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Molecular phylogeny of the nutmeg shells (Neogastropoda, Cancellariidae)

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ABSTRACT

Cancellariidae, or nutmeg shells, is a family of marine gastropods that feed on the body fluids and the egg cases of marine animals. The 300 or so living species are distributed worldwide, mostly on soft bottoms, from intertidal to depths of about 1000 m. Although they are a key group for the understanding of neogastropod evolution, they are still poorly known in terms of anatomy, ecology and systematics. This paper reports the first mitochondrial multi-gene phylogenetic hypothesis for the group. Data were collected for 50 morphospecies, representative of 22 genera belonging to the three currently recognized subfamilies. Sequences from three genes (12S, 16S and COI) were analyzed with Maximum Likelihood analysis and Bayesian Inference, both as single gene datasets and in two partitioned concatenated alignment. Largely consistent topologies were obtained and discussed with respect to the traditional subfamilial arrangements. The obtained phylogenetic trees were also used to produce Robinson–Foulds supertrees. Our results confirmed the monophyly of the subfamily Plesiotritoninae, while Admetinae and Cancellariinae, as currently conceived, were retrieved as polyphyletic. Based on our findings we propose changes to the systematic arrangement of these subfamilies. At a lower taxonomic rank, our results highlighted the rampant homoplasy of many characters traditionally used to segregate genera, and thus the need of a critical re-evaluation of the contents of many genera (e.g. *Nipponaphera*, *Merica*, *Sydaphera*, *Bivetia*), the monophyly of which was not recovered.

1. Introduction

The neogastropod family Cancellariidae Forbes and Hanley, 1851 (nutmeg shells) includes ca. 300 living species distributed worldwide, mostly in tropical and temperate region, prevalently soft bottoms dwelling, from subtidal to bathyal depths (Petit and Harasewych, 2005; Hemmen, 2007). The greatest diversity in Recent fauna occurs in the Indo-West Pacific and the Eastern Pacific (Harasewych and Petit, 1982); recent explorations indicate that diversity in tropical deep-sea is still underdocumented (see, e.g., Bouchet and Petit, 2002, 2008).

Conchologically, cancellariids are quite variable, having generally a solid, biconical shell with cancellate sculpture, an elliptical aperture usually with strong columellar plaits, and a short siphonal canal (see examples in Fig. 5), but there are many variations departing from that theme, that account for a number of species of Cancellariidae having originally been described in other families. The animal has no operculum. A peculiar anatomical synapomorphy is the nematoglossan radula, with one extremely elongated and flexible rachidian tooth per row (Fig. 1C). One central and two lateral cusps are responsible for a distal interlocking of the teeth, which is reversible and probably provides an efficient piercing action (Petit and Harasewych, 1986), allowing cancellariids to feed suctorially, as evidenced by several anecdotal observations of specimens feeding on cephalopods egg capsules (Talmadge, 1972), fish blood (O'Sullivan et al., 1987), and fluids from polychaetes, gastropods and bivalves (Loch, 1987; Bouchet and Oliverio, pers. observations). The presence of such a strong anatomical synapomorphy, together with previous molecular results (Modica et al., 2009), supports the monophyly of Cancellariidae, that will thus not be tested in the present paper.

The relationships of the Cancellariidae to other Caenogastropoda have been the subject of debate for a long time. Early workers included them in the Toxoglossa (Troschel, 1865; Tryon, 1882, 1885; Fischer, 1883) while later studies moved them to the Volutoidea (Thiele, 1929; Wenz, 1938–1944; Taylor and Sohl, 1962). The group was then ranked as an order Nematoglossa by Olsson (1970), later reduced to suborder by Golikov and Starobogatov (1975) and to a superfamily within the Neogastropoda by Ponder (1973). The state of the art of neogastropod classification (Bouchet and Rocroi, 2005) follows Ponder, although there is both anatomical and molecular evidence (Kantor, 1996, 2002; Oliverio and Modica, 2010; Cunha et al., 2009) that the Nematoglossa represent

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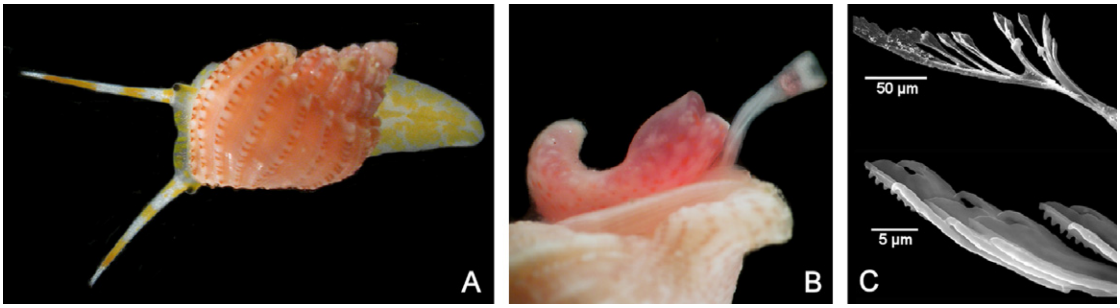

Fig. 1. Morphological characteristics of living cancellariids. (A) A crawling *Scalptia obliquata* (MNHN IM200732096) from Vanuatu, showing the elongated foot (note the absence of an operculum). (B) The head-foot of *Tritonoharpa antiquata*, from Vanuatu, with the long proboscis and, in transparency, the apical reddish buccal mass. (C) The nematoglossan radula of *Tritonoharpa* sp. from Mactan (Philippines; BAU00269: reprinted with permission after Modica et al., 2009), consisting in a tuft of elongated and flexible teeth (above), which are distally interlocked (below). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

one of the lineages that diverged first during the neogastropod radiation. Cancellariidae are well represented in the fossil record, with some 1200 Cretaceous to Neogene nominal fossil species.

Based on shell similarities it had been suggested that the Cancellariidae originated in the Upper Cretaceous from the Purpurinidae, an allegedly 'basal' neogastropod group (Taylor et al., 1980). A different hypothesis was proposed by Tracey et al. (1993) who suggested that the oldest fossils attributable to Cancellariidae were of *Palaeocancellaria hoelleitenensis* Kollmann, 1976 from the Albian (Lower Cretaceous) Losenstein Formation of Austria. The origin of the whole Neogastropoda was predated basing on the description of a muricoidean from the Lower Cretaceous (and probably earlier) by Kaim (2004).

Based on shell characters, cancellariids had been divided in three groups, ranked as subfamilies by Cossmann (1899): Cancellariinae, Admetinae and Trigonostomatinae. Cancellariinae possess shells with cancellate sculpture, three columellar plaits and a closed umbilicus; Trigonostomatinae have high shells with strong shoulder, open umbilicus and 2–3 columellar plaits; Admetinae possess thin shells, closed umbilicus and generally no columellar plaits. A fourth subfamily, the Plesiotritoninae, was erected by Beu and Maxwell (1987), to accomodate species with elongated shells, a long siphonal canal, an opisthocline and not collabral axial sculpture. The most recent gastropod classification (Bouchet and Rocroi, 2005) recognizes three subfamilies: Cancellariinae Forbes and Hanley, 1851 (including Trigonostomatinae Cossmann, 1899), Admetinae Troschel, 1865 (tentatively including the fossil Paladmetinae Stephenson, 1941), and Plesiotritoninae Beu and Maxwell, 1987. Genus-level systematics of Cancellariidae is rather confused, with about 100 fossil and recent nominal taxa with frequently overlap of the shell features used for generic classification (Verhecken, 1997).

Live cancellariids are rather infrequent, and most reference collections have very little – if at all – alcohol-preserved material. As a consequence, sequences from only eight species have been deposited in GenBank (Colgan et al., 2007; Hayashi, 2005; Cunha et al., 2009; Oliverio and Modica, 2010). Beside descriptions of radulae (e.g. Troschel, 1865; Barnard, 1958; Olsson, 1970; Oliver, 1982; Petit and Harasewych, 1986; Schremp and Richmond, 1983), there have been only a handful of papers describing the anatomy of Cancellariidae (Graham, 1966; Harasewych and Petit, 1982, 1984, 1986; Strong, 2003; Modica et al., 2009, 2011; Verhecken et al., submitted for publication). These data show that members of the Cancellariidae display a remarkable anatomical disparity, which does not make sense in the absence of a robust phylogenetic framework. Furthermore, cancellariids are plausibly the sister group to the rest of the neogastropods, probably representing a rather early off-shoot in the neogastropod radiation. Understanding the evolutionary forces having driven the diversification of this family may provide an insight into the early evolutionary history of the whole order Neogastropoda, the most successful living marine gastropod clade. The initial diversification of the Neogastropoda was apparently extremely rapid and contemporary for many internal lineages, hampering the reconstruction of the main evolutionary patterns (Ponder, 1973; Taylor et al., 1980, 1983; but see Jablonski (1979) for a contrasting interpretation). Moreover, the Cancellariidae include at least one hematophagous species that produces anticoagulant and probably anesthetic compounds, which may reveal useful pharmacological properties. A molecular phylogenetic framework, associated with ecological and anatomical data, would be a powerful tool to identify the species-groups to be targeted in search of new bioactive compounds (Olivera, 2006; Holford et al., 2009a,b; Modica and Holford, 2010; Puillandre and Holford, 2010).

2. Material and methods

2.1. Taxon sampling and collection

In the present study we have assayed 98 specimens of 49 putative morphospecies, representing 22 genera belonging to the three currently recognized subfamilies of the Cancellariidae. Most specimens were obtained from coral reefs biodiversity surveys (PANGLAO 2004, SANTO 2006) and deep-sea expeditions (PANGLAO 2005, NORFOLK2, SALOMON BOA3) in the West Pacific, or personally collected (East Pacific, Philippines) (see Table 1 for details, including locality and accession numbers for vouchers). Living specimens were anesthetized in $MgCl_2$ isotonic with seawater; a fragment of muscular tissue from the foot, or in some cases the whole specimen, was then fixed in 95% ethanol.

Specimens were identified based on shell morphology. Nominotypical genera were available for all subfamilies, and the type species were included for 10 of the 22 analyzed genera. The muricid neogastropod *Nucella lapillus* was chosen as outgroup.

All the sequences analyzed are original, with the exception of those from *Progabbia cooperi*, *Bivetiella cancellata, Plesiotriton* sp. and *N. lapillus* (Oliverio and Modica, 2010), *Tritonoharpa* sp. BAU270 (Modica et al., 2009), *Loxotaphrus deshayesii* and *L. rosadoi* (Modica et al., 2011), *Merica sinensis* (Hayashi, 2005) and *Sydaphera undulata* (Colgan et al., 2007).

To assess unambiguously the phylogenetic representativeness of our sample we used a formal approach recently proposed by Plazzi et al. (2010), based on the Average Taxonomic Distinctness (AvTD) (Clarke and Warwick, 1998). This method allows to test *a priori* whether our taxon sampling is representative of a reference taxonomy (see Plazzi et al. (2010) for methodological details). As reference taxonomy we used a Cancellariidae master list kindly