

RADIX RUFESCENS (J. E. GRAY, 1822)  
(GASTROPODA: LYMNAEIDAE), A NEW SPECIES  
FOR OMAN AND ARABIAN PENINSULA

ANDRZEJ FALNIOWSKI<sup>1</sup>, ALEKSANDRA JASZCZYŃSKA<sup>1,2</sup> and SEBASTIAN HOFMAN<sup>3</sup>

<sup>1</sup>Department of Malacology, Institute of Zoology and Biomedical Research,  
Jagiellonian University, ul. Gronostajowa 9, 30–387 Kraków, Poland

E-mail: andrzej.falniowski@uj.edu.pl; <https://orcid.org/0000-0002-3899-6857>

<sup>2</sup>Department of Invertebrate Evolution, Institute of Zoology and Biomedical Research,  
Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland

E-mail: a.jaszczynska@uj.edu.pl; <https://orcid.org/0000-0002-9395-9696>

<sup>3</sup>Department of Comparative Anatomy, Institute of Zoology and Biomedical Research,  
Jagiellonian University, ul. Gronostajowa 9, 30–387 Kraków, Poland;

E-mail: s.hofman@uj.edu.pl; <https://orcid.org/0000-0001-6044-3055>

The fauna of freshwater gastropods of the Arabian Peninsula, a desert land, is neither rich nor extensively studied. Only five species of the family Lymnaeidae have been recorded from this subcontinent so far. In February 2023, a few specimens of *Radix rufescens* (J. E. Gray, 1822) have been collected from the stream at Wadi Tiwi, NE Oman. The shell, female part of the reproductive organs, prostate, paraeputium and phalloteca are presented. Cytochrome oxidase subunit I (COI) sequences confirmed the assignment of these specimens to *R. rufescens*, known from Nepal, Bangladesh, and Myanmar, not from the Arabian Peninsula. Either *R. rufescens* is the sixth lymnaeid species inhabiting Arabia, or at least some of the records of *R. natalensis* (Krauss, 1848), whose shells are hardly discernible from the ones of *R. rufescens*, should be interpreted as misidentified *R. rufescens*. Thousands of years of the trade relations between Oman and India suggest accidental transportation of this snail by trade vessels.

Keywords: shell, reproductive organs, cytochrome oxidase subunit I (COI), mtDNA, molecular relationships

## INTRODUCTION

Lymnaeidae Rafinesque, 1815, a globally distributed family of freshwater heterobranch snails, for more than century have been widely known as showing incredible variability of their shells. This was the reason why the list of synonyms in this group is extremely long. The classic research of BAKER (1911) on the North American Lymnaeidae, and ROSZKOWSKI (1914) on the lymnaeids from the profundal of Lac Léman introduced the lymnaeid taxonomy based on the anatomical characters. Such studies were summarized and completed by HUBENDICK (1951), and continued (e.g., FALNIOWSKI 1980*a, b, c*, 1981). However, many ambiguities still remained in the lymnaeid taxonomy, most of them solved later with application of the molecular data (e.g., CORREAL *et al.* 2010, VINARSKI *et al.* 2020, SAITO *et al.* 2021).

The Arabian Peninsula inhabits a unique assemblage of animal species, the result of the geologic and climatic history of this region. Its fauna may originate from Eastern Africa, south-central Asia, or south-western Asia. The Arabian Peninsula, a desert land in general, inhabits a moderately rich freshwater fauna (GARCIA *et al.* 2015). From among the Lymnaeidae, only five species have been recorded so far: *Galba truncatula* (O. F. Müller, 1774), *Galba robusta* Vinarski, 2018, *Radix natalensis* (Krauss, 1848), *Radix auricularia* (Linnaeus, 1758), and *Stagnicola palustris* (O. F. Müller, 1774) (SMITH 1894, BROWN & GALLAGHER 1985, NEUBERT 1998, VINARSKI 2018). *Lymnaea arabica* E. A. Smith, 1894 from Saudi Arabia, studied by BIN DAJEM (2012), was recently considered a synonym of *Radix natalensis* (Krauss, 1848). In February 2023, a few specimens of *Radix* Montfort, 1810 and *Melanoides* Olivier, 1804 were collected in Wadi Tiwi in Oman.

## MATERIAL AND METHODS

The snails were collected 4th of February 2023 at the stream, from the stones and among the algae, at the lower part of Wadi Tiwi canyon in NE Oman, 22°48'15"N, 59°14'45"E, 20 m a.s.l. (Fig. 1). Wadi Tiwi is situated in Sharkiyah, Eastern Oman Moun-



**Fig. 1.** Wadi Tiwi, stream in its lower part

tains, rising to 2300 m altitude. Wadi (Arabic) means dry river valley. Wadi Tiwi is about eight km long. Its floor has eroded down through Eocene and Palaeocene to the top of the ophiolite which is composed of highly serpentinised mantle sequence harzburgite. The entire crustal sequence of the ophiolite has been eroded off, prior to deposition of the overlying Palaeocene limestones (SEARLE 2019). The lower part of the Wadi, where the snails have been found, has some small blue pools, lined with palms and reed-beds.

The snails were collected by hand and with a small sieve and fixed in 80% analytically pure ethanol, changed twice. The shells were photographed with Canon EOS 50D camera with Canon Macro Lens. The snails were dissected under a Nikon SMZ18 microscope with a dark field, the anatomical structures photographed with Canon EOS 50D camera under a Nikon SMZ18 microscope with a dark field. Measurements of the praeputium and penis sheath were done on the photograph with curvimeter. DNA was extracted from whole specimens; tissues were hydrated in TE buffer (3 × 10 min); then, total genomic DNA was extracted with the SHERLOCK extraction kit (A&A Biotechnology), and the final product was dissolved in 20 µl of tris-EDTA (TE) buffer. The extracted DNA was stored at -80 °C at the Department of Malacology, Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków (Poland). Mitochondrial cytochrome oxidase subunit I (COI) was sequenced. Details of PCR conditions, primers used, and sequencing were given by SZAROWSKA *et al.* (2016).

In the phylogenetic analysis, sequences from GenBank were used (Fig. 4). Sequences were initially aligned in the MUSCLE (EDGAR 2004) Program in MEGA 7 (KUMAR *et al.* 2016) and then checked in Bioedit 7.1.3.0 (HALL 1999). Uncorrected p-distances were calculated in MEGA 7. The estimation of the proportion of invariant sites and the saturation test for entire data sets (XIA 2000, XIA *et al.* 2003) were performed using DAMBE (XIA 2013). The data were analysed using approaches based on Bayesian inference (BI) and Maximum Likelihood (ml). For RAxML analysis, the jModelTest2 via the CIPRES Science Gateway (MILLER *et al.* 2010) was used to find the best-fitting model, the model HKY+G was used. The ML analysis was conducted in RAxML-NG v. 0.8.0 (KOZLOV *et al.* 2019) via web service available at <https://raxml-ng.vital-it.ch/>, with 10 random and 10 parsimony starting trees. In the BI analysis, the K81+G model of nucleotide substitution was applied in tree reconstruction. Model was selected using MrModelTest 2.4 (NYLANDER 2004). The analyses were run using MRBAYES v. 3.2.7a (RONQUIST *et al.* 2012) with defaults of most priors. Two simultaneous analyses were performed, each with 10,000,000 generations, with one cold chain and three heated chains, starting from random trees and sampling the trees every 1,000 generations. The first 25% of the trees were discarded as burn-in. The analyses were summarized as a 50% majority-rule consensus tree. Convergence was checked in TRACER v.1.7.1 (RAMBAUT *et al.* 2018), in all cases Effective Sample Size exceeded 200. FigTree v. 1.4.4 (RAMBAUT 2010) was used to visualize the trees.

## RESULTS

The shell (Fig. 2) with acute spire, high as for this genus, the aperture broad and variable in outline; the shell resembles the ones presented for *R. rufescens* in VINARSKI *et al.* (2020). The albuminoid gland (Fig. 3A) flat and rather broad, the corpus pyriformis short and bulky, bursa copulatrix small and without a discernible duct, prostate narrow, slightly broadened in its distal part, the vas deferens outlet close to the margin of the terminal part of

the prostate. The praeputium (Fig. 3B) proximally cylindrical, distally gradually narrowing, with spots of black pigment, the penis sheath pigmentless, with moderately broadened proximal end. The length proportion between the praeputium and penis sheath was 1.25, compared with 1.09–1.27 recorded for *R. rufescens* by VINARSKI *et al.* (2020).

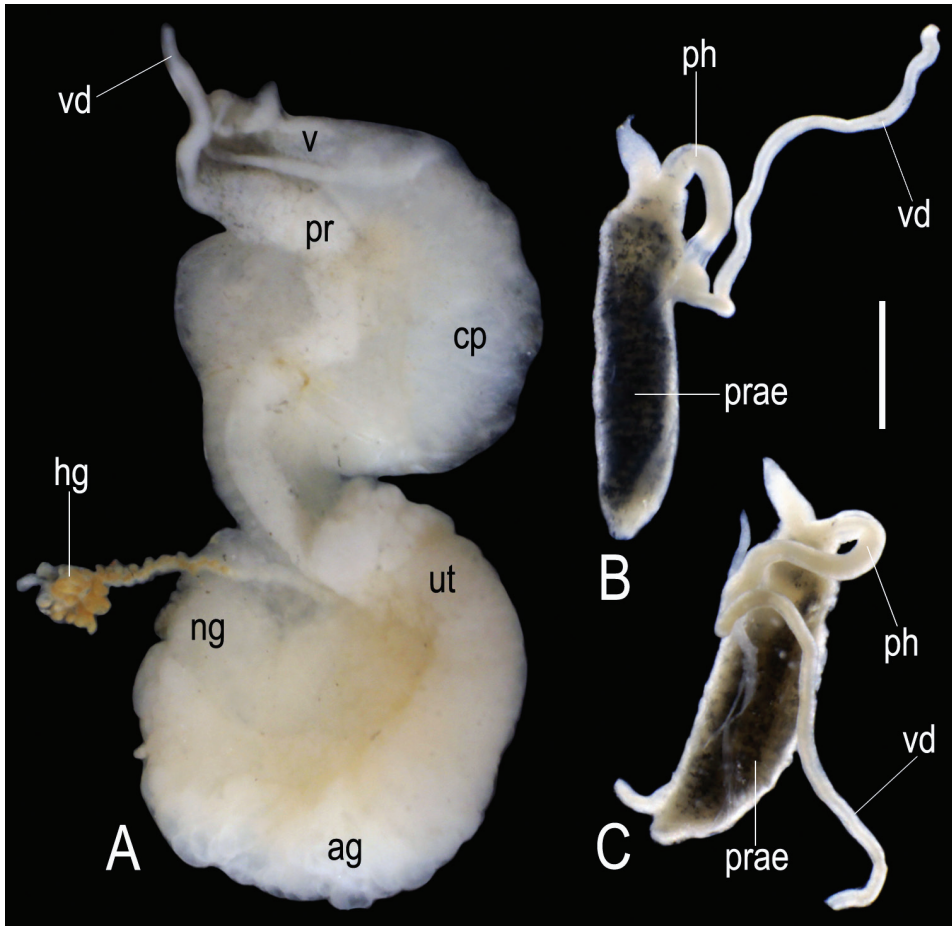
The maximum likelihood tree for COI (Fig. 4) clusters our Omani specimen (bootstrap support 94%, Bayesian probability 0.99) within *R. rufescens*, all the other specimens of this species are from Nepal, Bangladesh, and Myanmar. The p-distance within this group was 0.017. The Omani specimen differed from the other *R. rufescens* by p-distance 0.007–0.036. The sister species of *R. rufescens* is *R. natalensis*. p-distance between these two species equals 0.092.



**Fig. 2.** Shells and mantle pigmentation of *Radix rufescens*. Scale bar: 5 mm

DISCUSSION

Our data clearly identified our Omani specimens as belonging to *Radix rufescens*. Genetic differences between this specimen and the other sequences *R. rufescens* were much smaller than between *R. rufescens* and its sister species, *R. natalensis*. Also length proportion between the praeputium and penis sheath confirmed this species assignment. This is the first record of *Radix rufescens* not only in Oman, but in all Arabian Peninsula. Only five species of the Lymnaeidae, two of them representing the genus *Radix*, have been recorded (SMITH 1894, BROWN & GALLAGHER 1985, NEUBERT 1998): *R. natalensis*, molecu-



**Fig. 3.** Reproductive organs of *Radix rufescens*: A = ventrally, B–C = praeputium and phallotheca (ag – albuminoid gland, cp – corpus pyriformis, hg – haermaphrodite gland, ng – nidamental gland, ph – phallotheca (penis sheath), pr – prostate, prae – praeputium, ut – uterus, v – vagina, vd – vas deferens). Scale bar: 1 mm

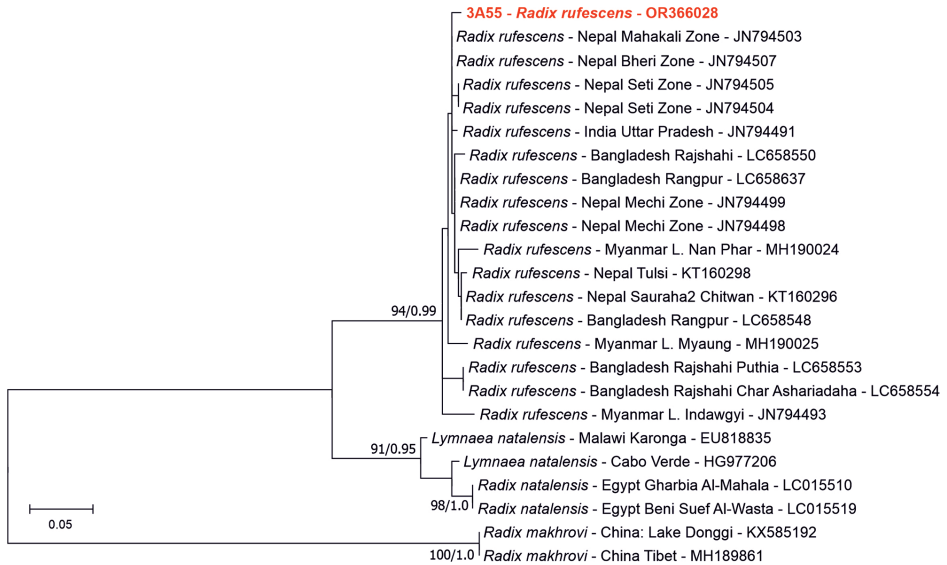


Fig. 4. Maximum likelihood tree computed for COI; bootstrap supports/Bayesian probabilities and GB numbers are given

larly identified so far only in Africa (SAITO *et al.* 2021), and *R. auricularia*, with a wide Eurasian range. Surprisingly, *R. euphratica* (Mousson, 1874), inhabiting Iran separated from Oman only by relatively narrow Ormuz Strait, has

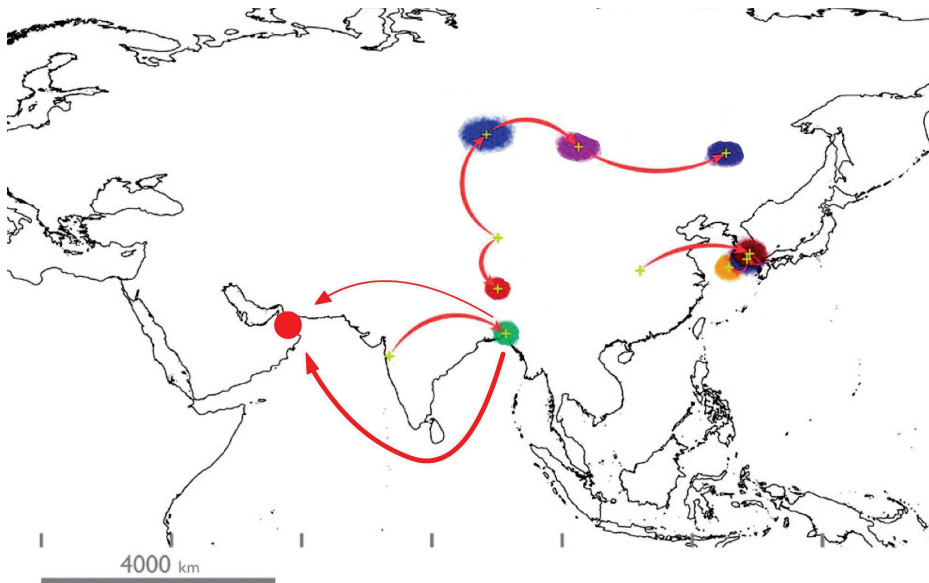


Fig. 5. Hypothetical way of invasion of *R. rufescens*, after SAITO *et al.* (2021), modified

not been recorded from the Arabian Peninsula. Thus *R. rufescens* would be the sixth lymnaeid species in the Arabia. However, the shells of *R. rufescens* are practically indistinguishable from the ones of *R. natalensis* (e.g., VINARSKI *et al.* 2020). Thus, either *R. rufescens* inhabits Wadi Tiwi (and some other still unstudied localities), or simply this is the first properly identified specimen of this species, and the records of *R. natalensis* (at least some of them) refer to *R. rufescens*. On the other hand, the supposed occurrence of *R. natalensis* in Oman may be the result of migration from the Horn of Africa Hotspot and the Eastern Afromontane Hotspot (MITTERMEIER *et al.* 2004).

The evolutionary history of *Radix* and main colonization routes are well known (SAITO *et al.* 2021). The first diversification area was situated in east India, the *rufescens/natalensis* group split then into two lines. The ancestor of *R. natalensis* probably moved into western India, and later to Africa, *R. rufescens* remained in Nepal and Bangladesh both NE of the Indian Subcontinent, as well as in Myanmar at Malay peninsula. How *R. rufescens* colonized Wadi Tiwi remains enigmatic. One of the excellent maps of SAITO *et al.* (2021) we have completed with our finding (Fig. 5). The known area of occurrence of *R. rufescens* may be much wider than the molecularly identified individuals confirm, thus the population discovered by us may be a part of the range covering perhaps the entire Indian Peninsula, southern Pakistan and Iran, and a large part of the Arabian Peninsula. Obviously, migration from the western coast of India would be more probable. Some older migrations may have been possible at the time when the Persian Gulf was reduced to a series of freshwater lakes (LAMBECK 1996).

However, small genetic differences between *R. rufescens* from Oman and from Nepal, Bangladesh, and Myanmar suggest much younger episodes of migration. Arabian Peninsula, especially Oman, since about 2,200 BC (PAINE 2013) was in trade relations with India. Arabian dhow sailed with monsoon winds (MILES 1919, LeBARON BOWEN 1949, VILLIERS 2018), conveying food, timber, etc., thus the accidental transportation of eggs or adult specimens of aquatic snails was probable. Especially the barrels and cans with drinkable water for the crew, rinsed before refilling in the stream in the outlet of Wadi Tiwi (situated not far from Sur, for centuries the main port at this region) were suitable for such transportation. The anthropogenic transportation of snails is extremely common (PRESTON *et al.* 2022). Also transportation by birds cannot be excluded (CHARALAMBIDOU & SANTAMARIA 2002, FIGUEROLA & GREEN 2002).

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