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Evolutionary diversification of the genus *Theba* (Gastropoda: Helicidae) in space and time: A land snail conquering islands and continents

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ABSTRACT

Among oceanic islands, the Canary Islands offer exceptional opportunities for studying speciation processes due to their habitat diversity and well documented geological history. Based on a combined COI + ITS1 data set for more than 140 specimens, we studied the diversification of the land snail genus *Theba* on the Canary Islands and adjacent African and European continental areas. Phylogenetic analyses resulted in the recognition of 18 genetically distinct clades including at least three new species. Divergence time estimates suggested an evolution of *Theba* in the Canarian archipelago and an initial radiation on the three eastern-most islands during the Late Oligocene/Early Miocene. Despite the close proximity of NW Africa to the Canary Islands, the main mode of diversification was intra-archipelago speciation rather than independent colonization of the islands from the mainland. Notably, species from Morocco are nested among species from the Canary Islands, indicating re-colonization of the continent from the islands. The re-colonization of NW Africa occurred during the Middle Miocene and led to a remarkable continental radiation.

1. Introduction

Gittenberger and Ripken (1987) described a remarkable radiation of the land snail genus *Theba* on the Canary Islands and adjacent African continental areas. They showed that this radiation can be traced back to the Miocene with an extensive fossil record of *Theba*, as did other authors such as Hutterer (1990), Hutterer and Groh (1997), and Bank et al. (2002). *Theba* is thus an ideal model organism to study speciation processes on islands.

Whereas oceanic archipelagos such as the Galapagos and Hawaiian Islands have played pivotal roles in evolutionary biology (Cowie and Holland, 2008; Parent et al., 2008), the Canary Islands have only lately moved into the focus of evolutionary research (Juan et al., 2000). Compared to other oceanic islands the Canary Islands are characterized by a close proximity to potential continental source areas, considerable habitat diversity, a comparatively broad range of geological ages from 1 to 30 million years, and a well documented geological history (Juan et al., 2000; Fernández-Palacios and Whittaker, 2008). Radiations in many animal and plant lineages have inspired an increasing number of phylogeographic studies on this archipelago (Juan et al., 2000; Francisco-Ortega et al., 2001; Moore et al., 2002; Carine et al., 2004; Arnedo et al., 2008; Dimitrov et al., 2008; Hochkirch and Görzig, 2009; Stüben and Astrin, 2010).

The volcanic archipelago of the Canary Islands consists of eight sub-aerial main islands (including the Selvagens Islands) and was formed in a sequence from east to west along a volcanic hotspot track in the Atlantic Ocean. All current islands were formed in the past 30 million years with the Selvagens Islands (30 mya) being the oldest, followed by Fuerteventura and Lanzarote (24 mya), Gran Canaria (15 mya), Tenerife (12 mya), La Gomera (9 mya), La Palma (2 mya) and El Hierro (1 mya) (Carracedo, 2008). The archipelago is located in the east Atlantic Ocean with Fuerteventura lying approximately 100 km off the West Saharan coast of NW Africa. It was never connected to continental landmasses (Meco et al., 2007; Carracedo, 2008). The climate of the Canary Islands is predominantly regulated by the humid north-eastern trade winds with the more mountainous central and western islands enjoying subtropical, rather moist climate. The two eastern-most, flatter islands Lanzarote and Fuerteventura have a more arid climate, because they are less affected by the trade winds and are more under the continental influence of the Saharan desert. Up to five vegetation zones can be distinguished on the central and western islands while Lanzarote and Fuerteventura are dominated by arid scrub vegetation (Juan et al., 2000). Active volcanism and erosion

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are still important in shaping geomorphology as well as habitats of the islands (Juan et al., 2000; Carracedo and Day, 2002).

Non-marine snails represent excellent model organisms for biogeographic and evolutionary studies, because they preserve ancestral distribution patterns especially well due to their low vagility. *Theba* comprises 12 extant species (Gittenberger and Ripken, 1987; Hutterer, 1990; Hutterer and Groh, 1997; Bank et al., 2002) distributed mainly on the Canary Islands, in NW Africa, and on the Iberian Peninsula. Before the revision of Gittenberger and Ripken (1987), all populations of *Theba* in the archipelago were regarded as a single species, *T. pisana* (Hutterer and Groh, 1997). With five currently described species endemic to the three eastern-most islands (Gittenberger and Ripken, 1987; Hutterer and Groh, 1997; Bank et al., 2002), five extinct species with a Holo- to Miocene fossil record (Hutterer, 1990), and a further species confined to the Selvagens Islands, the Canarian archipelago represents a diversity hotspot for this genus. Other species are restricted to Morocco and the Iberian Peninsula; only *T. pisana* has a wider, most likely anthropogenic distribution. *T. pisana* is found throughout the Mediterranean region and northwards along the Atlantic coast to the British Isles (Fig. 1). It has also been introduced in parts of the United States, South Africa and Australia (Gittenberger and Ripken, 1987).

All but one species occur in semi-arid to arid environments such as sand dunes and rocky areas with sparse vegetation. Only a single, undescribed species occurs in a moist habitat on an ocean-facing, wind-exposed, steep slope on Fuerteventura (Hutterer, unpubl. data). The fossil record of *Theba* is excellent, e.g. four of the recent Canary Islands endemics can be traced back into the Holo-, Pleisto-, or Miocene (Hutterer, 1990; Hutterer and Groh, 1997).

Species of *Theba* are largely diagnosed by characters of shell morphology including shell shape, keel presence, umbilicus width, surface microstructure (Fig. 2), and by genital system anatomy (Gittenberger and Ripken, 1987). Several species are highly variable (*T. subdentata* and *T. pisana*), however, casting doubt on their taxonomic status. It remained unclear whether the island species of *Theba* can be traced back to one colonization event, whether speciation events on the Canary Islands can be correlated with the geological history of the islands, or whether species occurring in continental Africa are the immediate ancestors. We used mitochondrial and nuclear markers to resolve phylogenetic relationships within the genus *Theba*, to clarify their taxonomic status, and to shed light on their colonization patterns and evolutionary history. The rich fossil record (Gittenberger and Ripken, 1985; Hutterer, 1990; Hutterer et al., 2010) in combination with the excellent geological record and extensive data on extant species offers the possibility to estimate ages and rates of diversification. Additionally, historical geographic ranges were assessed with a recently developed maximum likelihood (ML) approach (Ree et al., 2005; Ree and Smith, 2008). The application of the mentioned tools allowed us to develop a clear picture of the biogeographic processes responsible for shaping the current species diversification and distributional areas.

2. Materials and methods

2.1. Sampling

One hundred and forty-eight specimens, representing all described *Theba* were collected from 76 localities (Table 1 and Table A, electronic supplement; Fig. 1). Each snail was separately stored in absolute ethanol for DNA extraction and was assigned a distinct sample code, indicating individual sample plus geographic locality (Table 1 and Table A, electronic supplement). In search of an appropriate outgroup, we sequenced a panel of helicid genera. Based on preliminary analysis (not shown), we finally selected *Hemicycla paeteliana* and *Cornu aspersum* as outgroup species. Both outgroup species belong to the subfamily Helicinae, which is the sister group to the subfamily Ariantinae (Schileyko, 2006). Voucher specimens are deposited at the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), the Senckenberg-Museum, Frankfurt (SMF), in the collection Alonso-Ibanez at the Department of Zoology, University of La Laguna, Tenerife (AIT), and in the collections of K. Groh, Hackenheim (CGH) and R. Hutterer, Bonn (CHB). Other comparative material was studied in Naturalis, the National Museum of Natural History, Leiden (RMNH), the Museum National d'Histoire Naturelle, Paris (MNHN), and the Zoological Museum of the University of Zurich (ZMZ).

2.2. DNA isolation, PCR and sequencing

Total genomic DNA was extracted from the foot muscle of each snail following the manufacturer's protocol of the QIAGEN DNeasy® Blood & Tissue Kit. Polymerase chain reaction (PCR) was used to amplify a fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) with the primer combination LCO-1490 [5′-GGTCAACAAATCATAAAGATATTGG-3′ (Folmer et al., 1994)] and C1-N-2191 [5′-CCCGGTAAAATTAAAATATAAACTTC-3′ (Simon et al., 1994)]. To avoid possible contamination of the COI data set with nuclear mitochondrial DNA (numt), we followed the suggested control protocol for numts of Song et al. (2008). The nuclear ribosomal internal transcribed spacer 1 (ITS1) was PCR-amplified using the forward primer, 1sITS1f [5′-GCTCGAACTCGATCGCTTGG-3′] and the reverse primer, 1s5.8r [5′-GCGTTCAAGATGTCGATGTTCAATG-3′] from Kameda et al. (2007). PCR reactions were carried out in a total volume of 10 µl using the QIAGEN Multiplex PCR Kit. Thermal cycling conditions for COI were as follows: 95 °C for 15 min, 15 cycles of touchdown PCR (94 °C for 0:35 min, 55–40 °C annealing for 1:30 min and 72 °C extension for 1:30 min) followed by 25 cycles (94 °C for 0:35 min, 40 °C annealing for 1:30 min and 72 °C extension for 1:30 min) and a final extension step at 72 °C for 10 min. Reaction conditions for ITS1 amplification were the same as for COI, except for the annealing temperature which was set to 65–50 °C in the 15 cycles of touchdown PCR and held at 50 °C in the following 25 cycles. PCR products were purified using ExoSAP-IT® (USB).

Purified PCR products were then used as templates for direct sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), with PCR primers (see above) serving also as sequencing primers. Sequencing was carried out on an ABI 3130xl Genetic Analyser automated DNA sequencer (Applied Biosystems). Gene products were sequenced in both directions and the two strands were assembled in SeqMan II (DNASTAR Lasergene software). Sequences were deposited in GenBank (see Table 1 and Table A, electronic supplement).

2.3. Phylogenetic analyses

We analyzed a combined COI + ITS1 dataset as ITS1 sequences were quite conservative and resolved only deep splits within the genus *Theba*, whereas COI sequences were much more variable and yielded resolution of terminal nodes (see Figs. A–B, electronic supplement). Sequences were aligned with MUSCLE v3.7 (Edgar, 2004) using default parameter settings. Unambiguously misaligned positions were adjusted manually in Bioedit v7.0 (Hall, 1999).

Homogeneity of base frequencies among sequences was checked with the $\chi 2$ – test implemented in PAUP* v4.0b10 (Swofford, 2002). With a χ^2 – value of 1395.45 (df = 534) and $p < 0.001$, the base composition analysis over 148 ingroup sequences showed that the 3rd COI codon position was compositionally extremely