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Native Relict versus Alien Molluscs in Thermal Waters of North-Western Romania, Including the First Record of *Melanoides tuberculata* (O. F. Müller, 1774) (Gastropoda: Thiaridae) from Romania

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Abstract: Some rare relict (i.e *Theodoxus prevostianus*) or even endemic freshwater molluscs (*Melanopsis parreyssii*) occurred until recently in several small and scattered thermal spring-fed brooks and wetlands in the north-western part of Romania. Owing to human pressure, geothermal aquifer exploitation, disturbance of habitats and invasion of alien species, significant changes in the freshwater mollusc fauna and communities have been recorded over the last two decades. These species, as well as others from the same habitats, have become extinct during the last years. Among other alien intruders found in the mentioned area during these years is *Melanoides tuberculata.* The present paper documents its first record in the Peţea Brook (nature reserve and site of community interest), pointing to a new threat to these highly imperilled ecological systems and their native communities.

Key words: thermal freshwaters, eco-insular systems, human pressure, alien species, range expansion, *Melanoides tuberculata*

Introduction

Anthropogenic stressors like introduction of non-indigenous species, eutrophication and pollution may devastate severely the ecology of aquatic systems. According to Van Bocxlaer & Albrecht (2014), biotic invasions, in synergy with other anthropogenic stressors that affect ecosystem stability, can have far-reaching effects in isolated eco-insular systems (such as the African Great Lakes). Such effects may propagate throughout the food web. Multiple anthropogenic stressors regularly interact, resulting in a hastened deterioration of ecosystem stability beyond the anticipated effects of individual stressors. The same assertions could be considered valid also for thermal springs and brooks from the temperate regions, because some of the species with certain preferences for higher water temperature are trapped in isolated small ranged thermal spots. Such ecological systems are among the most imperilled, because of human-related needs and use of the geothermal resources for a wide range of purposes. These usually small and scattered habitats shelter some of the rarest, relict or endemic taxa, which are subject to exponentially growing human pressures but also to invasions of alien species, which might interfere with other stressors.

This paper presents some of the main changes in the structure of mollusc communities occurring in thermal areas in north-western Romania, especially in the natural reserves at Răbăgani and Peţea (1 Mai Baths), located in the Bihor County, highlighting the decline or even extinction of native and relict elements as a result of human pressure, habitat destruction and alien species invasion.

Materials and Methods

In the last two decades, severe changes in the mollusc fauna of Romanian thermal waters, owing to anthropogenic pressure, were documented in several

papers (Sîrbu 2006; Glöer & Sîrbu 2006; Sîrbu & Benedek 2009; Sîrbu *et al.* 2013). In 2015, we carried out two field campaigns in order to assess the conservation state of the thermal Peţea Lake and Brook, as well as of the two freshwater mollusc species of conservation interest: the local endemic thermal freshwater gastropod *Melanopsis parreyssii* (Philippi, 1847), whose range and natural habitat is Peţea Lake, and the thick shelled river mussel *Unio crassus* Phillipson, 1788, recorded in the creek originating from the lake. The Peţea Creek flows together with the warm Hidişel Creek, coming from the neighbouring Felix Baths. In the last rivulet and downstream their confluence, on August 22nd, we found a very abundant colony of the tropical alien species *Melanoides tuberculata* (O.F. Müller, 1774). This is its first record in Romanian freshwaters (Fig. 1); the site's coordinates are 47.00627^0 N, 21.97931^0 E and the altitude is 142 m a.s.l. Seven quantitative samples were taken from the microhabitats where the species was recorded, in order to assess the ecological density and biomass. The counts were log-transformed and statistical ecological parameters were computed accordingly (Fowler *et al*. 2006). Fifty individuals were randomly selected and measured and descriptive and inferential statistics were calculated (Table 1). The codes used are as follows: W – weight or total wet biomass (in g); H – height of the shell, WID1 – shell`s main width with the aperture facing forward, WID2 – the small width (perpendicular on the former), HAP – height of the aperture, and WAP – width of the aperture, all in mm. In order to assist further research, the relation between biomass and biometrical parameters were subjected to two types of regression analysis. The level of significance for the parameters and r^2 – determinant coefficient values are given beside the statistical equations. In the mathematical expressions Exp(x) means $e^{(x)}$, where e is the basis of natural logarithms.

Fig. 1. The Peţea Creek downstream its confluence with the Hidişel Creek (the latter bringing thermal waters from Felix Baths), Sânmartin Village (Bihor County, Romania), where the first individuals of *Melanoides tuberculata* were identified in August 2015

Results

Changes in the native and alien mollusc fauna of two thermal areas in north-western Romania (Răbăgani and Peţea - 1 Mai Baths) are hereby discussed and updated according to the last field studies carried out between the summer and winter in 2015. In both areas the native species have either gone extinct or have a decreasing trend with respect of population parameters, distribution and quality of their habitats, while alien molluscs are increasing in terms of range, abundance and species richness.

There was an obvious decline of the thermal Peţea Lake and its water supply during the last two decades, because of drilling and exploiting the main resource of the area, the geothermal waters, mainly for touristic and household purposes. Consequently, the lake surface shrank, the water level decreased and the thermal spring reduced its flow until December 2011 when it dried out. Accordingly, a constant decline of the single population of the endemic *M. parreyssii* was documented (Sîrbu *et al.* 2013) until July 2015 when the authors did not found any living specimen. During the same year, several other biologists also strived to find this species, without any success. Therefore, although not long ago *M. parreyssii* was assessed by Z. Fehér (2013) in the *IUCN Red List of Threatened Species* as Critically Endangered (CR B1ab(iii,v)+2ab(iii,v), v. 3.1), now it has to be declared "Extinct in the Wild". There are still few specimens sampled from Peţea Lake and kept artificially in Hungary, but their future is highly uncertain. Welter-Schultes (2012: 35) considers that *Melanopsis parreyssii* is synonymous with *Melanopsis praemorsa* (Linnaeus, 1758), an opinion that is not supported by any evidence. Some debates during the last years concerned its taxonomic status, whether it should be ascribed to the genus *Melanopsis, Microcolpia* or *Fagotia.* This snail probably diverged during the Holocene from its closest living relative *Fagotia acicularis* or *Esperiana* (*Microcolpia*) *daudebartii acicularis* (Férussac, 1823). According to Smoleń & Falniowski (2009), who analysed partial sequences of ribosomal 18S and mitochondrial COI, *Fagotia* should be synonymised with *Melanopsis*. The application of a molecular clock with one point calibration for COI for the Hydrobiidae estimated the time of divergence as

Fig. 2. Typical habitus of *Melanoides tuberculata* sampled from the Peţea Creek

Fig. 3. Blackish shells of *Melanoides tuberculata* in the Hidişel and Peţea Creeks, due to deposits of peculiar physical and chemical loads of the thermal water

2.53±0.56 Mya for *M. parreyssii* and *F. acicularis*. This age coincides with the beginning of the glacial period in Europe (idem). According to Sümegi *et al*. (2012a, b), the morphological variation along the lineage suggests that *M. parreyssii* is (or was) an eco-form of *F. acicularis*. In case we accept that they were distinct taxa, then *M. parreyssii* was the youngest endemic to have evolved in the Carpathian Basin. Neubauer *et al.* (2014) speculates that the last living taxon was, comprising all the ribbed morphologies, *Microcolpia parreyssii parreyssii* (Philippi, 1847) and that its evolution was triggered mainly by genetic drift following a bottle-neck effect induced by environmental changes. Despite all debates, the main common results are that it was (1) most probably a distinct, young (i.e. recently evolved), local endemic species but (2) sadly it lives no longer in its former natural habitat (it became extinct in the wild).

The species of community interest *U. crassus* was found in the summer of 2015 only in two short sectors with non-thermal water of the Peţea Creek, being highly endangered and close to collapse. In Hidişel Creek and downstream their confluence, in the warm waters brought by the former brook, we found a very abundant colony of the tropical alien species *M. tuberculata*. In December 2015, the density was lower, as expected, but the species inhabited the same sector as in summer. Considering the seven quantitative samples taken from different microhabitats, the ecological density ranged between 614 and 2741 ind./m^2, with a mean of 1444.3 individuals/m^2 and SD = 903.8. The population density lies at a 95% probability between 806.6 and 2586.1 ind./m^2, these values being intermediate between those quoted in the literature (Van Damme 2014). The mean biomass was 151.4 g/m^2 (SD = 27.7, 95% Cl: 83.582 - 219.238 g/m^2).

Melanoides tuberculata has a high, elongate, conical shell (Fig. 2), which is usually light brown or horny, marked with rust-coloured spots, and streaks (Welter-Schultes 2012; Glöer 2015). In the thermal waters of Bihor County most snails have a blackish organic coated shell (Fig. 3), and not the typical spotted pattern (Fig. 2), because of deposits related to the peculiar physical and chemical water loads of the stream. However, the black coverage can be mechanically or chemically removed. The shells have about ten to 15 slightly convex whorls, each of them marked by a number of spiral grooves and by costae (Duggan 2002; Welter-Schultes 2012).

Table 1. Descriptive statistics of 50 randomly selected individuals of *M. tuberculata* from the Peţea Creek (variable codes are described in the text).

Statistical parameter	W (g)	H (mm)	WID1 (mm)	WID2 (mm)	HAP (mm)	WAP (mm)
Minimum	0.005	7.100	2.200	2.100	2.500	1.600
Maximum	0.580	26.600	8.000	7.600	8.100	5.000
Range	0.575	19.500	5.800	5.500	5.600	3.400
Mean	0.245	18.592	5.772	5.478	5.486	3.598
95% CI Upper	0.282	19.727	6.112	5.797	5.837	3.796
95% CI Lower	0.207	17.457	5.432	5.159	5.135	3.400
Std. Error	0.019	0.565	0.169	0.159	0.174	0.099
Standard Deviation	0.134	3.992	1.198	1.123	1.233	0.698
Coeff. of Variation	0.546	0.215	0.207	0.205	0.225	0.194

The descriptive statistics of the 50 randomly selected individuals of *M. tuberculata* from the samples (Table 1) are comparable with those published in other sources (Glöer 2015; Welter-Schultes 2012). The statistical model between the weight (W) and the height of the shell (H) is expressed as: $W = 0.0176 * \text{Exp}(0.1347 * H)$; $r^2 = 0.979$, $p < 0.001$ for both parameters. Another significant multivariate relation was found, namely: $W = 0.008858 + 0.357 * 10^{-3} * H * WID1 * WID2$; $r^2 = 0.988$, $p < 0.001$ for both parameters.

Discussion

The main changes in the thermal waters from northwestern Romania are related to geothermal aquifer overexploitation for human use, habitat debasement or even the disappearance of the (semi)natural environmental conditions. These changes have caused the extinction of some relict or endemic species, while colonisation and proliferation of some alien taxa were recorded.

Until recently, two small creeks with meso-thermal waters near Răbăgani Village supported isolated relict populations of *Theodoxus prevostianus* (C. Pfeiffer, 1828) and *Esperiana* (*Microcolpia*) *daudebartii acicularis*. The only population of *Theodoxus prevostianus* has gone extinct from Romania in 2007 because of the habitat destruction - a company has illegally excavated the whole area, captured the thermal rivulets and built a series of fishponds, without any reaction of the environmental authorities. The alien *Biomphalaria tenagophila* (D'Orbigny, 1835) was first identified in Romania (46.750361^0 N and 22.212444^o E) from the remaining waters in 2005 (Majoros *et al*. 2008). At that time, it was new for the European fauna. According to the authors this represented the furthest self-sustaining population of unknown origin and possibly a starting point for further dispersal in Europe, which might easily be accomplished by migrating birds or more likely with plants used in aquaria.

In December 2015 we found near Răbăgani, at the same place as the original recording, a highly abundant colony of *B. tenagophila*. *Esperiana daudebartii acicularis* still lives in a short sector of the single remaining rivulet, isolated and critically endangered because the area of the former nature reserve was claimed by a villager, and the spring as well as the upper sector of the creek now lies on private territory. As stated by the owner, there are already offers from at least one firm to buy the land and build fishponds, as it happened to the second nearby spring. Thus, besides the extinction of the single *T. prevostianus* population, the remnant relict communities are also on the brink of extinction. The authorities and official institutions were, and still are, unable to prevent or hinder this environmental disaster.

The changes in the thermal habitats at 1 Mai Baths were registered already during the 19th century, when an endemic subspecies (*serratilinea*) of *Theodoxus danubialis* (C. Pfeiffer, 1828) inhabiting the thermal waters, went extinct probably around the 1870s (Soós 1943). The human impact intensified during the last two decades leading both to extinctions, like the one of the previously mentioned endemic gastropod *Melanopsis parreyssii* and to alien invasions. In the thermal Peţea Lake several other alien species have been found during the last decade (Glöer & Sîrbu 2006), i.e. *Pseudosuccinea columella* (Say, 1817), *Planorbella anceps anceps* (Menke, 1830) and *Sinanodonta woodiana* (Lea, 1834). While *P. columella* has disappeared from the lake during the last years, *S. woodiana* was still found in 2015 as the single living aquatic mollusc, inhabiting both the remnants of the thermal lake and some sectors of the Peţea Creek. *Planorbella anceps anceps*

has vanished from the lake but we found it again in 2015 in the Peţea Creek, in the sectors inhabited by *M. tuberculata*. The introduction and colonisation of *M. tuberculata* were most likely the result of one or more releases from aquaria.

The range expansion of the alien mussel *Corbicula fluminea* (O.F. Müller, 1774) is also of interest. Besides the Danube River and Delta, the first finding of this species in the Romanian inland waters was in 2011 along the lower Timiş River in Banat (Sîrbu & Sîrbu 2013). Its dispersal in north-western Romania in the three Criş Rivers, both on Hungarian and Romanian territory, and mainly along the Crişul Repede River, the collector of the Peţea Creek, was documented by Cupşa (2014). The other findings on the Crişul Negru and the Crişul Alb River were on Hungarian territory (idem). In July 2015 we found *C. fluminea* also on Romanian territory in the lower sector of the Crişul Negru River, in Tămaşda and Zerind Villages, upstream the locations where Cupşa (2014) identified it in Hungary. Considering its distribution in inland waters in Bulgaria and along some tributaries of the Danube river, Hubenov *et al.* (2013) stated that the most probable dispersal vector of *C. fluminea* is passive upstream transport, facilitated probably by some human activities, such as fishing, fish stocking, recreational activities, sand and gravel extraction.

The rapid dispersal of *C. fluminea* along the Criş Rivers demonstrates its high ability of range expansion in this area as well. It also raises the questions of when will it reach the thermal waters in the area and what effect will it have on the present communities.

The recent introduction of *M. tuberculata* in the Peţea Creek was also most likely the result of one or more releases from aquaria, although it is commonly dispersed by birds (Van Damme 2014). *Melanoides tuberculata* is a cosmopolite, both parthenogenetic and ovoviviparous, prosobranch snail, inhabiting freshwaters but also brackish waters. This gastropod is native to Northern Africa and Southern Asia, artificially introduced likely by aquarists in many areas around the world, including in thermal and artificially heated waters even in colder regions, in botanical gardens and aquaria, living on the bottom of standing or flowing waters (Welter-Schultes 2012; Glöer 2015). This species was found few years ago in Serbia (Milenković & Gligorijević 2012) and recently in Bulgaria (Gashtarov & Georgiev 2016). It lives in waters with temperatures between 18^0C and 31^0C, avoiding low pH values (Duggan 2002) and is able to survive in relatively alkaline and saline waters (Van Damme 2014). *Melanoides tuberculata* feeds on detritus, epiphytic microalgae, decaying plant matter and dead animals and is likely resistant to most threats except droughts (idem). In Romania it is still confined to sectors with warm water brought by Hidişel Creek from Felix Baths. In the cold Peţea rivulet, upstream the confluence, it is absent. In the survey area *M. tuberculata* inhabited all kinds of available substrata, but the highest abundance was found on hard and stable surfaces (artificial concrete, boulders, riverbanks, dams etc.) in faster flowing water. Although restricted in the temperate areas of the world to thermal or artificially heated waters, its presence and dispersal should not be underestimated. In Romania there are many thermal springs and streams, spread across the country, thus this species is prone to a wide dispersal. According to Mehler & Acharya (2014), even at low abundances the exotic species can have a significant impact on ecosystem processes, especially through nitrogen recycling in systems with very low ambient nutrient concentrations. Significant anthropogenic disturbances, like eutrophication, pollution and sedimentation, are known to increase abundance and biomass of opportunistic molluscs including *M. tuberculata*. Anthropologically stressed aquatic systems and high human densities in sub-Saharan Africa indicate that its colonisation success is strongly related to humans (Van Bocxlaer *et al.* 2015). *Melanoides tuberculata* has often been found to have superior competitive abilities compared to other snail species and its introduction was correlated with declines in native gastropods (Duggan 2002). Considering the highly invasive nature of this species, it represents a threat to aquatic biodiversity (Van Damme 2014). Thus, monitoring the local colony in Bihor County, as well as periodic surveys of the thermal waters in Romania and other countries facing similar problems, could aid the conservation of these rare and imperilled ecological systems. *Melanoides tuberculata* is a first intermediate host for different Trematoda, including some parasites of humans, fish and domestic animals (Besprozvannykh *et al*. 2013; Van Damme 2014), thus its survey and dispersal control has also epidemiological implications. This is valid also for other alien species, like *B. tenagophila*, which is considered to be a potential risk to the public health in the conditions of the global climate change and its role as the host of schistosomes (Majoros *et al*. 2008).

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