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

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ABSTRACT

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

1. Introduction

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

The gastropod family Conidae, commonly known as cone snails, includes the widely distributed, mainly tropical *Conus*, a relatively young genus first appearing in the Early Eocene. The family Conidae is one of the most diverse in the marine environment (Kohn, 1990), with 761 valid Recent species currently (21th

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January 2014) recognized in the World Register of Marine Species (WoRMS, 2013) and new species descriptions are published each year. It is also the most rapidly diversifying marine molluscan genus (Kohn, 1990; Stanley, 2008, 1979) and is ecologically important especially in coral reef environments where up to 36 species, specialized predators on worms, other molluscs, and fishes, co-occur on a single reef (Kohn, 2001).

These latter attributes all likely relate to the extremely diverse peptide venoms that cone snails use to overcome and capture prey and that also make the Conidae a most promising source for neurobiologic and therapeutic applications (Biass et al., 2009; Lluisma et al., 2012; Olivera, 2006). Molecular geneticists, evolutionary biologists, pharmacologists, and toxicologists thus all require a robust phylogeny and taxonomy for this group. New drug discovery is particularly likely to benefit from a clear phylogenetic context that permits targeting divergent lineages and thus potential novel toxins (Biggs et al., 2010; Olivera, 2006).

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

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

Cone snails experienced several episodes of enhanced diversification since their origin (Duda and Kohn, 2005; Kohn, 1990; Williams and Duda, 2008) and exhibit the highest rate of diversification of any marine gastropod or bivalve group (Stanley, 1979), a remarkable radiation that was likely driven by ecological speciation (Stanley, 2008). Currently they occur mostly throughout tropical regions of our world's oceans, although the overwhelming majority of species, both fossil and recent, are restricted to single marine biogeographic provinces (e.g., Indo-Pacific, East Pacific, West Atlantic, East Atlantic and South Africa) (Duda and Kohn, 2005). Results from previous molecular phylogenetic analysis suggest that three major lineages arose shortly after the origination of the group: one with extant species mostly occurring in the present-day Indo-Pacific, another with most extant species found in the present-day East Pacific and West Atlantic, and a third that today consists of a single species that is restricted to the East Pacific (Duda and Kohn, 2005). Based on the geographic distributions of species in these clades, there has apparently been very little interchange of lineages among the major marine biogeographic provinces (Duda and Kohn, 2005; Duda and Lessios, 2009). Nonetheless, this work included analyses of sequence data from only one-fifth of the recognized cone snail species and the authors caution that their results are preliminary and the patterns that they observed may change with more complete taxonomic coverage (Duda and Kohn, 2005). Here we examine the biogeography of this group with a much more exhaustive taxonomic and geographic coverage than available previously.

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

We propose here a molecular phylogeny of the Conidae sensu Bouchet et al. (2011), based on three mitochondrial genes (COI, 12S, 16S) sequenced for 320 species (>40% of the known species diversity), and including representatives from the main lineages defined in previous DNA studies: *C. californicus*, the Small Major Clade and the Large Major Clade (Duda and Kohn, 2005). Tucker and Tenorio (2009) classified the Small Major Clade as the Family Conilithidae – it included *C. californicus* – and the Large Major Clade as the family Conidae (see Table 1 for a comparison of the recent classifications of cone snails and related species). We then analyse the evolution of three character sets: diet category, biogeographic province and toxin diversity. Previous molecular phylogenetic studies analysed the main evolutionary diet shifts (from worms to fishes or molluscs) (Duda and Kohn, 2005; Duda and Palumbi, 2004; Duda et al., 2001), but never on such a large dataset. Disentangling the evolution of these traits throughout this hyperdiverse taxon should help to generate and critically examine hypotheses of the factors that promoted its exceptional ecological and evolutionary diversification.

2. Material and methods

2.1. Sampling

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

Nine vouchers from GenBank were only identified at the genus level (as "*Conus* sp."). For various reasons, the voucher specimens were not available for all the non-GenBank specimens, but in some cases digital images of shells were available (unpublished data) for confirmation of identifications. In most cases, the morphological identification was double- or triple-checked by several taxonomic specialists of the group. We followed the cone snail taxonomy provided in the World Register of Marine Species (WoRMS, version of 14th May 2013) in applying species names to the vouchers: only species names considered valid in WoRMS were applied. All other species-level names that could have been attributed to the specimens were considered subspecies, form or variety names, or as synonyms. In total, the 2107 specimens were attributed to 320 species names, representing >40% of the total number of cone snail species considered valid in WoRMS (Supplementary data 2). Additionally, we recognize nine morphospecies as potentially corresponding to undescribed species (numbered from a to i). In total, 1740 COI, 928 16S and 599 12S sequences were analysed, of which 1523 are newly published (Supplementary data 1).