animals. *Toxicon* 52, 264–
373 276.

374 Kohn, A.J., 1959. The ecology of *Conus* in Hawaii. *Ecol. Monogr.* 29, 47–90.

375 Kohn, A.J., 2001. Maximal species richness in *Conus*: diversity, diet and habitat on reefs of
376 northeast Papua New Guinea. *Coral Reefs* 20, 25–38.

377 Koua, D., Laht, S., Kaplinski, L., Stöcklin, R., Remm, M., Favreau, P., Lisacek, F., 2013.
378 Position-specific scoring matrix and hidden Markov model complement each other for
379 the prediction of conopeptide superfamilies. *Biochim. Biophys. Acta BBA - Proteins*
380 *Proteomics* 1834, 717–724. doi:10.1016/j.bbapap.2012.12.015

381 Kozminsky-Atias, A., Bar-Shalom, A., Mishmar, D., Zilberberg, N., 2008. Assembling an
382 arsenal, the scorpion way. *BMC Evol. Biol.* 8, 333.

383 Krug, P.J., 2011. Patterns of speciation in marine gastropods: A review of the phylogenetic
384 evidence for localized radiations in the sea. *Am. Malacol. Bull.* 29, 169–186.

385 Moolenbeek, R.G., Zandbergen, A., Bouchet, P., 2008. *Conus* (Gastropoda, Conidae) from the
386 Marquesas Archipelago: description of a new endemic offshore fauna. *Vita Malacol.*
387 6, 19–34.

388 Nascimentoa, D.G., Ratesa, B., Santosa, D.M., Verano-Bragaa, T., Barbosa-Silva, A., Dutra,
389 A.A.A., Biondi, I., Martin-Eauclaire, M.F., De Lima, M.E., Adriano, M.C., 2006.
390 Moving pieces in a taxonomic puzzle: Venom 2D-LC/MS and data clustering analyses
391 to infer phylogenetic relationships in some scorpions from the Buthidae family
392 (Scorpiones). *Toxicon* 47, 628–639.

393 Olivera, B.M., 2006. *Conus* peptides: biodiversity-based discovery and exogenomics. *J. Biol.*
394 *Chem.* 281, 31173–31177.

395 Puillandre, N., Koua, D., Favreau, P., Olivera, B.M., Stöcklin, R., 2012. Molecular
396 phylogeny, classification and evolution of conopeptides. *J. Mol. Evol.* 74, 297–309.

397 Quenouille, B., Hubert, N., Bermingham, E., Planes, S., 2011. Speciation in tropical seas:
398 Allopatry followed by range change. *Mol. Phylogenet. Evol.* 58, 546–552.

399 Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. Available from
400 <http://beast.bio.ed.ac.uk/Tracer>.

401 Röckel, D., Korn, W., Kohn, A.J., 1995. *Manual of the Living Conidae*. Vol. I, Indo-Pacific.
402 Christa Hemmen Verlag, Wiesbaden.

403 Tucker, J.K., Tenorio, M.J., 2013. Illustrated catalog of the living cone shells. MDM
404 Publishing, Wellington, USA.
405 Violette, A., Biass, D., Dutertre, S., Koua, D., Piquemal, D., Pierrat, F., Stöcklin, R., Favreau,
406 P., 2012. Large-scale discovery of conopeptides and conoproteins in the injectable
407 venom of a fish-hunting cone snail using a combined proteomic and transcriptomic
408 approach. *J. Proteomics* 75, 5215–5225.
409

410 **Figure caption**

411

412 Figure 1: Illustrations of shell and radula morphology. A-C: *Conus conco* n. sp., holotype
413 MNHN 23355; D-E: *Conus conco* n. sp. radula (scale = 1 mm and 100µm respectively). F:
414 *Conus conco* n. sp., paratype 7. G: *Conus conco* n. sp., paratype 8. H: *Conus conco* n. sp.,
415 paratype 9. I: *Conus lividus*, MNHN IM-2007-30891, Santo, Vanuatu. J: *Conus lividus*, 39.8
416 mm, Ile Ste Marie, Madagascar, coll. Monnier. K: *Conus sanguinolentus*, 42.2 mm, Ile Ste
417 Marie, Madagascar, coll. Monnier. L: *Conus sanguinolentus*, 52.8 mm, Taiohae, Nuku Hiva,
418 coll. Monnier.

419

420 Figure 2: Bayesian phylogenetic trees (MrBayes) based on (A) the COI gene and (B) the 28S
421 gene. Labels of the specimens correspond to the sample_ID in the the Supplementary Data 2
422 (GB = GenBank). PP (> 0.80) are indicated for each node. Geographic distribution (when
423 known) is indicated as vertical bars: C = Chesterfield Islands; Mg = Madagascar; Mq =
424 Marquesas Islands; P = Philippines; SA = South Africa, T = Tahiti; V = Vanuatu. (C): map
425 showing the different sampling localities in the Indo-West Pacific.

426

427 Figure 3: Bayesian phylogenetic tree (BEAST) based on a concatenation of the COI and 28S
428 genes with estimate of the age of each node (with confidence interval). PP (> 0.95) are
429 indicated for each node.

430

431 Figure 4: Phylogenetic tree obtained by coding the presence/absence of each toxin mass for
432 the three species *C. sanguinolentus* (light grey lines), *C. lividus* (dark grey lines) and *C. conco*
433 (black lines).

434

435

436 **Supplementary Data**

437

438 Supplementary Data 1: Description of *Conus conco* new species

439

440 Supplementary Data 2: List of analyzed specimens. Sample ID (museum voucher ID when
441 available), species name, locality, COI and 28S GenBank accession numbers. Specimens used
442 for MALDI-TOF and transcriptomic analyses are marked with an “x” in the last two columns.

443

444 Supplementary Data 3: Conotoxin sequences found in the transcriptomes of *C. lividus*, *C.*
445 *sanguinolentus* and *C. conco*.

446

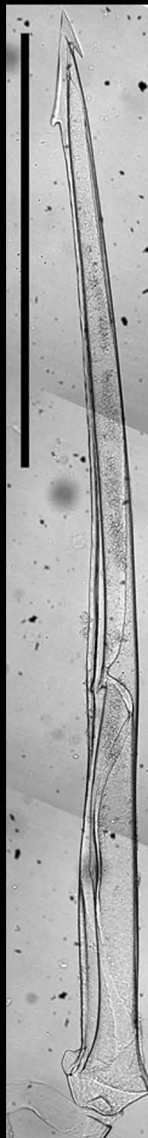
447 Supplementary Data 4: Description of type specimens. Measurements are in millimeters,
448 rounded to nearest 0.1 mm. Measurements are made according to the method of Röckel *et*
449 *al.*(1995). L: shell length; RD: relative diameter; MD: maximum diameter; PMD: position of
450 maximum diameter; HMD: height of maximum diameter; RSH: relative spire height; AH:
451 aperture height. A: *Conus conco*; B: *Conus sanguinolentus*; C: *Conus lividus*.

452

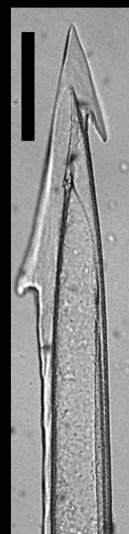
453 Supplementary Data 5: Maximum diameter (MD) plotted against Aperture height (AH) for *C.*
454 *lividus* (n=16; diamonds), *C. sanguinolentus* (n=15; squares) and *C. conco* (n=17; triangles).

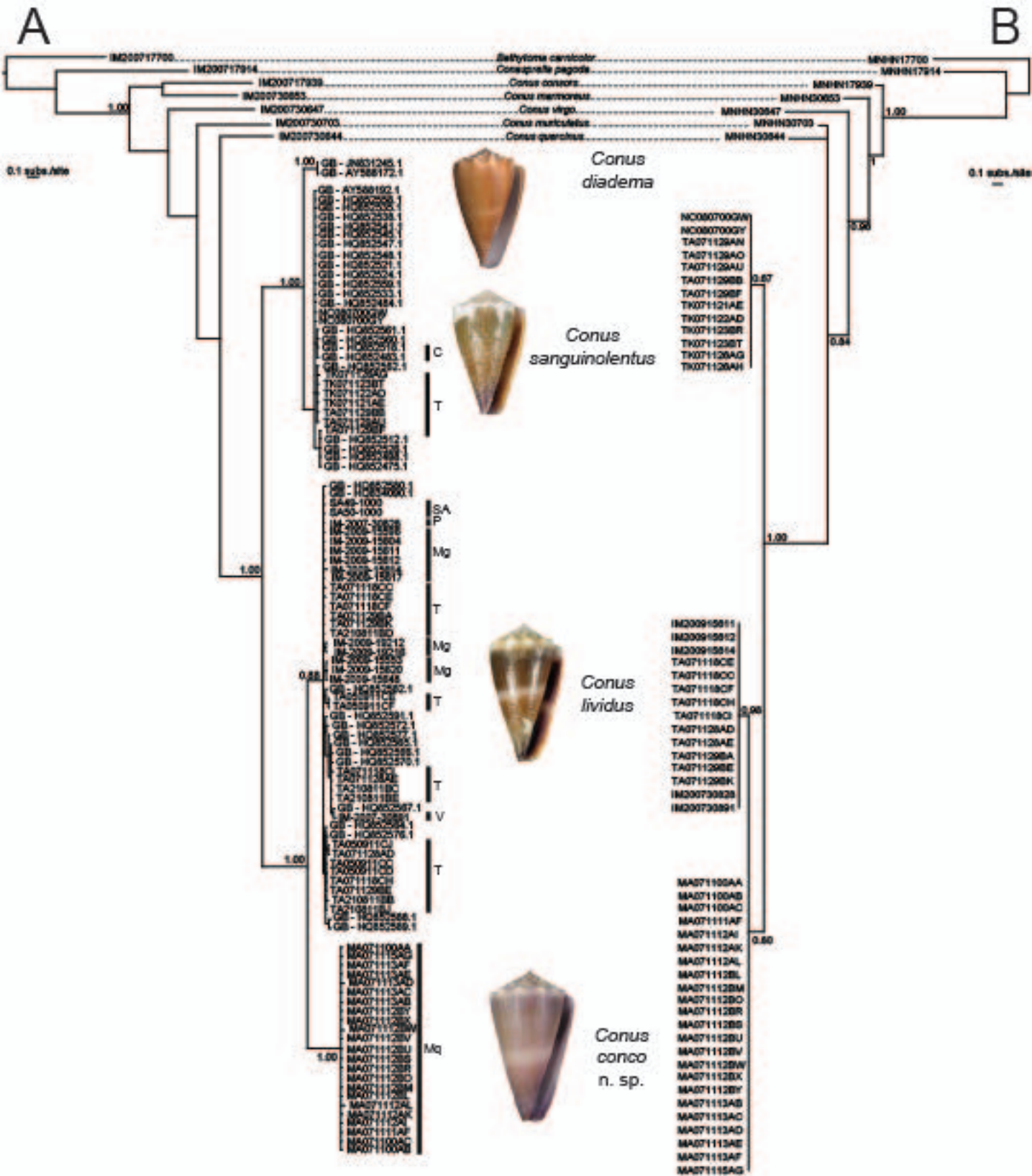


D



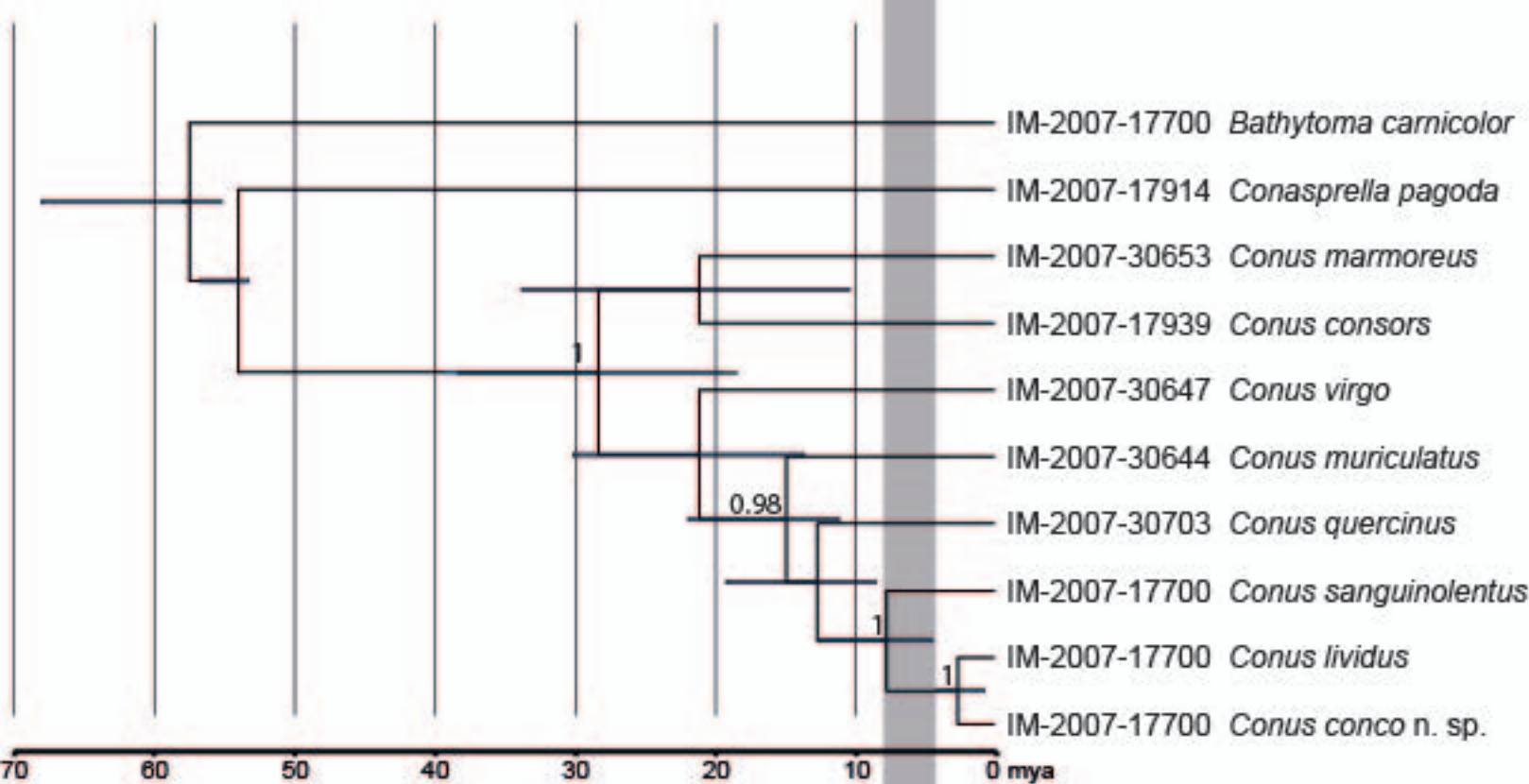
E



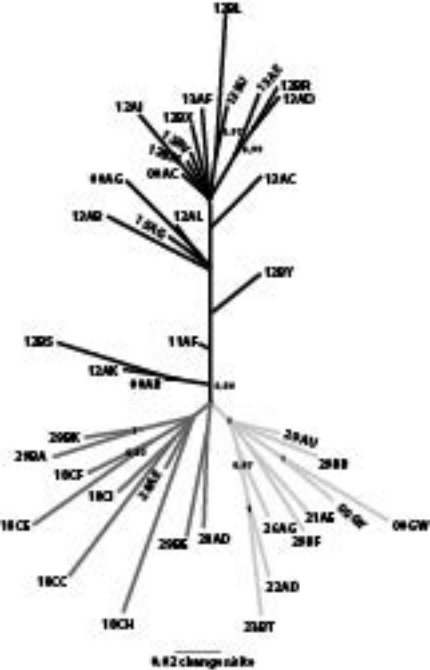


C





Emergence of the
Marquesas
archipelago



Corrigendum

Corrigendum to ‘When everything converges: Integrative taxonomy with shell, DNA and venom data reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea)’ [Molecular Phylogenetic and Evolution Vol. 80 (2014) 186-192]

Puillandre Nicolas^{a,b}, Stöcklin Reto^b, Favreau Philippe^b, Bianchi Estelle^b, Perret Frédéric^b, Rivasseau Audrey^c, Limpalaër Loïc^d, Monnier Eric^e, Bouchet Philippe^f.

^a Museum National d’Histoire Naturelle, Département Systematique et Evolution, ISyEB Institut (UMR 7205 CNRS/UPMC/MNHN/EPHE), 43, Rue Cuvier, 75231 Paris, France

^b Atheris Laboratories, Case postale 314, CH-1233 Bernex-Geneva, Switzerland

^c UMS 2700, Museum National d’Histoire Naturelle, Département Systematique et Evolution, 43, Rue Cuvier, 75231 Paris, France

^d 11, rue de la Poste 60510 Haudivillers

^e Conservatoire National des Arts et Métiers, Département Chimie, Alimentation, Santé, Environnement et Risque, 292, rue Saint-Martin, 75003 Paris, France

^f Museum National d’Histoire Naturelle, Département Systematique et Evolution, ISyEB Institut (UMR 7205 CNRS/UPMC/MNHN/EPHE), 55, Rue Buffon, 75231 Paris, France

The article by Puillandre et al. (2014) included the description of the new species *Conus conco*. The formal description appeared in the online Supplementary Material only, and was not included in the printed version of the article. This description does not meet the requirements of the International Code of Zoological Nomenclature (ICZN) for electronic publications, and the name *Conus conco* Puillandre et al., 2014, is not a nomenclaturally available name.

To meet the ICZN requirements and make the name *Conus conco* available, the description (formerly Supplementary Material) is now published as a standard part of *Molecular Phylogenetics and Evolution*. The date of publication of the new species will be the date of publication of the printed edition of the journal.

Description of *Conus conco* new species

Conus conco new species

Type data: Holotype MNHN IM-2000-23355 (specimen Atheris MA071111AF) in Muséum National d'Histoire Naturelle, collected by Atheris Laboratories, under Reto Stöcklin (Fig. A1 A-C). Associated COI and 28S sequences deposited in GenBank under accession numbers KJ550027 and KJ603257, respectively.

Paratypes: 16 specimens (Puillandre et al., 2014, Supplementary data 4, Fig. A1 F-G).

Type locality: Taioha'e bay, Nuku Hiva, Marquesas Islands, French Polynesia.

Etymology: The specific name is a noun in apposition. It is taken from the “Conco” European project.

Description: Shell of medium size, ranging from 37.4 mm to 56.8 mm. Shape of the last whorl conical, constant in PMD (average 0.958) and RD (average 0.625). Spire low, with strongly tuberculated shoulder. Average RSH is 0.118. Number of tubercles on the last whorl varies from 10 to 11. Protoconch eroded in all studied specimens and number of its whorls unknown; deep pink in color from observation of the best preserved ones. Adult shell with 11 - 12 teleoconch whorls. Spire outline straight with pointed apex. Sutural ramp shows up to six irregular shallow spiral striae. Last whorl profile very slightly convex in the upper part of the last whorl and straight below.

Aperture slightly widening abapically. Holotype shell smooth on the adapical half of the last whorl and sculptured by narrow widely separated cords on the abapical part. In some specimens shell almost completely smooth, in others cords may be granulose. Shell color barely variable. Spire lavender to purple. First teleoconch whorls may have brown dots between the tubercles. Last whorl with lavender to mauve ground color overlaid with a plain beige coat missing in a wide, diffuse, mid-body and at periphery. Anterior extremity darker than the rest of the body whorl. Aperture deep violet or blue becoming lighter towards the interior and with a paler band in the middle. Periostracum golden brown, smooth and translucent. The radula (Fig. A1 D, E) shows no difference with the radula of *C. lividus* (Tucker and Tenorio, 2013).

Geographical range and habitat: Beside Nuku Hiva, *Conus conco* has also been collected in Ua Pou, Tahuata (this paper), and Ua Huka (Moolenbeek et al., 2008, as *C. lividus*), and appears to be endemic to the Marquesas archipelago. It has been collected under stones in 8 meters in Tahuata and in crevices between 15 and 30 m in Nuku Hiva (Alain Gaspard, pers. comm.).

Remarks: *Conus conco* belongs to the *Conus lividus* (Hwass, 1792) species complex together with *C. sanguinolentus* (Quoy & Gaimard, 1834) (Fig. A1 I-L). The three species cannot be separated based on their overall contours (Puillandre et al., 2014, Supplementary Material 5), and the only significant morphometric parameter that separates them is the adult size: *C. sanguinolentus* has the smallest shells (average size of 35.2 mm, sample range from 24.9 mm to 44.9 mm); *C. lividus* shells reach 73 mm (in our sample) and even 81 mm (Röckel et al., 1995) (minimum = 28.2 mm and average = 44.9 mm); our sample of *C. conco* is quite homogeneous with an average size of 48 mm, a minimum of 37.4 mm and a maximum of 56.8 mm (one specimen illustrated by Röckel et al. (1995), plate 4 fig. 12, reaches 61 mm). *Conus lividus* differs from *C. conco* by its colour. The former is olive or yellowish brown; the spire and shoulder are completely white. No shell of *C. lividus* shows lavender to purple background color. *Conus sanguinolentus*, which is sympatric with the new species in the Marquesas, has brown spots between the spire tubercles but it never has a lavender to purple background color; pustules are more frequently present on the abapical cords.

Conus conco has been known for long and confused with *C. sanguinolentus* (Röckel et al., 1995) or *C. lividus* (Moolenbeek et al., 2008). It is also sometimes available in the specimens shell trade under the name *Conus unicolor* G.B. Sowerby I, 1833. The latter was named without description and type locality, no type specimen exists, and the type figure cannot be unambiguously recognized. Kohn (1992) concluded that it is a *nomen dubium*, an opinion followed by Filmer (2001). A different opinion was expressed by Richard (1985), who found similarities between the type figure of *C. unicolor* and shells originating from the Marquesas that are generally assigned to *C. moreleti* Crosse, 1858. Our opinion is that the type figure does not agree neither with *C. conco* nor with *C. moreleti* which has crowded nodules on the spire. Furthermore, no marine mollusc endemic to the Marquesas seems to have reached European conchologists until the early mid-1840s (Tröndle and Cosel, 2005). The voyage of the "Sulphur", which called in Nuku Hiva in January 1840, was the first British source that sampled the Marquesas. Thus Sowerby could not have Marquesan shells before that date.

Conus sanguinolentus Quoy & Gaimard, 1834 was established based on the soft parts of a young specimen collected in Carteret [today Kavieng, New Ireland, Papua New Guinea]. This shell is not present in MNHN (where the Quoy and Gaimard material is preserved); Kohn (1992) designated the specimen illustrated by Quoy and Gaimard (1833, pl. 53 fig. 18) as the lectotype, and treated *C. sanguinolentus* as a valid species. By contrast, Richard (1985) considered *C. sanguinolentus* a synonym of *C. lividus*. *Conus lividus* Hwass, 1792 is defined by the lectotype in MHNG, and none of its accepted synonyms (Röckel et al., 1995) is applicable to the new species.

References

- Filmer, R.M., 2001. A Catalogue of Nomenclature and Taxonomy in the Living Conidae 1758–1998. Backhuys Publishers, Leiden.
- Kohn, A.J., 1992. A chronological taxonomy of *Conus*, 1758-1840. Smithsonian Institution Press, Washington.
- Moolenbeek, R.G., Zandbergen, A., Bouchet, P., 2008. *Conus* (Gastropoda, Conidae) from the Marquesas Archipelago: description of a new endemic offshore fauna. *Vita Malacol.* 6, 19–34.
- Puillandre, N., Stöcklin, R., Favreau, P., Bianchi, E., Perret, F., Rivasseau, A., Limpalaër, L., Monnier, E., Bouchet, P., 2014. When everything converges:

Integrative taxonomy with shell, DNA and venom data reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea). *Mol. Phylogenet. Evol.* 80, 186–192. doi:10.1016/j.ympev.2014.06.024

Quoy, J.R.C., Gaimard, J.P., 1833. Voyage de découvertes de l’Astrolabe. Zoologie, Atlas. J. Tastu, Editeur-Imprimeur, Paris.

Richard, G., 1985. Conidae de Polynésie Française (III). *Xenophora* 28, 9–20.

Röckel, D., Korn, W., Kohn, A.J., 1995. Manual of the Living Conidae. Vol. I, Indo-Pacific. Christa Hemmen Verlag, Wiesbaden.

Tröndle, J., Cosel, R. v., 2005. Inventaire bibliographique des mollusques marins de l’archipel des Marquises (Polynésie Française). *Atoll Res. Bull.* 542, 267–340.

DOI of original article: 10.1016/j.ympev.2014.06.024

Corresponding author: Nicolas Puillandre, Museum National d’Histoire Naturelle, Département Systematique et Evolution, ISyEB Institut (UMR 7205 CNRS/UPMC/MNHN/EPHE), 43, Rue Cuvier, 75231 Paris, France. Tel: +33 1 40 79 31 73

Corresponding author email: puillandre@mnhn.fr