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A question of rank: DNA sequences and radula characters reveal a new genus of cone snails (Gastropoda: Conidae)

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ABSTRACT

Molecular phylogenies of cone snails have revealed that the *c.* 350 sequenced species are divided into four main lineages, *Conus*, *Conasprella*, *Californiconus* and *Profundiconus*. In a recent study, minute species (less than 8 mm) were for the first time included in a molecular phylogenetic tree and were shown to correspond to deep lineages, of similar status to the four previously recognized, and sister group to *Californiconus*. They were attributed to the available generic names *Lilliconus* and *Pseudolilliconus*. In this article, we analyse, using morphological (shell and radula) and molecular characters (*cox1* gene), several species of minute cone snails, and we conclude that the species considered as *Pseudolilliconus* in the previous study should actually be placed in a new genus, *Pygmaeconus*. By comparing the *cox1* genetic distances calculated among the species of *Lilliconus*, *Pygmaeconus* and *Californiconus* with the genetic distances calculated among other cone snails species included in different subgenera and genera, and by comparing the estimated ages of *Lilliconus* and *Pygmaeconus* with the ages of other caenogastropod genera, we conclude that *Lilliconus* and *Pygmaeconus* can be considered at the genus rank.

INTRODUCTION

The Linnean ranks above the species level are hardly comparable between taxa, whether in terms of genetic distance, morphological divergence, species diversity or age (Johns & Avise, 1998; Hedges *et al.*, 2015; Giribet *et al.*, 2016). Even when the taxa are defined following the clear criterion of monophyly, deciding which clades will be named and at which rank they will be placed is often arbitrary and taxonomist dependent. Consequently, some authors simply argue that ranks should be abandoned (Zachos, 2011; Lambertz & Perry, 2016), while others state that ranks remain important for communication and that they convey information (Giribet, Hormiga & Edgecombe, 2016).

In cone snails (Gastropoda, Conoidea), many classifications have been proposed in the past two centuries, but only in the last 15 years have phylogenetic approaches been used to test whether the groups defined mainly by shell characters are compatible with independently evolving lineages (e.g. Espiritu *et al.*, 2001; Duda & Kohn, 2005). In fact, the cladistic analyses of shell and radula characters and of the growing amount of DNA sequence data have led to the conclusion that groups above the species level defined only by shell morphology and without regard to a phylogenetic framework are meaningless as evolutionary hypotheses (Tucker & Tenorio, 2009; Puillandre *et al.*, 2014). Based on these recent works, two competing classifications have been proposed (Tucker & Tenorio, 2009; Puillandre *et al.*, 2015). Overall, the taxa defined in these two classifications are similar and compatible, and the discrepancies mostly concern species for which the radula remains unknown and/or no DNA sequences are available. However, these two classifications contradict each other regarding the ranks at which the taxa are considered. Since the work of Duda & Kohn (2005), living cone snails have been known to consist of a few main lineages, of which one in particular has radiated into several hundred species. At first, two lineages were recognized (the 'large major clade' and the 'small major clade'; Duda & Kohn, 2005; Williams & Duda, 2008) and subsequently the single-species lineage represented by *Californiconus californicus* (Reeve, 1844) was added (Tucker & Tenorio, 2009; Biggs *et al.*, 2010). These three lineages were recognized at the level of family or subfamily (Conidae, Conilithinae and Californiconinae, respectively; Tucker & Tenorio, 2009) or at the level of genus (*Conus*, *Conasprella* and *Californiconus*, respectively; Puillandre *et al.*, 2015). Puillandre *et al.* (2015) added a fourth lineage to the list, *Profundiconus* (previously recognized as one of the genera of Conilithidae by Tucker & Tenorio, 2009).

A criterion has been proposed to standardize the ranks of taxa, namely temporal banding, where temporal ranges are attributed to each rank (Avise & Liu, 2011). Applying this criterion could eventually allow discrimination between the two alternate cone snail classifications, by comparing the ages of their main lineages with the ages of the families and genera of

other conoideans. Although a family-level classification of the Conoidea based on a molecular phylogeny has been proposed (Bouchet *et al.*, 2011), only a few genera have been revised, and a large number of them are probably nonmonophyletic (e.g. Castelin *et al.*, 2012b; Puillandre *et al.*, 2012). Furthermore, the published phylogenies are not dated. Consequently, it is difficult to apply this criterion to all the cone snails. However, it can be used tentatively to attribute a rank to new lineages of cone snails. In a recent article, Uribe, Puillandre & Zardoya (2017) published a phylogeny of cone snails based on full mitogenomes. The four main lineages of cone snails (*Profundiconus*, *Conus*, *Californiconus* and *Conasprella*) were recovered, plus two new lineages, sisters to *Californiconus*, which were revealed for the first time. These are minute cone snails and were tentatively attributed to two taxa placed at the genus level (thus at the same rank as the four main lineages of cone snails), *Lilliconus* and *Pseudolilliconus*.

In the present study, we aimed to test two hypotheses. First, we tested whether the two species sequenced by Uribe *et al.* (2017) can be attributed to *Lilliconus* and *Pseudolilliconus*, by analysing the morphological (shell and radula) and molecular (cytochrome oxidase subunit I gene, *cox1*) variability of additional specimens and species of minute cone snails. Second, we used two criteria to determine at which rank the taxa of minute cone snails should be considered. To do so, the genetic distances (calculated for the *cox1* gene) among and within the main lineages of cone snails were compared with the genetic distances among the minute cone snails and their closest relative, *Californiconus*. To avoid the effect of homoplasy in the *cox1* gene, which is significant at the family level, we refrained from comparing genetic distance with other families. In addition, the estimated ages of the lineages of cone snails were compared with ages of other caenogastropod taxa, obtained from the literature.

MATERIAL AND METHODS

Samples

Minute cone snails used for molecular analyses were collected during two expeditions of the Museum National d'Histoire Naturelle, Paris (MNHN): Atimo Vatae in Madagascar in 2010 and Kavieng 2014 in Papua New Guinea (Table 1). During the Atimo Vatae expedition, specimens were treated with an isotonic solution of magnesium chloride until relaxed (i.e. showing no response to touch) and then a tissue clip was cut; during the Kavieng 2014 expedition, specimens were processed using a microwave oven (Galindo *et al.*, 2014). Tissue samples were preserved in 96% ethanol and voucher shells are kept in MNHN. Additional material was studied for morphological characters (shell and radula), most of it previously deposited in institutional repositories as indicated.

Shell and radula analyses

We describe shell morphology using the terminology established by Röckel, Korn & Kohn (1995). Descriptions are based on shells orientated in the traditional way, spire uppermost and with the aperture facing the viewer. Maximum shell length (SL) was measured using a digital caliper and measurements rounded to the nearest 0.1 mm.

Specimens of shells containing the dried animal inside were digested in concentrated aqueous potassium hydroxide for 24 h. These included specimens MNHN IM-2009-31328 and MNHN IM-2013-47253, which were sequenced, plus four additional specimens that were not sequenced. The contents were flushed out of the shell by injecting distilled water through the aperture by means of a syringe with an incurved needle. The resulting mixture was then placed in a Petri dish and examined under a binocular

Table 1. List of specimens analysed.

MNHN ID	BOLD ID	Expedition	Family	Genus	Species	GenBank accession number
			Conidae	*Californiconus*	*californicus*	DQ885848.1
IM-2007-17914	CONO313-08	Panglao 2005	Conidae	*Conasprella*	*pagoda*	EU015729
IM-2007-17921	CONO296-08	Panglao 2005	Conidae	*Conasprella*	*orbignyi*	EU015721
IM-2007-34849	CONO1508-14	Terrasses	Conidae	*Conasprella*	*alisi*	KJ550113
			Conidae	*Conasprella*	*arcuata*	KJ549861
IM-2007-30639	CONO1403-14	Santo 2006	Conidae	*Conus*	*striatus*	KJ550458
IM-2007-30646	CONO999-10	Santo 2006	Conidae	*Conus*	*distans*	KJ550204
IM-2007-30653	CONO1004-10	Santo 2006	Conidae	*Conus*	*marmoreus*	KJ550367
IM-2007-30860	CONO1460-14	Santo 2006	Conidae	*Conus*	*chiangi*	KJ550172
IM-2009-31325	CONO1979-17	Atimo Vatae	Conidae	*Lilliconus*	*sagei*	KY570905
IM-2009-31328	CONO1980-17	Atimo Vatae	Conidae	*Lilliconus*	*sagei*	KY570904
IM-2007-30760	CONO1027-10	Ebisco	Conidae	*Profundiconus*	*barazeri*	KJ550111
IM-2009-18243	CONO1477-14	Terrasses	Conidae	*Profundiconus*	*vaubani*	KJ550517
IM-2013-18551	CONO1807-15	Papua Niugini	Conidae	*Profundiconus*	*teramachii*	KT874757
IM-2013-47253	CONO1981-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570911
IM-2013-47254	CONO1982-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570910
IM-2013-47769	CONO1984-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570908
IM-2013-47770	CONO1985-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570907
IM-2013-47771	CONO1986-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570906
IM-2013-50753	CONO1987-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570913
IM-2013-53787	CONO1983-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570912
IM-2013-54883	CONO1988-17	Kavieng	Conidae	*Pygmaeconus*	*traillii*	KY570909
IM-2007-17700	CONO147-08	Boa 1	Borsoniidae	*Bathytoma*	*carnicolor*	EU015643
IM-2007-17934	CONO372-08	Salomon 2	Borsoniidae	*Borsonia*	*sp.*	EU015746
IM-2007-42331	CONO602-08	Norfolk 2	Conorbidae	*Benthofascis*	*lozoueti*	HQ401574
IM-2007-40991	FRANZ462-08	Santo 2006	Turridae	*Turris*	*condei*	EU820787

microscope. The entire radula was removed with fine tweezers and rinsed with distilled water, then mounted on a slide using Aquatex (Merck) mounting medium and examined under an optical microscope. Photos were obtained with a CCD camera attached to the microscope. Samples of individual radular teeth for scanning electron microscopy (SEM) were rinsed with distilled water, allowed to dry in air and then mounted on stubs covered with double-sided carbon tape. SEM studies were carried out at the Museo Nacional de Ciencias Naturales-Consejo Superior de Investigaciones Científicas (MNCN-CSIC) on a FEI INSPECT SEM equipped with a secondary and retro-dispersed electron detector and an analytical-INCA integrated analysis system (Oxford Instruments). We used the terminology for radular teeth of Tucker & Tenorio (2009) and the abbreviations of Kohn, Nishi & Pernet (1999).

Molecular analyses

DNA was extracted using the Epmotion 5075 robot (Eppendorf), following the manufacturer's recommendations. A fragment of the *cox1* gene was amplified using universal primers LCO1490/HCO2198 (Folmer *et al.*, 1994). PCRs were performed in 25 µl, containing 3 ng of DNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.26 mM dNTP, 0.3 mM each primer, 5% DMSO and 1.5 units of Qbiogene Q-Bio Taq. Amplification consisted of an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C, followed by extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. PCR products were purified and sequenced by the Eurofins sequencing facility. Specimens and sequences were deposited in BOLD and GenBank (Table 1).

Cox1 sequences from representative specimens of the four main deep lineages of cone snails, together with four non-cone snail conoideans, chosen from closely related (Borsoniidae and Conorbidae) or more distant groups (Turridae) (Puillandre *et al.*, 2011) were added to the newly sequenced specimens (Table 1). Most sequences were obtained from specimens in MNHN, except two (*Californiconus californicus* and *Conasprella arcuata*), which were downloaded from GenBank (Table 1). All the sequences were aligned using Muscle v. 3.8.31 (Edgar, 2004). The dataset was analysed using a Bayesian approach as implemented in MrBayes v. 3.2 (Huelsenbeck, Ronquist & Hall, 2001), with two runs consisting of four Markov chains of 20,000,000 generations each, with a sampling frequency of one tree 1,000 generations each. Each codon position of the *cox1* gene was treated as an unlinked partition, each following a general time reversible (GTR) model, with a gamma-distributed rate variation across sites approximated in four discrete categories and a proportion of invariable sites. Convergence of each analysis was evaluated using Tracer v. 1.6 (Rambaut & Drummond, 2014) and analyses were terminated when estimated sample size (ESS) values were all >200. A consensus tree was then calculated after omitting the first 25% trees as burn-in. Statistical support was evaluated as Bayesian posterior probability (PP).

Comparison of genetic distances

We compile a dataset of *cox1* genetic distances (the most commonly sequenced gene in cone snails), combining all the sequences available in GenBank with unpublished *cox1* sequences obtained from specimens preserved in the MNHN collections. Only one sequence per species was retained, giving a total of 349 sequences. All the sequences were trimmed to the 'barcode' fragment (defined by the Folmer primers; Folmer *et al.*, 1994) and aligned using Muscle v. 3.8.31 (Edgar, 2004). Tamura-Nei genetic distances were computed using MEGA v. 6 (Tamura *et al.*, 2013) and the pairwise distributions of the intergeneric, intrageneric/intersubgeneric and intrasubgeneric distances (following the classification of Puillandre *et al.*, 2015) were visualized separately.

Comparison of clade ages

A review of the literature was performed to identify articles that included dated phylogenies of groups within caenogastropods. For each dated tree, the stem ages of the genera were estimated using the time scale of the published phylogenetic trees. Only monophyletic groups (thus including at least two representatives) were taken into account. These ages were then compared with the stem ages of the six main lineages of cone snails, as estimated by Uribe *et al.* (2017).

Abbreviations of museums and institutions

AMS	Australian Museum, Sydney
CSIC	Consejo Superior de Investigaciones Científicas, Spain
INHS	Illinois Natural History Survey, Illinois
MJT	Manuel J. Tenorio reference collection, Jerez, Spain
MNCN	Museo Nacional de Ciencias Naturales, Madrid
MNHN	Muséum National d'Histoire Naturelle, Paris
NBC	Naturalis Biodiversity Centre, Leiden
NHMUK	Natural History Museum, London
SMNS	Staatliches Museum für Naturkunde, Stuttgart
WAM	Western Australian Museum, Perth
ZMA	Zoological Museum, Amsterdam (collection now in NBC)

RESULTS

Phylogenetic analyses and comparison of genetic distances and clade ages

Our *cox1*-based phylogenetic tree including several specimens for each sequenced species of minute cone snails (Fig. 1) is mostly congruent with the results obtained by Uribe *et al.* (2017), using only one specimen per species but full mitogenomes. All cone snails form a monophyletic group, although without statistical support (PP = 0.37). Three main lineages of cone snails previously reported in the literature (*Profundiconus*, *Conasprella* and *Conus*) each correspond to a highly supported clade (PP > 0.98), with shorter within-clade branches and longer between-clade branches. *Conus* and *Conasprella* are sister groups (PP = 0.99), in contradiction to the tree based on full mitogenomes, in which *Conasprella* is more closely related to *Californiconus* and relatives (see below). *Californiconus californicus* is an independent lineage, sister to the two lineages of minute cone snails revealed by Uribe *et al.* (2017) (PP = 1), each characterized by relatively long branches. One of them includes two specimens identified as *Lilliconus sagei* (Korn & Raybaudi Massilia, 1993) (Fig. 2D, E). The other lineage includes eight individuals of a species that had been tentatively placed in the genus *Pseudolilliconus* by Tucker & Tenorio (2009), an opinion followed by Uribe *et al.* (2017), namely *P. traillii* (Adams, 1855) (Fig. 2I–L). As discussed below, morphological comparison suggests that *traillii* and *boschorum* (Fig. 2G; type species of *Pseudolilliconus*) do not belong to the same genus or subgenus and, therefore, we introduce a new taxon, *Pygmaeconus* (below), to include *traillii* and other related species.

The distribution of the genetic distances calculated among genera, within genera but among subgenera, and within subgenera (following the classification of Puillandre *et al.*, 2015) are distinct, but largely overlapping (Fig. 3). In comparison, the genetic distances calculated between specimens of *Californiconus*, *Lilliconus* and *Pygmaeconus* are between 0.24 and 0.26; this is clearly out of the within-subgenera range of genetic distances for the cone snails, in the upper part (highest 10%) of the within-genera/among-subgenera range and in the middle of the among-genera range.

The stem ages of *Lilliconus* and *Pygmaeconus* are clearly younger that the stem ages of the four other main lineages of cone snails

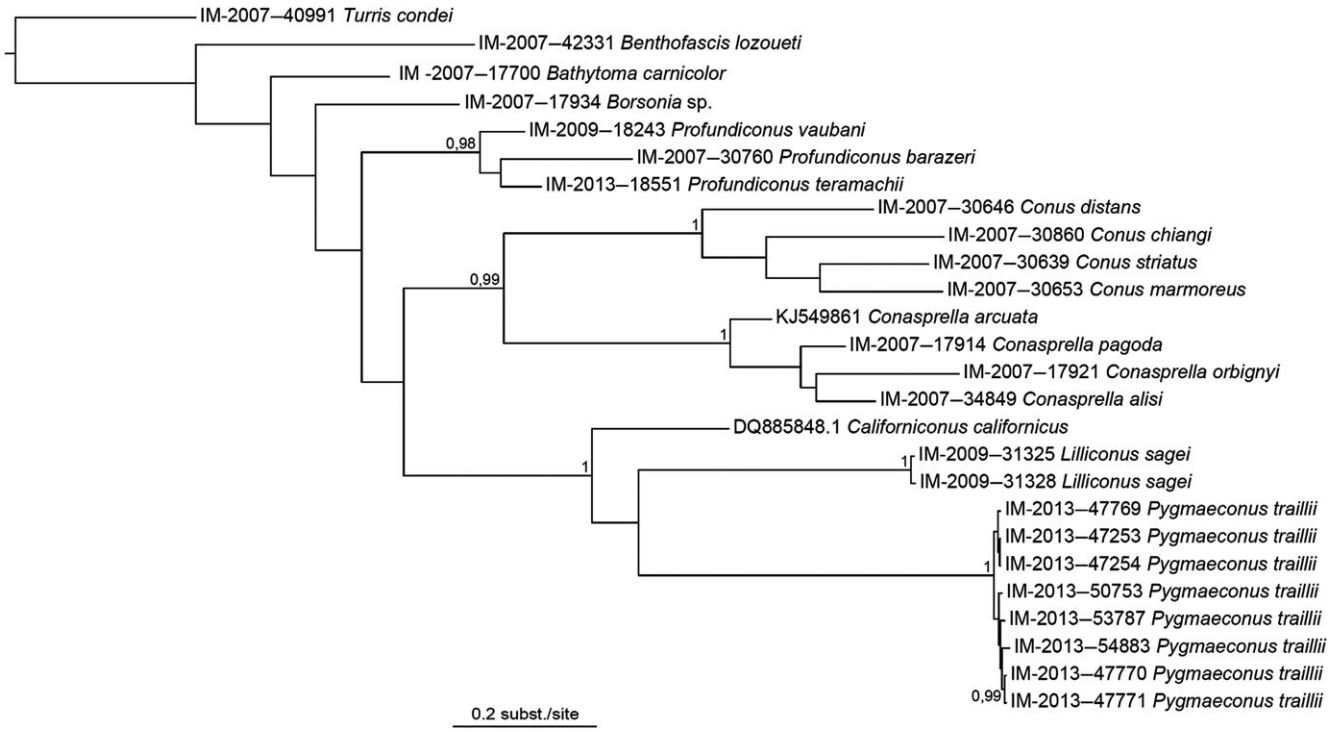

Figure 1. Bayesian phylogenetic tree obtained with the *cox1* gene. Posterior probabilities (if >0.95) are shown above nodes.

(30 Ma *vs* 38–56 Ma; Fig. 4). Compared with other ages of genera in Muricidae, Bursidae and Littorinidae (Castelin *et al.*, 2012a; Reid, Dyal & Williams, 2012; Claremont *et al.*, 2013), the stem ages of *Lilliconus* and *Pygmaeconus* would be among the youngest genera, with only four genera of Rapaninae (Muricidae) that are younger.

Based on these results, we conclude that *Pygmaeconus* and *Lilliconus* (and potentially *Pseudolilliconus*) are at least two different subgenera. Considering them as two different genera is also compatible with the distribution of genetic distances among the four main lineages of cone snails, considered here as different genera, and with the estimated ages for genera of Muricidae.

SYSTEMATIC DESCRIPTION

Family CONIDAE Fleming, 1822
Genus *Pygmaeconus* new genus
(Figs 2, 5)

Type species: *Pygmaeconus traillii* (Adams, 1855) (Fig. 2I–L).

ZooBank registration: urn:lsid:zoobank.org:act:C73D6E30-2BA7-494F-9303-CF3B9951B82C.

Etymology: The name combines *Conus* and *pygmaeus*, pertaining to a pygmy or dwarf (Latin), in reference to the very small size of the species in this genus.

Material examined: More than 50 specimens of *Pygmaeconus* species from Philippines, Papua New Guinea and Indonesia (MNHN, MJT).

Diagnosis: Shell very small, squat, rounded with high spire; protoconch paucispiral; sutural ramp flat or convex; surface of last whorl sculptured with variable number of equally spaced raised minute spiral cords; operculum small; radular tooth of relatively large size, anterior portion much shorter than posterior portion, tooth armed with 1 barb, 3 blades and 1 small denticle, shaft fold blunt, basal spur absent.

Shell (Fig. 2I–Q): Shell very small (SL 3–9 mm), broadly and ventricosely conical, often squat and rounded, with high spire of straight or slightly convex profile. Nodules usually absent, but a few large knobs, often rounded and obsolete, may be present. Protoconch paucispiral. Sutural ramp flat or convex, often smooth, occasionally with 1–2 grooves or furrows; deeply incised suture. Shoulder rounded; sides of last whorl convex; surface of last whorl sculptured with variable number of equally spaced raised minute spiral cords leaving flat ribbons between; these cords usually cover entire last whorl and may reach spire, but often absent on shoulder region. Anal notch shallow; anterior notch absent. Operculum small. Periostracum yellowish, smooth, translucent.

Radula (Fig. 5): Radular tooth relatively large for size of shell (SL/tooth length = 17.4–19.4); anterior portion of tooth much shorter than posterior portion. Tooth with 1 barb and 3 blades (terminology of Tucker & Tenorio, 2009), plus 1 small denticle on under side of tooth in middle of adapical opening; the small apical barb opposes the short, pointed blade; wrapped around the shaft is a tusk-shaped posterior blade; third blade is a pointed structure that terminates the sheet that rolls around the shaft, located at level of waist of tooth; blunt shaft fold present; basal spur absent.

Distribution and habitat: The included species occur in the western Pacific and eastern Indian Oceans (known from Australia, Papua New Guinea, Indonesia, Philippines, Malaysia and Thailand). They have been collected so far in relatively shallow water, intertidally to 40 m depth, among seaweed and algae (live) and among fine coral rubble and shell grit (dead).

Remarks: These morphologically unusual cone snails constitute a group of species characterized by a very small shell (usually less than 8 mm), which initially was placed in the genus *Lilliconus* Raybaudi, 1994 (type species: *Lilliconus biraghii* (Raybaudi, 1992)) (Fig. 2A–E). Apart from the very small size, the shells of species of *Lilliconus* have bicoloured, paucispiral, often sculptured protoconchs and distinctly angulate or carinate shoulders. The radula of individuals of *Lilliconus*, as thus defined, is composed of numerous, relatively large teeth with a complex

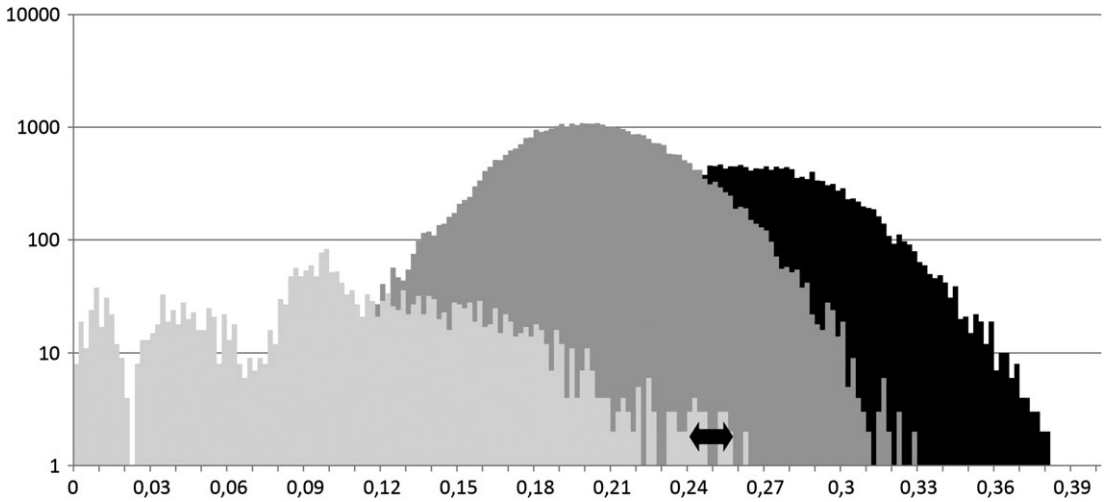

Figure 3. Pairwise distribution of the *cox1* genetic distances within subgenera (light grey), among subgenera but within genera (dark grey) and among genera (black) for species of Conidae. Black arrow, genetic distances calculated between specimens of *Californiconus*, *Lilliconus* and *Pygmaeconus*.

armature of barbs and blades, and absence of serrations in the strict sense and of the basal spur (Fig. 6A, B). These features are remarkably similar to those exhibited by the radular tooth of *Californiconus californicus* from the Eastern Pacific (Figs 2F, 6C), a recognized generalist feeder that is known to prey on worms, molluscs, fish and even shrimps (Stewart & Gilly, 2005; Biggs *et al.*, 2010).

Two species initially placed in the genus *Lilliconus*, namely *Conus boschorum* Moolenbeek & Coomans, 1993 (Fig. 2G) and *C. (Leptoconus) korni* Raybaudi Massilia, 1993 (Fig. 2H), exhibit a remarkably distinct radular morphology. The tooth of *C. boschorum* (Fig. 7) is relatively large and bears three short and flat apical barbs, one of them wrapping around the tooth shaft. There is no waist and the shaft has an unusually large central lumen. The most striking feature of this tooth is the rounded apex with a central, rugose nucleus of unknown function. The tooth of *C. korni* (Fig. 8) is narrow and elongated with a conical instead of rounded apex and also has three apical barbs, one of them articulated on a basal membrane, giving a 'winged' aspect. These most unusual radular morphologies led to the introduction of the genus *Pseudolilliconus* Tucker & Tenorio (2009) (type species: *P. boschorum*). The species in *Pygmaeconus*, *Lilliconus* and *Californiconus* show similarities in the general aspect of their radular teeth, which can be considered synapomorphies shared by the three genera, i.e. the anterior portion much shorter than the posterior portion of the tooth, the presence of the barb and multiple blades on a sheet rolling around the shaft, the presence of a shaft fold and the lack of a basal spur. The main difference of the radular tooth in *Pygmaeconus* species compared with the tooth of species of the other two genera is the size of the single denticle on the under side of the tooth in the middle of the adapical opening. In *Pygmaeconus*, this structure is

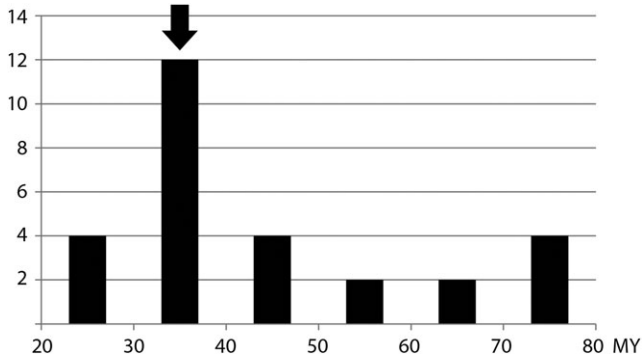

Figure 4. Number of genera found for each time frame in the literature review. Black arrow, *Lilliconus* and *Pygmaeconus*.

reduced to a small denticle (Fig. 5), whereas in *Lilliconus* and *Californiconus* (Fig. 6) is of about the same size as the blade present on the over side of the tooth. Additionally, the third blade near the waist forms a pointed structure that is more developed in *Lilliconus* and even more so in *Californiconus*. There are conchological differences and similarities among the species in these genera. *Lilliconus* and *Pygmaeconus* have in common the very small size of their shells, but the general rounded, rotund shape of most *Pygmaeconus* species (Fig. 2I–Q) resembles that of *C. californicus* (Fig. 2F). On the contrary, the shells of *Lilliconus* species have scalariform spires that are often nodulose, with angulate or sharply angulated shoulders (Fig. 2A–E). Additionally, the tree in Figure 1 indicates that species in these three genera are highly divergent,

Figure 2. A. Holotype of *Leptoconus (Thoraconus) biraghii* Raybaudi, 1992 (SMNS ZI8843), Obja, 600 km N of Mogadishu, Somalia; SL 10.5 mm. **B.** Holotype of *Conus biraghii omanensis* Moolenbeek & Coomans, 1993 (NBC ZMA Moll. 3.92.003), Masirah I., Oman; SL 7.7 mm. **C.** Holotype of *Conus (Lilliconus) kuiperi* Moolenbeek, 2006 (NBC ZMA Moll. 4.05.17), Masirah I., Oman; SL 5.9 mm. **D.** *Lilliconus sagei* (INHS), Tegeta, Tanzania; SL 7.9 mm. **E.** *Lilliconus sagei* (MNHN IM-2009-31328), Lavanono, S Madagascar; SL 6.4 mm. **F.** Holotype of *Conus californicus* Reeve, 1844 (NHMUK), California; SL 23.5 mm. **G.** Holotype of *Conus boschorum* Moolenbeek & Coomans, 1993 (NBC ZMA Moll. 3.92.001), Masirah I., Oman; SL 11.0 mm. **H.** Paratype of *Conus (Pseudolilliconus) korni* (NBC), Aden Gulf, off N Somalia; SL 11.0 mm. **I.** Lectotype of *Conus traillii* Adams, 1855 (NHMUK), Malacca, Malaysia; SL 7.0 mm. **J.** *Pygmaeconus traillii* (MNHN IM-2013-47253), Kavieng, Papua New Guinea; SL 6.3 mm. **K.** *Pygmaeconus traillii* (MJT), Mactan I., Philippines; SL 6.4 mm. **L.** *Pygmaeconus traillii* (MJT), Mactan I., Philippines; SL 6.3 mm. **M.** Holotype of *Conus (Pseudolilliconus) molaerivus* Dekkers, 2016 (NBC RMNH.5004022), Mactan I., Cebu, Philippines; SL 4.6 mm. **N.** Paratype of *Pygmaeconus wallacei* (SMNS), Taka Bulango, SW Sulawesi, Indonesia; SL 7.4 mm. **O.** Holotype of *Conus micarius* Hedley, 1912 (AMS), Cape York, Australia; SL 6.2 mm. **P.** Holotype of *Conus visseri* Delsaerdt, 1990 (NBC ZMA 137077), Ka Lhim Beach, Patong Bay, Phuket I., Thailand; SL 8.8 mm. **Q.** Lectotype of *Conus papalis* Weinkauff, 1875 (NHMUK), Ticao I., Philippines; 9.0 mm. Scale bars = 5 mm.

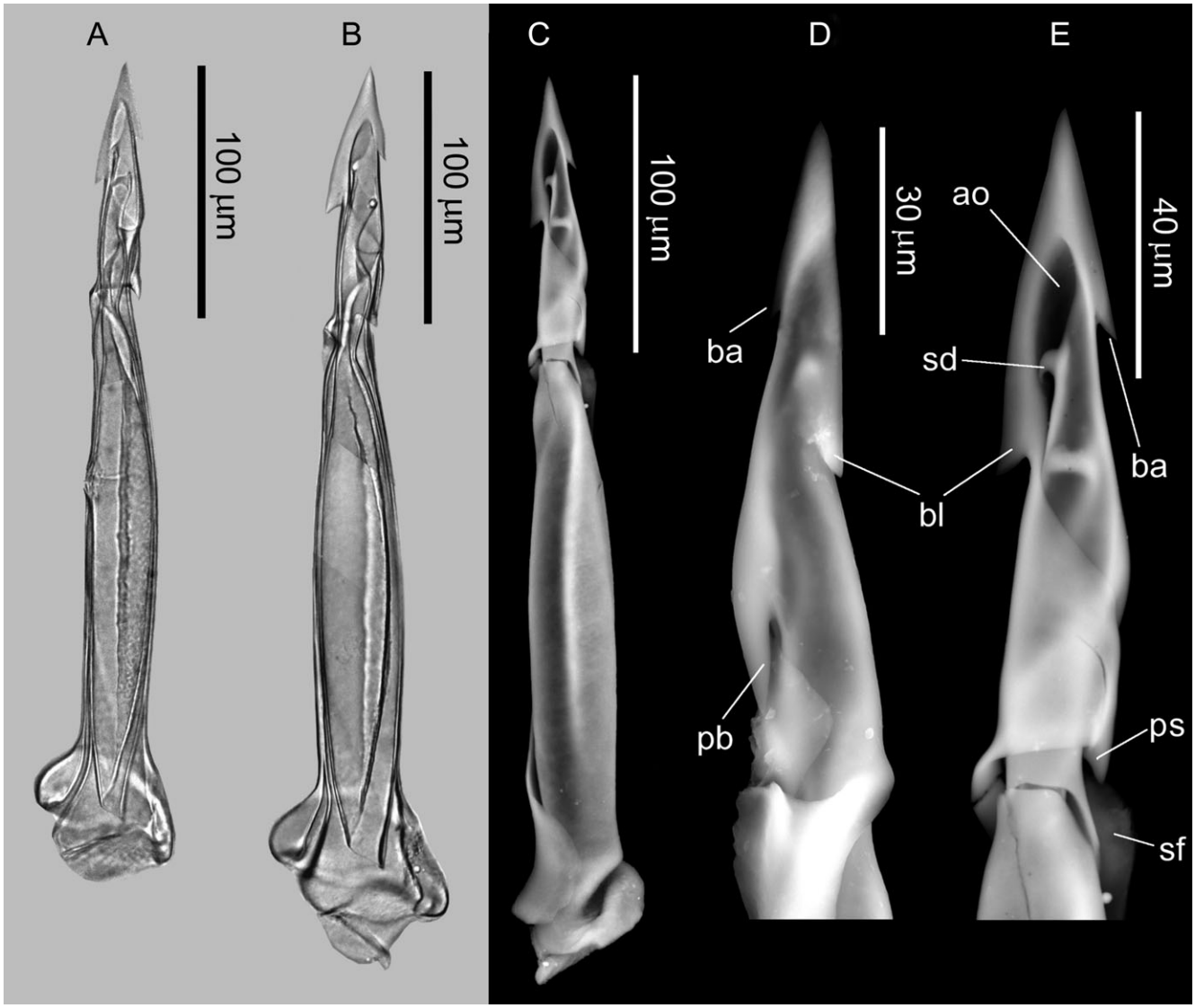

Figure 5. A–E. Radular teeth of *Pygmaeconus traillii* with major parts labelled. **A, B.** Optical microscopy. **A.** Kavieng, Papua New Guinea (MNHN IM-2013-47253); SL 6.3 mm. **B.** Cebu, Philippines (MJT); SL 6.3 mm. **C–E.** SEM; Cebu, Philippines (MJT); SL 6.4 mm. Abbreviations: ao, adapical opening; ba, barb; bl, blade; pb, tusk-shaped posterior blade; ps, pointed structure terminating sheet that rolls around shaft; sd, single small denticle; sf, shaft fold.

with genetic distances similar to those found between the other main lineages. The species in genus *Pseudolilliconus* (Fig. 2G, H) have not been examined molecularly, but they display evident differences in radular morphology (Figs 7, 8). Additionally, the shell of *Pseudolilliconus* species has features very different from those of species of *Pygmaeconus*. These differences are mainly the lower, stepped spire, with a straight to slightly concave profile, canaliculated teleoconch whorls and a sharply angulated shoulder. The presence of a groove on the body whorl just below the shoulder seems to be characteristic of species of *Pseudolilliconus* (Moolenbeek & Coomans, 1993). Table 2 summarizes the most relevant differences in shell and radular morphology among the genera *Pygmaeconus*, *Lilliconus*, *Californiconus* and *Pseudolilliconus*.

Included species:
Pygmaeconus molaerivus (Dekkers, 2016) **new combination**
Pygmaeconus wallacei (Lorenz & Morrison, 2004) **new combination**
Pygmaeconus visseri (Delsaerdt, 1990) **new combination**
Pygmaeconus micarius (Hedley, 1912) **new combination**
Pygmaeconus papalis (Weinkauff, 1875) **new combination**

DISCUSSION

Based on molecular and morphological data (both of the shell and radula), we described here a new taxon of cone snails, *Pygmaeconus*. We currently recognize six species in this new genus. The recently described taxon *Conus (Pseudolilliconus) molaerivus* Dekkers, 2016 (Fig. 2M) is more correctly placed in *Pygmaeconus*, if it is indeed considered a valid species and not a synonym (colour form) of *Pygmaeconus traillii*. The species *P. wallacei* (Fig. 2N) and *P. micarius* (Fig. 2O) were considered by Moolenbeek & Goud (2008) to be synonyms of *P. traillii*. However, given the paucispiral protoconch in all *Pygmaeconus* species, which indicates nonplanktonic larvae with limited dispersal abilities, and the observed differences in shell pattern and structure, we rather consider these two species as valid. The inclusion of *Conus visseri* Delsaerdt, 1990 (Fig. 2P) in the genus is only provisional and requires further confirmation. This species, known from Phuket Island, Thailand, shares with other *Pygmaeconus* species the small size and rounded shape. However, it has a lower spire and, instead of displaying equally spaced raised minute spiral cords on the body whorl, it is sulcated with spiral

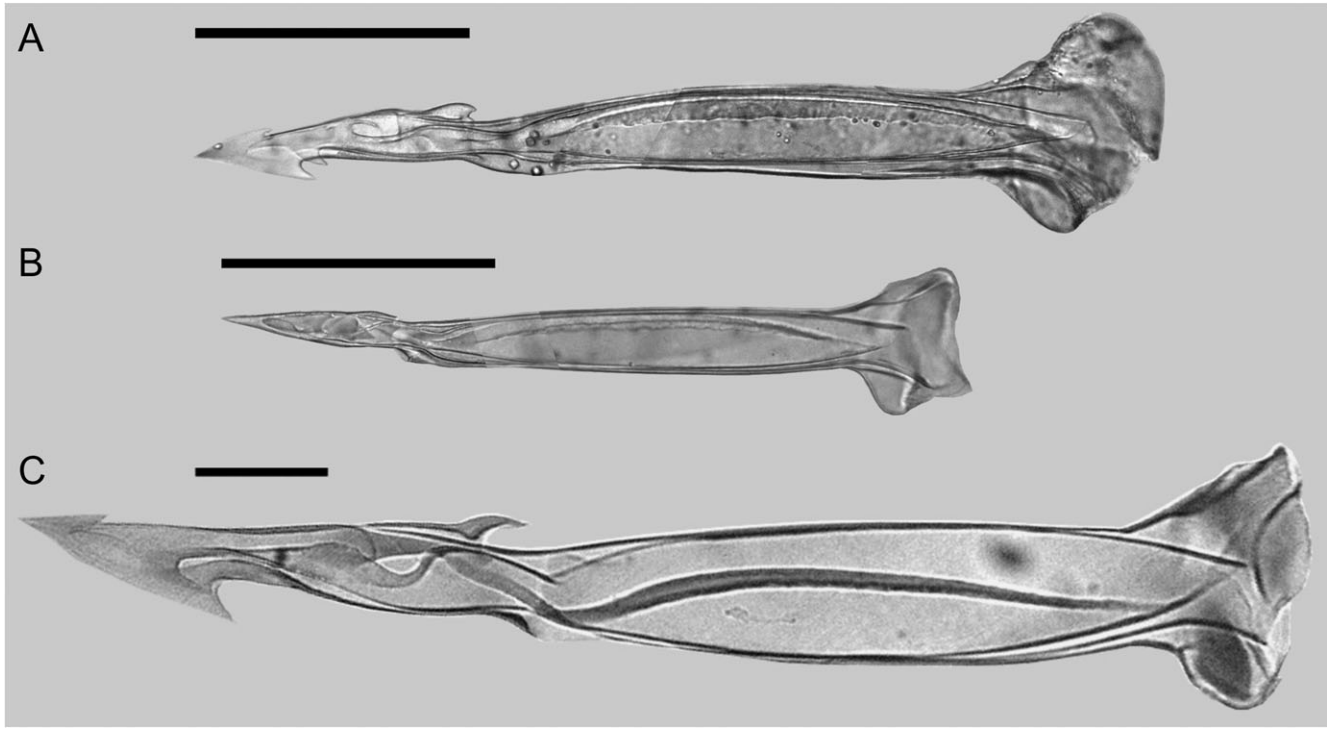

Figure 6. Radular teeth. **A.** *Lilliconus biraghii omanensis*, Masirah I., Oman (MJT); SL 6.3 mm. **B.** *Lilliconus sagei*, Lavanono, S Madagascar (MNHN IM-2009-31328); SL 6.4 mm. **C.** *Californiconus californicus*, CA, USA (ex-coll. J. Nybakken); SL 40.3 mm. Scale bars = 100 μm.

grooves containing fine axial riblets. This feature has been also observed in *Lilliconus kuiperi* (Moolenbeek, 2006) (Fig. 2C).

The very small size of species in the genus *Pygmaeconus* may mean that many species in this group have been overlooked and remain undescribed. Dead specimens of *P. traillii* have often been found in samples of shell grit, but finding living specimens is a difficult task due to their small size. The unusual size and shape combination displayed by species in this genus has also led others to conclude that certain species were not conids. For instance, *Conus micarius* Hedley, 1912 has been considered a member of genus *Mitromorpha*, in the conoidean family Mitromorphidae (WoRMS Editorial Board, 2017), i.e. *Mitromorpha micaria*. However, the holotype of *C. micarius* (Fig. 2O) shows strong conchological similarities to *P. trailli*. This might indicate a close relationship of the two taxa, consistent with the inclusion of *micarius* in *Pygmaeconus*. *Conus papalis* Weinkauff, 1875 (new name for *Conus coronatus* Reeve, 1849) (Fig. 2Q) has been also treated as a member of *Mitromorpha* (WoRMS Editorial Board, 2017), despite its inclusion in *Lilliconus* following the designation of a lectotype for this species (Raybaudi Massilia, 1994; Lorenz, 1997). The unusually high and nodulose spire indeed resembles features observed in species of *Lilliconus*. However, the geographical distribution of this species (Philippines) and other conchological characters such as the presence of equally spaced, minute, raised spiral cords on the last whorl would fit better with its placement in *Pygmaeconus*. Further studies are needed in order to clarify the status of this species, which we provisionally consider a member of *Pygmaeconus* (i.e. *P. papalis*) and not a *Mitromorpha*.

The food habits of the *Pygmaeconus* species remain unknown. However, the similarities of their radular teeth with that of *Californiconus californicus* suggest that species of *Pygmaeconus*, and possibly those of *Lilliconus* as well, might be generalist feeders, but this hypothesis requires confirmation. Direct observation of such minute shells will be difficult, but recent articles have demonstrated the feasibility of sequencing DNA of prey contained in the gut of cone snails (Duda *et al.*, 2009).

The recognition of *Lilliconus* and *Pygmaeconus*, both including relatively well-known species, as deep lineages of cone snails, similar in status to the four previously recognized main lineages of cone snails, would suggest that some species that are currently classified as genera within (sub)families (Tucker & Tenorio, 2009) or subgenera within genera (Puillandre *et al.*, 2015), and that show unusual radula

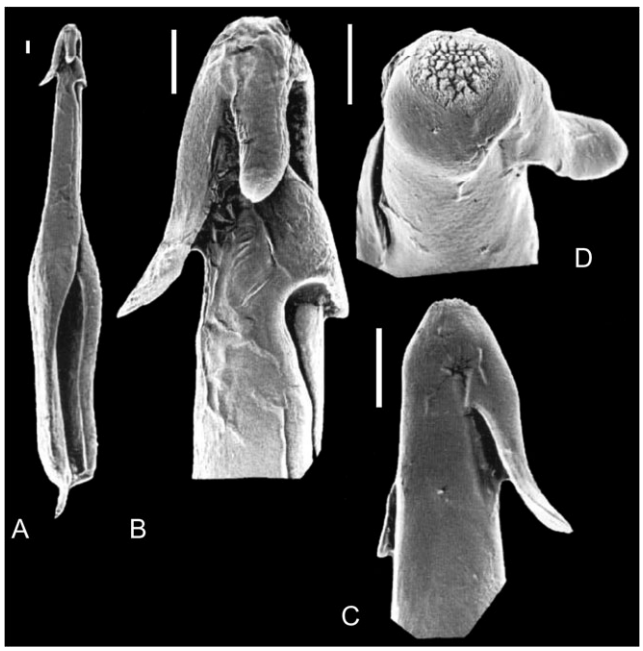

Figure 7. SEM of radular tooth of *Conus (Pseudolilliconus) boschorum* (reproduced from Rolán & Raybaudi Massilia, 1994, with permission). **A.** Entire tooth. **B.** Laterodorsal view of apical part, showing two prominent barbs and third lower barb, close to shaft. **C.** Opposite view, showing two lower barbs. **D.** Apical view showing unusually rounded apex with central rough area. Scale bars = 10 μm.

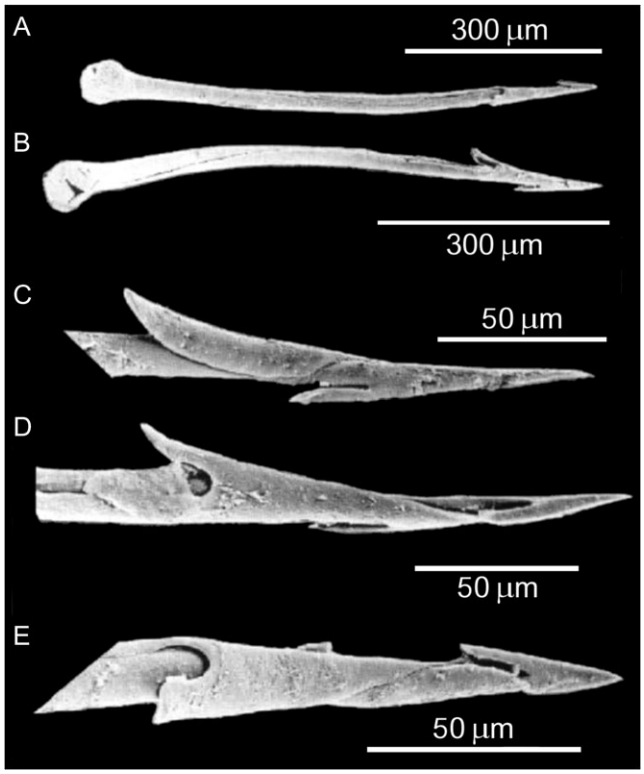

Figure 8. SEM of radular tooth of *Conus (Pseudolilliconus) korni* (reproduced from Rolán & Raybaudi Massilia, 1994, with permission). **A, B.** Complete tooth, two views. **C–E.** Enlarged view of apical portion showing barbs, adapical opening and enrolled lower, flat barb, externally recurved.

and/or shell morphology, potentially represent additional deep lineages that remain to be recognized. One good candidate is *Pseudolilliconus*, considered here as a separate taxon from *Lilliconus* and *Pygmaeconus*. This hypothesis is supported by morphological characters only and whether it actually constitutes a third lineage of minute cone snails needs to be tested with molecular data.

Molecular data are now routinely used to clarify phylogenetic relationships. However, turning a molecular phylogeny into an operational classification remains largely arbitrary—not to identify which groups will be named (the clades) or how they will be named (following the rules of the International Code of Zoological Nomenclature), but to decide at which ranks they will be considered. In their revision of the classification of cone snails, Puillandre *et al.* (2015) appealed to the prevailing usage of the familiar designation 'Conidae' for all the cone snails in order to support their classification as a single family. However, this criterion cannot be applied to the taxon *Lilliconus* (or, of course, to the new taxon *Pygmaeconus*), which has been cited in only a few publications. We therefore analysed the genetic divergence within cone snails, the clade ages of closely related taxa and, finally, the morphological divergence of other minute cone snails and *Californiconus*. Given the results obtained, we decided to place *Lilliconus* and *Pygmaeconus* at the rank of genus, following the ranking chosen for the four other main lineages of cone snails.

Nevertheless, we also acknowledge that taxonomic ranks may need to be re-evaluated in the future. First, the molecular phylogenies currently available for cone snails include at best less than 40% of the known species. Inclusion of the missing species in a phylogenetic tree may drastically change the inferred pattern. Second, the available molecular phylogenies are all based on mitochondrial markers alone and, once again, the inferred pattern may be different using nuclear genes. Third, accelerated

Table 2. Summary of diagnostic traits for the genera *Pygmaeconus*, *Lilliconus*, *Californiconus* and *Pseudolilliconus*.

Genus	Shell features							Radula features			Distribution
	SL (mm)	Protoconch	RSH*	Spire profile	Nodules	Teleoconch whorls	Shoulder	Operculum	Waist	No. of barbs + blades	Single-serration size	
Pygmaeconus	3–9	Paucispiral	0.31–0.32	Straight or convex	Absent or obsolete	Convex, smooth or with one or two grooves	Rounded	Small	Present	5	Very small	W Pacific and E Indian O.
Lilliconus	6–12	Paucispiral	0.12–0.25	Straight, stepped	Present	Flat or slightly concave, with subsutural ridge	Angulated	Small	Present	5	Large	W Indian O.
Californiconus	15–40	Multispiral	0.14–0.19	Convex	Absent	Flat, smooth	Rounded	Large	Present	5	Large	E Pacific O.
Pseudolilliconus	9–13	Paucispiral	0.13–0.28	Concave, stepped	Absent	Concave, canaliculated	Sharply angulated	Small	Absent	3	Absent	NW Indian O.

*RSH, relative spire height (= spire height/SL).

rates of evolution (i.e. mutation rates) may lead to overestimation of the genetic distances and estimated ages, and thus change our conclusions regarding the ranks within cone snails. More complete phylogenies with many more genes, and in particular nuclear genes, would smooth such heterogeneity in diversification rates. Integrating the cone snail diversity within a more general framework, with estimated divergences and ages for the whole Conoidea, would also clarify the situation.

To conclude, the available data for cone snails support our hypothesis of recognizing *Lilliconus* and *Pygmaeconus* as genera, but a more complete phylogeny of the cone snails and Conoidea, based on more genes and particularly nuclear markers, is required. The final decision may, nevertheless, ultimately be based on subjective thresholds of divergence.

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