

THE BROWN RAT *RATTUS NORVEGICUS* BERKENHOUT, 1769 (MAMMALIA, RODENTIA), A NEW ENTRY FOR THE MAMMAL FAUNA OF SYMI ISLAND (DODECANESE, GREECE)

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Abstract

The presence of the brown rat *Rattus norvegicus* Berkenhout, 1769 in the Aegean Island of Symi is reported here for the first time. Molecular analysis of a specimen collected in the island confirms this finding.

Introduction

The brown rat *Rattus norvegicus* is one of the most common and widely distributed mammals around the globe, since it is found in almost all areas inhabited by humans, on all continents except for Antarctica. Native in northern China and Mongolia, this rat did not appear in Europe until the 1500 s. (Puckett et al. 2016). Originally when the brown rat lived only in Asia, used to occupy forests and brushy areas. Nowadays, it is able to live in a wide variety of environments such as open fields, woodlands, basements, garbage dumps and sewers. In the Mediterranean islands, *Rattus norvegicus* is not so widespread as its relative *Rattus rattus*, because it is presumably unable to successfully colonize the dry environments, due to its dependence on water (Masseti 2012). In the Ionian and Aegean islands, as far as is presently known, brown rat has been found on Corfu, Cephalonia, Zakynthos, Kythera, Euboea, Atalandi (north Euboean Gulf), Crete, Karpathos, Rhodes, Kos, Ikaria, Chios, Skiathos, Lemnos, and Gökçeada (Masseti 2012).

Symi was firstly faunistically explored in 1926 during the period of Italian rule (Ghigi et al. 1929) but no data concerning the mammals (only invertebrates and herpetofauna) were collected (De Beaux 1929). In the literature concerning the herpetofauna of Symi, it has been referred that a large rat was regurgitated by a specimen of *Hemorrhois nummifer*, when captured (Clark 1972) while *Rattus* sp. and *Apodemus* cf. *mystacinus*, are reported among the prey of *Montivipera xanthina* (Cattaneo 2007). Data on the mammalian fauna of the island of Symi are not included in the monographic work of Masseti (2012) about the mammals of the Ionian and Aegean islands. Only recently new evidence

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regarding the mammalian fauna of the island has been published, highlighting the presence for Symi of taxa as *Suncus etruscus*, *Apodemus mystacinus*, *Rattus rattus*, *Rattus* sp., *Mus* sp. and *Talpa* sp. (Nappi et al. 2019).

Materials and Methods

Symi belongs to the Dodecanese Archipelago. It is located 22 km N of Rhodes and approximately 5 km W of the Turkish coast. Its nearest land neighbors are the Datça and Bozburun peninsulas of Muğla Province in Turkey. Its geographical coordinates are: 36°35'39.36" N, 27°50'11" E. It covers a surface area of 58.1 km². The coastline perimeter (85 km) is articulated with numerous bays, capes and steep cliffs. Symi is mountainous and the highest relief is Mt. Vigla (620 m a.s.l.). Symi is made up mainly of limestone rock, with some areas characterized by flysch (Desio 1923, 1924a, 1924b, 1928). Due to its rocky nature, cultivated areas are very few, except for Pedi and Niborios valleys. Symi lacks of a superficial hydrography. There are only wells and cisterns. From a climatic point of view, the climate of the island is a semi-arid Mediterranean, with short, mild and wet winters, followed by long, hot and dry summers. The island is extremely arid, being largely composed of rocky terrain (Ghigi 1929). Symi is mainly characterized by phrygic communities that are more developed in the northern part of the island while in the central and southern part the island is covered by a pre-existing conifer woodland of *Cupressus sempervirens* with stands of *Pinus brutia*. Niborios valley is rather narrow with a dry river bed used for the cultivation of fruit, vegetables and olives. The slopes of the valley are steep and rocky and are characterized by phrygic vegetation constituted mainly of *Origanum onites*, *Thymbra capitata*, *Salvia fruticosa* and *Sarcopoterium spinosum*. The island was visited by two of the authors (M.G., C.C.) in July-August 2017, April 2018 and April 2019. During these field expeditions some live animals were observed along a drain in Pedi locality which in terms of size, morphological aspect and habitat could be ascribed to brown rat, but it was not possible to collect samples. A specimen was found dead in April 2018, hit by a car near Panormitis. Tissue sample from this specimen were collected, stored in 95% ethanol and was used for molecular analysis.

Genomic DNA was obtained using the Quick-DNA Microprep Kit by Zymo Research. Mitochondrial DNA cytochrome b sequence (CYTB) (~1100bp) was amplified by PCR (in a Labnet Thermocycler) using the primers L14723 (5'-ACCAATGACATGAAAATCATCGTT-3') and H15915 (5'-TCACCATTTCTGGTTTACAAGAC-3') (Irwin et al. 1991). The reaction was performed in a 50µl volume containing 3µl (100ng) DNA, 2µl each primer (10 µM), 1.5µl dTNP mix (10mM), 5µl reaction buffer, 3.5µl Mg (50mM) and 1 unit of TAQ Polymerase (Bioline). The amplification was performed at 94°C for 1 min followed by 35 cycles at 94°C for 1min, 52°C for 1 min, 72°C for 1 min and a final extension step at 72°C for 10 min. The amplified product was purified using the Sure Clean (Bioline) and sequenced on both strands by external service (Bio-Fab Research, Italy). Electropherograms were manually checked using Chromas 1.45 (Technelysium Pty Ltd, Tewantia, Queensland, Australia) and derived sequences were aligned with the ClustalW algorithm in MEGA 6 program (Tamura et al. 2013). Molecular identification of the specimen was performed with the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) using, as query, the obtained haplotype and searching for highly similar sequences (Mega BLAST) on the entire nucleotide collection database.

Results and Discussion

The retrieved haplotype was 600bp length and was submitted to GenBank (accession number MN379690). The BLAST algorithm retrieved, unequivocally, the CYTB sequences of *R. norvegicus* as the most similar sequences (99.8% identity). More specifically, the obtained haplotype showed a single substitution in respect to three haplotypes belonging to brown rats from China (accession numbers KT808634, KY356124 and GU592991). Previous phylogeographic analysis identified at least three independent colonization event in Europe although the routes remained an open question (Puckett et al. 2016). More detailed analysis, aimed to identify the possible source area for the Symi specimen, is not feasible since no sequence data are available from Anatolia, the closest mainland area. The presence of brown rat in Symi and in some other small islets close to Crete (Theodorou and Dhia), characterized by a dry and barren environment, apparently quite unsuited to the species, demonstrates the great adaptive capacities of this species that normally depends on water to settle stable populations. Therefore its presence on other islands of Aegean archipelago, especially in harbor areas, is likely.

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