

## *Microcondylaea bonellii* as a new host for the European bitterling *Rhodeus amarus*

Ronaldo Sousa<sup>1,\*</sup>, Arthur E. Bogan<sup>2</sup>, Duarte V. Gonçalves<sup>3</sup>, Jasna Lajtner<sup>4</sup>, Vincent Prié<sup>5</sup>, Nicoletta Riccardi<sup>6</sup>, Spase Shumka<sup>7</sup>, Amílcar Teixeira<sup>8</sup>, Maria Urbańska<sup>9</sup>, Simone Varandas<sup>10</sup> and Manuel Lopes-Lima<sup>3,11</sup>

<sup>1</sup> CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus Gualtar, Braga, Portugal

<sup>2</sup> North Carolina State Museum of Natural Sciences, 11 West Jones Street, Raleigh, USA

<sup>3</sup> CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

<sup>4</sup> Division of Zoology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

<sup>5</sup> Institut de Systématique, Évolution, Biodiversité ISYEB – Museum National d’Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France

<sup>6</sup> CNR-IRSA Institute of Water Research, Verbania Pallanza (VB), Italy

<sup>7</sup> Faculty of Biotechnology and Food, Agricultural University of Tirana, Tirana, Albania

<sup>8</sup> Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>9</sup> Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

<sup>10</sup> CITAB-UTAD – Centre for Research and Technology of Agro-Environment and Biological Sciences, University of Trás-os-Montes and Alto Douro, Forestry Department, Vila Real, Portugal

<sup>11</sup> CIBIO/InBIO – Research Center in Biodiversity and Genetic Resources, University of Porto, Campus Agrário de Vairão, Vairão, Portugal

Received: 8 November 2019 / Accepted: 13 December 2019

**Abstract** – We report for the first time that the freshwater mussel *Microcondylaea bonellii* (Férussac, 1827) functions as a suitable host for the European bitterling *Rhodeus amarus* (Bloch, 1782). Given the recent expansion of *R. amarus* in Europe, the possible physiological cost (*e.g.* competition for oxygen, reduction in water circulation, and consequent impairment of filter-feeding) of this interaction may further affect the already poor conservation status of *M. bonellii* populations.

**Keywords:** Conservation / ecology / freshwater mussels / non-native species / threat

**Résumé** – *Microcondylaea bonellii*, un nouvel hôte pour la bouvière *Rhodeus amarus*. Nous signalons pour la première fois que la moule d’eau douce *Microcondylaea bonellii* (Férussac, 1827) sert d’hôte à la bouvière *Rhodeus amarus* (Bloch, 1782). Étant donné l’expansion récente de *R. amarus* en Europe, le coût physiologique possible de cette interaction (par exemple la compétition pour l’oxygène, la réduction de la circulation de l’eau et l’altération consécutive de l’alimentation par filtration) pourrait affecter davantage le statut de conservation déjà mauvais des populations de *M. bonellii*.

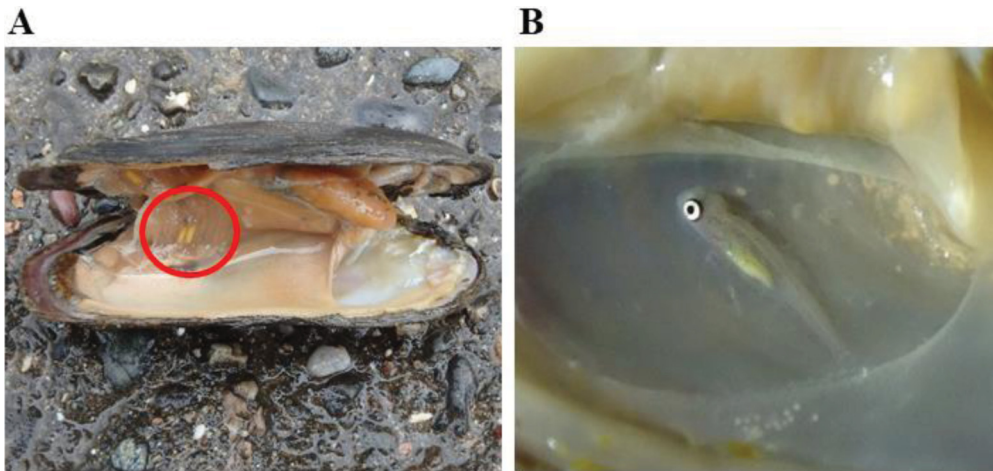
**Mots-cles :** Conservation / écologie / moules d’eau douce / espèces non indigènes

### 1 Introduction

Bitterlings (Fish: Cyprinidae) and freshwater mussels (Bivalvia: Unionida) have an interesting life cycle given that

the embryos need a suitable host to complete their development. In bitterlings, embryos are initially brooded in a suitable freshwater mussel (for a review see Smith *et al.*, 2004). Conversely, freshwater mussels need a suitable fish as a host to complete their life-cycle (for a review see Modesto *et al.*, 2018). In bitterlings, females possess long ovipositors to

\*Corresponding author: [rg.eco.sousa@gmail.com](mailto:rg.eco.sousa@gmail.com)



**Fig. 1.** A. Two embryos of European bitterling in *Microcondylaea bonellii*. B. An almost developed bitterling juvenile inside *Microcondylaea bonellii*.

deposit their eggs in the gills of freshwater mussels (*via* the exhalant aperture) and males fertilize these eggs releasing sperm into the mussels' inhalant aperture (Smith *et al.*, 2004). The fertilized eggs will then develop inside the suitable freshwater mussel, leaving the host around one month later as an active juvenile (Smith *et al.*, 2004). On the other hand, freshwater mussels have a myriad of different strategies to infest their hosts (*e.g.* using mantle lures, broadcasting masses of larvae, release of larvae packages in conglutinates that resemble food items, development of a lure to capture the fish host between the valves, among others) and larvae (glochidia) will then spend some time, from a few days to several months, depending on species, on the fins and/or gills of the suitable host until complete metamorphosis into juvenile mussels (Barnhart *et al.*, 2008; Modesto *et al.*, 2018).

Despite some controversy about the number of bitterling species colonizing Europe, *Rhodeus amarus* (Bloch, 1782) is considered the most widespread species on the continent (Bartáková *et al.*, 2019). In Europe, freshwater mussel species described as possible viable hosts for bitterlings are: *Anodonta anatina* (Linnaeus, 1758), *Anodonta cygnea* (Linnaeus, 1758), *Potomida littoralis* (Cuvier, 1798), *Pseudanodonta complanata* (Rossmässler, 1835), *Pseudunio auricularius* (Spengler, 1793), *Unio crassus* Philipsson, 1788, *Unio mancus* Lamarck, 1819, *Unio pictorum* (Linnaeus, 1758) and *Unio tumidus* Philipsson, 1788 (revised in Soler *et al.*, 2019). Therefore, only 7 of the 16 currently recognised European freshwater mussel species (Lopes-Lima *et al.*, 2017) are not documented as suitable hosts for bitterlings, possibly due to the lack of overlapping distribution (Prié, 2017; Soler *et al.*, 2019).

In this report, we describe for the first time, that the freshwater mussel *Microcondylaea bonellii* (Férussac, 1827) may also be considered a suitable host for *R. amarus* (Fig. 1A and B). During a survey in July 2019 in the Skadar Lake basin (Albania) bitterling embryos (identified visually) in different stages of development were found inside *M. bonellii*. In Drin River (41.890205, 19.571525), of a total of 7 *M. bonellii* analysed, 4 had bitterling embryos (57.1%) and in Gjadër River (41.905293, 19.581752), 8 of 14 *M. bonellii* analysed had bitterling embryos (57.1%). These levels of infestation are

much higher than the ones described for *U. crassus* in Poland (4.25%) (Tatoj *et al.*, 2017) but lower than the 100% described for *Anemina globosula* (Heude, 1878) individuals infested by *Rhodeus ocellatus* (Kner, 1866) embryos (Reichard *et al.*, 2007). These high levels of infestation, in comparison to other European studies, may translate in a higher energetic cost for *M. bonellii* (Methling *et al.*, 2019 and see below further discussion). In addition to *M. bonellii*, *A. anatina* and *U. crassus* were also present in Drin and Gjadër Rivers, respectively. *Anodonta anatina* was also parasitized by *R. amarus* (1 of 5 individuals; 20% of infestation) but no *U. crassus* was found with bitterlings embryos in a total of 5 individuals analysed.

*Rhodeus amarus* is rapidly expanding its distribution and increasing their abundance in Europe mostly due to the increasing number of accidental (*e.g.* waterways between different systems) and/or deliberate (hobbyists and anglers) introductions by humans, and the rise in temperature in response to climate change (Van Damme *et al.*, 2007). Albania seems to be no exception to this recent increase in spatial distribution due to human activities because recent molecular data showed that the specimens colonizing Skadar and Prespa Lakes were introduced from the Danube (Bartáková *et al.*, 2019). In fact, a recent study reported the rapid spread and very high densities of *Pseudorasbora parva* and *R. amarus* in Greater Prespa Lake, mainly near the shore (Shumka and Apostolou, 2018). It is possible that *R. amarus* will further expand their spatial distribution in Albania and negatively impact freshwater mussels in this region. *Microcondylaea bonellii* is classified as Vulnerable by the IUCN Red List and major threats to its conservation include pollution and natural systems modifications, mainly the transformation of lotic to lentic habitats due to the presence of dams or other physical obstacles (Lopes-Lima *et al.*, 2017). Given this threatened status the additional physiological stress caused by the infestation of bitterling embryos may be problematic. In fact, this biotic relationship between freshwater mussels and bitterlings was first classified as mutualistic, given that the bitterlings use mussel gills as spawning sites and mussels use bitterlings as hosts for glochidia (Smith *et al.*, 2004). However, recent studies suggest that European bitterlings should be

regarded as parasites of mussels since *R. amarus* rarely hosts glochidia of freshwater mussels (Reichard *et al.*, 2006) and the presence of bitterling embryos in mussels has a physiological cost (*e.g.* competition for oxygen and reduction in water circulation and consequent impairment of filter-feeding) to the host (Smith *et al.*, 2001; Mills *et al.*, 2005; Prié, 2017; Methling *et al.*, 2019). These negative effects may reduce growth and affect fecundity of freshwater mussels (Reichard *et al.*, 2006). In addition, freshwater mussels parasitized with multiple clutches of bitterling eggs experienced high mortality in mesocosms; although almost no data exist for wild populations (Smith *et al.*, 2000; Reichard *et al.*, 2010).

The recent expansion of *R. amarus* into new regions and countries, including Albania, has led to an increasing contact with a larger number of freshwater mussel species, including *M. bonellii*. Our results confirm for the first time that *R. amarus* may parasitize *M. bonellii* and this situation appears to confirm the recent hypothesis raised by Soler *et al.* (2019) that this fish species is able to spawn in any European unionid mussel. In addition, freshwater mussels are actively developing anti-bitterling adaptations (*e.g.* decreasing the success of oviposition and expelling bitterling eggs and embryos; Smith *et al.*, 2000; Reichard *et al.*, 2010). From this point of view, in European areas historically without any bitterling species, these adaptations are possibly missing and *R. amarus* may benefit from exploiting novel hosts, which may not possess appropriate adaptive responses to parasitism (Rouchet *et al.*, 2017). This situation should be further investigated given the high imperilment faced by several European freshwater mussels, including *M. bonellii*.

**Acknowledgments.** We acknowledge the two anonymous referees for the helpful suggestions that improve the clarity of our manuscript. This research was funded by FCT under project ConBiomics N° NORTE-01-0145-FEDER-030286, cofinanced by COMPETE 2020, Portugal 2020 and the European Union through the ERDF.

## References

- Barnhart MC, Haag WR, Roston WN. 2008. Adaptations to host infection and larval parasitism in Unionoida. *J N Am Benthol Soc* 27: 370–394.
- Bartáková V, Bryja J, Šanda R, *et al.* 2019. High cryptic diversity of bitterling fish in the southern West Palearctic. *Mol Phylogenet Evol* 133: 1–11.
- Lopes-Lima M, Sousa R, Geist J, *et al.* 2017. Conservation status of freshwater mussels in Europe: state of the art and future challenges. *Biol Rev* 92: 572–607.
- Methling C, Douda K, Reichard M. 2019. Intensity-dependent energetic costs in a reciprocal parasitic relationship. *Oecologia* 191: 285–294.
- Mills SC, Taylor MI, Reynolds JD. 2005. Benefits and costs to mussels from ejecting bitterling embryos: a test of the evolutionary equilibrium hypothesis. *Anim Behav* 70: 31–37.
- Modesto V, Ilarri M, Souza AT, *et al.* 2018. Fish and mussels: Importance of fish for freshwater mussel conservation. *Fish Fish* 19: 244–259.
- Prié V. 2017. Newly overlapping ranges: First records of *Potomida littoralis* (Cuvier, 1798) infestation by the European Bitterling *Rhodeus amarus* (Bloch, 1782). *J. Conchol* 42: 381–382.
- Reichard M, Liu H, Smith C. 2007. The co-evolutionary relationship between bitterling fishes and freshwater mussels: insights from interspecific comparisons. *Evol Ecol Res* 9: 1–21.
- Reichard M, Ondrackova M, Przybylski M, Liu H, Smith C. 2006. The costs and benefits in an unusual symbiosis: experimental evidence that bitterling fish (*Rhodeus sericeus*) are parasites of unionid mussels in Europe. *J Evolution Biol* 19: 788–796.
- Reichard M, Polačik M, Tarkan AS, *et al.* 2010. The bitterling–mussel coevolutionary relationship in areas of recent and ancient sympatry. *Evolution* 64: 3047–3056.
- Rouchet R, Smith C, Liu H, *et al.* 2017. Avoidance of host resistance in the oviposition-site preferences of rose bitterling. *Evol Ecol* 31: 769–783.
- Shumka S, Apostolou A. 2018. Current Knowledge on the Status of the Most Common Non-Indigenous Fish Species in the Transboundary Greater Prespa Lake (Albanian Side). *Acta Zool Bulgar* 70: 203–209.
- Smith C, Reichard M, Jurajda P, Przybylski M. 2004. The reproductive ecology of the European bitterling (*Rhodeus sericeus*). *J Zool* 262: 107–124.
- Smith C, Reynolds JD, Sutherland WJ, Jurajda P. 2000. Adaptive host choice and avoidance of superparasitism in the spawning decisions of bitterling (*Rhodeus sericeus*). *Behav Ecol Sociobiol* 48: 29–35.
- Smith C, Rippon K, Douglas A, Jurajda P. 2001. A proximate cue for oviposition site choice in the bitterling (*Rhodeus sericeus*). *Freshwater Biol* 46: 903–911.
- Soler J, Wantzen KM, Araujo R. 2019. *Rhodeus amarus* (Bloch, 1782): a new potential threat for *Margaritifera auricularia* (Spengler, 1793) (Unionoida, Margaritiferidae). *Freshw Sci* 38: 406–411.
- Tatoj K, Ćmiel AM, Kwaśna D, Lipińska AM, Zając K, Zając T. 2017. The endangered thick-shelled river mussel (*Unio crassus*): a new host species for the European bitterling (*Rhodeus amarus*). *Biodivers Conserv* 26: 1217–1224.
- Van Damme D, Bogutskaya N, Hoffmann RC, Smith C. 2007. The introduction of the European bitterling *Rhodeus amarus* to west and central Europe. *Fish Fish* 8: 79–106.

**Cite this article as:** Sousa R, Bogan AE, Gonçalves DV, Lajtner J, Prié V, Riccardi N, Shumka S, Teixeira A, Urbańska M, Varandas S, Lopes-Lima M. 2020. *Microcondylaea bonellii* as a new host for the European bitterling *Rhodeus amarus*. *Knowl. Manag. Aquat. Ecosyst.*, 421, 4.