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Phylogenetic relationships elucidate colonization patterns in the intertidal grazers *Osilinus* Philippi, 1847 and *Phorcus* Risso, 1826 (Gastropoda: Trochidae) in the northeastern Atlantic Ocean and Mediterranean Sea ☆

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ABSTRACT

Snails in the closely related trochid genera *Phorcus* Risso, 1826 and *Osilinus* Philippi, 1847 are ecologically important algal grazers in the intertidal zone of the northeastern Atlantic Ocean and Mediterranean Sea. Here we present the first complete molecular phylogeny for these genera, based on the nuclear 28S rRNA gene and the mitochondrial 16S rRNA and COI genes, and show that the current classification is erroneous. We recognize nine species in a single genus, *Phorcus*: estimated by BEAST analysis, this arose 30 (±10) Ma; it consists of two subgenera, *Phorcus* and *Osilinus*, which we estimate diverged 14 (±4.5) Ma. *Osilinus kotschyi*, from the Arabian and Red Seas, is not closely related and is tentatively referred to *Priotrochus* Fischer, 1879. Our phylogeny allows us to address biogeographical questions concerning the origins of the Mediterranean and Macaronesian species of this group. The former appear to have evolved from Atlantic ancestors that invaded the Mediterranean on several occasions after the Zanclean Flood, which ended the Messinian Salinity Crisis 5.3 Ma; whereas the latter arose from several colonizations of mainland Atlantic ancestors within the last 3 (±1.5) Ma.

1. Introduction

The Mediterranean and adjacent seas, with their well documented geological history, are ideal for addressing a number of biogeographic questions. This history is complex (Krijgsman et al., 1999; Harzhauser et al., 2007); the Atlantic and Pacific Oceans were originally connected by the wide Tethys Sea, but with the rise of the Arabian plate in the late Burdigalian (around 17 Ma) this connection closed (Harzhauser et al., 2007), separating the Indian Ocean from the Mediterranean Sea. Since its formation, the Mediterranean Sea, which comprises several deep basins, has been variously connected to both the Atlantic and Indian Oceans (Por, 1978). Around 6 Ma all connectivity between the Mediterranean and any other ocean was lost resulting in the biologically critical Messinian Salinity Crisis (MSC), ~6.0–5.3 Ma, during which the vast majority of the Mediterranean dried out (Krijgsman et al., 1999). The MSC ended at the start of the Pliocene (5.3 Ma) with the Zanclean Flood and the reconnection of the Mediterranean to the Atlantic Ocean via the Strait of Gibraltar (Harzhauser et al., 2007).

The Macaronesian Islands are volcanic with fairly recent origins and have never been connected to the African mainland. Both the Canaries and the Cape Verde Islands were formed in an east to west progression: the Canaries forming from 20 to 1 Ma (Juan et al., 2000) and the Cape Verde Islands forming from 25 to 5 Ma (Cunha et al., 2005), while Madeira has been dated at 14–11 Ma (Geldmacher et al., 2000). The Azores are both more remote and younger still with Santa Maria being formed no more than 10 Ma and most of the remaining islands being between 3 and less than 1 million years old (Azevedo et al., 1991). Ocean currents in this area are complex, re-inforcing isolation from the mainland; the main current is the southerly flowing Canaries Current, which flows past the Azores, then Madeira, towards the Canaries

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(Domingues et al., 2007). However eddies and upwellings also occur sporadically in the opposite direction, including an upwelling between the African mainland and the Canaries (Barton et al., 1998) and eddies which cause sporadic water transport north through the Macaronesian Islands (Santos et al., 1995; Molina et al., 1996).

The geological history of the Mediterranean and adjacent seas leads to several questions about the evolution of marine taxa in this region: (i) are there sister relationships, which pre-date the closure of the Tethyan Seaway, between Indian and Atlantic Ocean taxa? (ii) has there been recolonization of the Mediterranean from the Atlantic Ocean following the MSC? and (iii) what is the colonization history of the shallow-water fauna of the Macaronesian islands?

As currently recognized, the intertidal topshell genus *Osilinus* Philippi, 1847 is distributed from the northwestern Indian Ocean, across the Mediterranean Sea, to the coasts of North Africa, mainland western Europe, Britain and Ireland and several Atlantic Islands, including Madeira, Selvagens, the Canaries and the Cape Verde Islands but excluding the Azores (Hawkins et al., 2000). The related genus *Phorcus* Risso, 1826 is believed to be restricted to the Mediterranean.

The classification of *Osilinus* has been historically confused, with species often included within the genus *Monodonta* (Gofas and Jabaud, 1997), in the Gibbulini tribe of the subfamily Trochinae (Hickman and Mclean, 1990). Nevertheless, *Osilinus* is morphologically (Hickman and Mclean, 1990) and genetically (Donald et al., 2005) distinct from *Monodonta* and, based on molecular evidence, *Osilinus* has recently been moved into the subfamily Cantharidinae, separate from the Monodontinae and Trochinae (Williams et al., 2010). Species classification within *Osilinus* has also been unstable (see Section 4). In this study, therefore, we include all seven most commonly recognized *Osilinus* species: *Osilinus articulatus*, *Osilinus atratus*, *Osilinus sauciatus*, *Osilinus kotschyi*, *Osilinus lineatus*, *Osilinus punctulatus* and *Osilinus turbinatus*, as well as both species of *Phorcus*, *Phorcus richardi* and *Phorcus mutabilis*.

We aim to use molecular data to reconstruct the phylogenetic relationships of all recognized *Osilinus* and *Phorcus* species, collected from a wide range of locations throughout the Mediterranean, Persian Gulf and Atlantic. Performing a BEAST analysis (Drummond and Rambaut, 2007) permits us to estimate the dates of cladogenic events, and to address three specific biogeographic questions deriving from the more general ones above. First, we test the monophyly of *Osilinus*, including the Arabian *O. kotschyi*, hypothesized by Herbert (1994) to be a relic of a former Tethyan distribution that became isolated when the connection between Indian Ocean and Mediterranean Sea was severed 16–14 Ma. Second, we test the hypothesis that the Mediterranean species were derived by post-MSC invasions from the Atlantic and estimate the number and age of such invasions. Third, we test the hypothesis that the Atlantic Island *Osilinus* species were derived by sequential dispersal events from the mainland.

2. Materials and methods

2.1. Sample collection

Trochid snails, representing all nine species currently recognized in *Phorcus* and *Osilinus*, were collected from intertidal sites

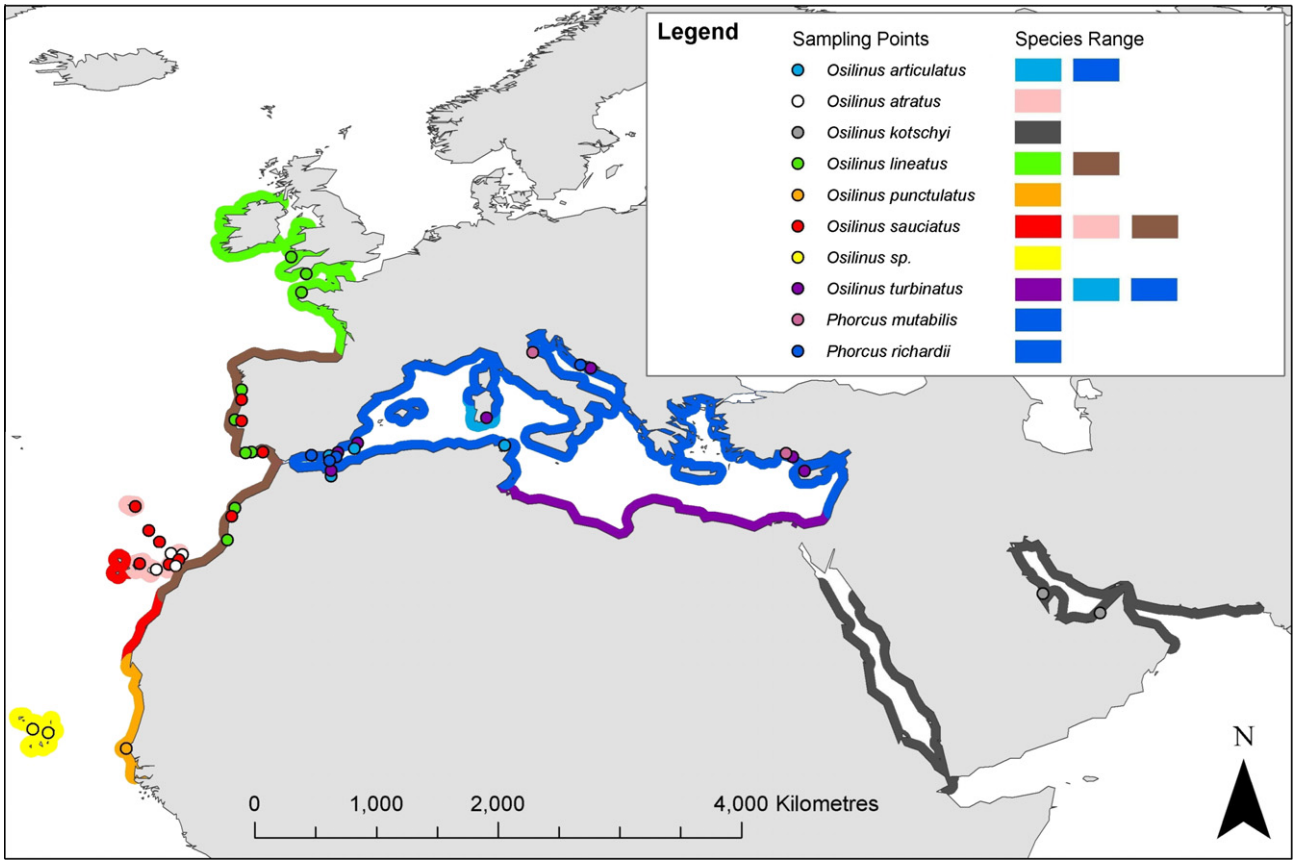

Fig. 1. Approximate sampling locations and distributions of *Osilinus* and *Phorcus* species. Solid circles refer to sampling sites and solid lines denote species range (colors of species sampling sites and ranges are noted in the legend).