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

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

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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](http://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.

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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

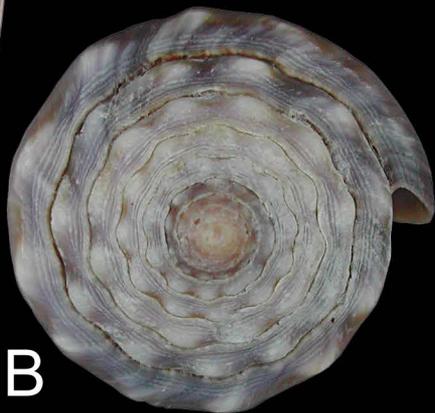
444 Supplementary Data 3: Conotoxin sequences found in the transcriptomes of *C. lividus*, *C.*  
445 *sanguinolentus* and *C. conco*.

446

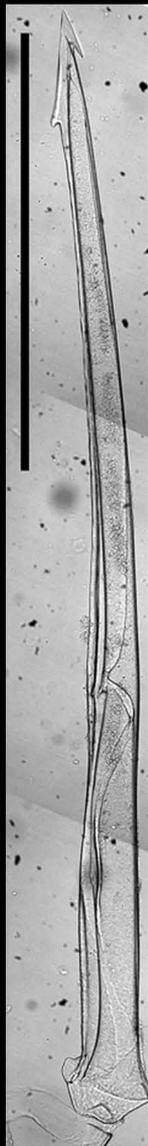
447 Supplementary Data 4: Description of type specimens. Measurements are in millimeters,  
448 rounded to nearest 0.1 mm. Measurements are made according to the method of Röckel *et*  
449 *al.*(1995). L: shell length; RD: relative diameter; MD: maximum diameter; PMD: position of  
450 maximum diameter;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

452

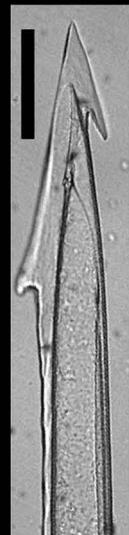
453 Supplementary Data 5: Maximum diameter (MD) plotted against Aperture height (AH) for *C.*  
454 *lividus* (n=16; diamonds), *C. sanguinolentus* (n=15; squares) and *C. conco* (n=17; triangles).



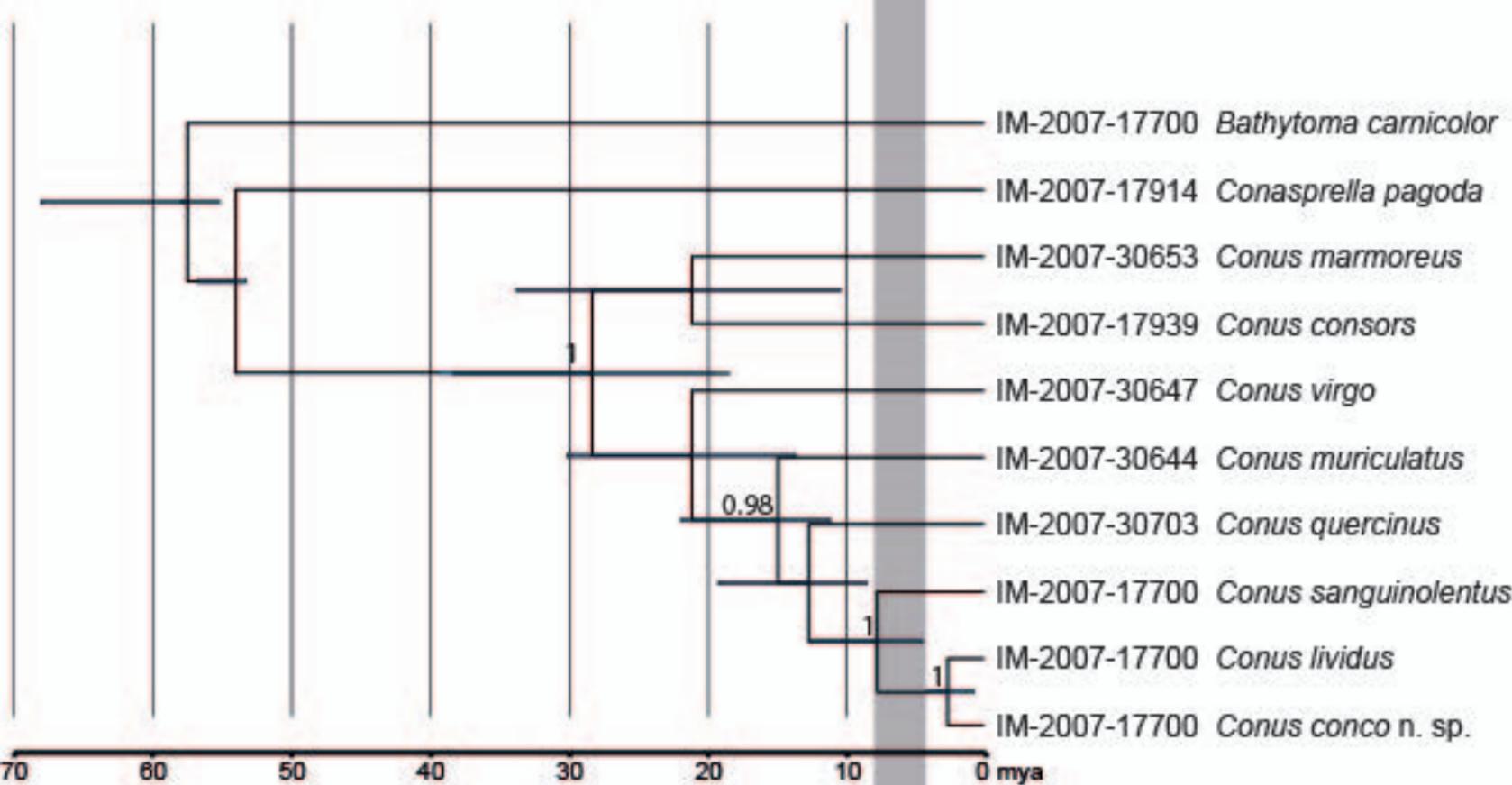
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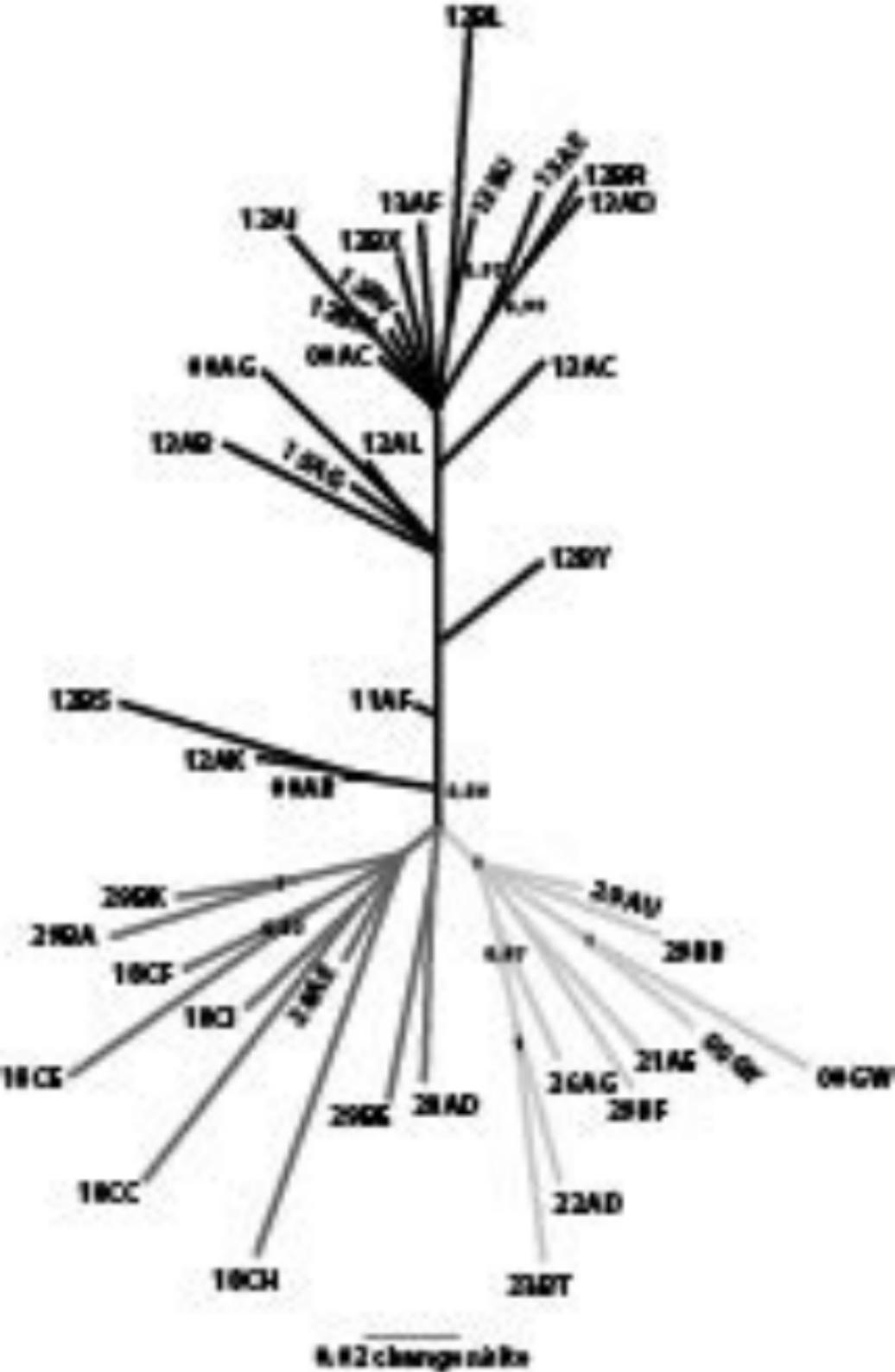
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Emergence of the  
Marquesas  
archipelago



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]

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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.

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