e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 70 (2022), No 4
https://doi.org/10.3409/fb_70-4.26

What Does the Haired Keel on the Shell Whorls of *Potamopyrgus antipodarum* (Gastropoda, Tateidae) Mean?

Anna STANICKA, Kamila Stefania ZAJĄC, Dorota LACHOWSKA-CIERLIK, Kinga LESIAK, Monika LEWALSKA, Anna CICHY, Janusz ŻBIKOWSKI, and Elżbieta ŻBIKOWSKA

Accepted November 21, 2022 *Published online December 06, 2022* *Issue online December 06, 2022*

Short communication

STANICKA A., ZAJĄC K.S., LACHOWSKA-CIERLIK D., LESIAK K., LEWALSKA M., CICHY A., ŻBIKOWSKI J., ŻBIKOWSKA E. 2022. What does the haired keel on the shell whorls of *Potamopyrgus antipodarum* (Gastropoda, Tateidae) mean? Folia Biologica (Kraków) **70**: 237-242.

In several ecosystems, *Potamopyrgus antipodarum* (Gray, 1853) (Gastropoda, Tateidae) is considered among the worst invasive species. Its tolerance to a broad range of environmental conditions has favoured its success in colonising new environments worldwide. However, population crashes may occur, leading to significant fluctuations in snail densities. Such crashes might be linked to morphological changes in the shell whorls, like the emergence of a haired keel (carinatus morphotype). In this study, we investigated the link between the appearance of the carinatus morphotype and the crashes in population densities over three years, based on field observations. The presented results show that after the emergence of the so-called carinatus morphotype, the population of *P. antipodarum* collapsed and did not recover for the next two years. This may indicate that the carinatus morphotype is a defensive reaction to extremely unfavourable environmental conditions.

Key words: New Zealand mud snail, carinatus morphotype, invasive species, shell, lake.

Anna STANICKA✉, Kinga LESIAK, Monika LEWALSKA, Anna CICHY, Elżbieta ŻBIKOWSKA, Faculty of Biological and Veterinary Sciences, Department of Invertebrate Zoology and Parasitology, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Toruń, Poland.
E-mail: anna.marszewska@umk.pl
Kamila Stefania ZAJĄC, Nature Education Centre, Jagiellonian University, Gronostajowa 5, 30-387 Kraków, Poland.
Dorota LACHOWSKA-CIERLIK, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland.
Janusz ŻBIKOWSKI, Faculty of Biological and Veterinary Sciences, Department of Ecology and Biogeography, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Toruń, Poland.

In several European ecosystems, the New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1853) (Gastropoda, Tateidae) has been included among the worst invasive species (NENTWIG *et al.* 2018). GEIST *et al*. (2022) reported that this invader is currently listed in the aquatic systems of at least 39 countries from five non-native continents. Furthermore, it has been present in Europe for 160 years (BOYCOTT 1936). A wide range of *P. antipodarum* densities have also been reported in non-native areas; in extreme cases, these densities reach as many as several hundred thousand snails per square metre (LEVRI *et al.* 2007; GEIST *et al*. 2022). Such highly abundant populations of *P. antipodarum* may impact the structure and functioning of the invaded ecosystem (GEIST *et al*. 2022). The success of *P. antipodarum* in colonising new areas is due to its tolerance to unfavourable physiochemical conditions (e.g. dissolved oxygen, pH and water temperature), as well as a lack of native enemies and the peculiar features of the shell morphology (ALONSO & CASTRO-DÍEZ 2008, 2012). For example, a solid operculum and a hard shell allow the snails to survive in the digestive systems of fish (ALONSO & CASTRO-DÍEZ 2012). However, the European populations of *P. antipodarum* show significant fluctuations in their occurrence; in particular, high densities and subsequent extinctions can be observed. It has mainly been postulated that this is the result of low genetic diversity among the individuals (STANICKA *et al.* 2020).

Little is known about the role of a haired keel on the shell whorls appearing in some populations of *P. antipodarum*. This morphological variation in *P. antipodarum* may be stress-induced by changes in environmental conditions, such as variations in the water depth, flow or the appearance of predators and parasites (VERHAEGEN *et al*. 2018). Based on three years of field research, we would like to consider the presence of a haired keel on the shell whorls of *P. antipodarum* as an adaptation to stressful environmental conditions.

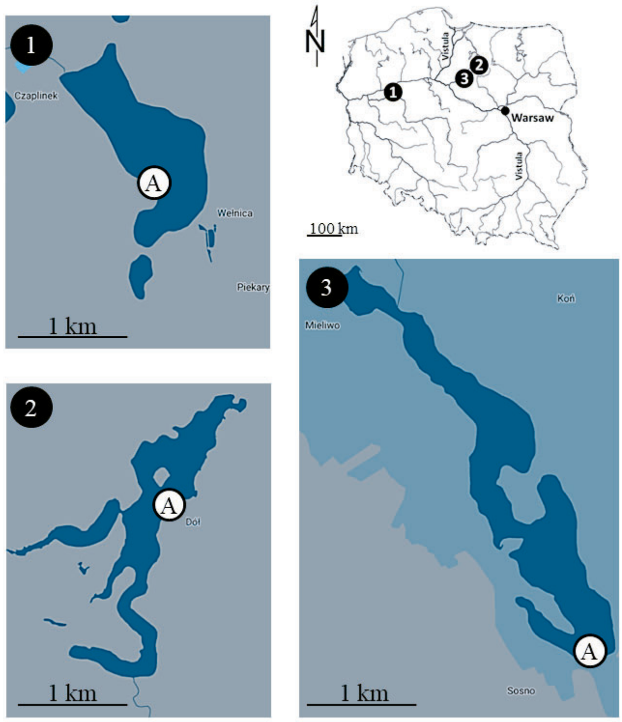

Fig. 1. Sampling sites (A): 1 – Lake Czaplino, 2 – Lake Iławskie, 3 – Lake Sosno.

Materials and Methods

The research areas were three Polish water bodies – Lake Czaplino (53°32′59″N, 16°14′59″E), Lake Iławskie (53°35′37″N, 19°36′54″E) and Lake Sosno (53°20′15″N, 19°20′55″E) – where we have been controlling the presence of *P. antipodarum* for many years (Fig. 1). The general characteristics of the sampling sites are summarised in Table 1. The water parameters were measured with a core sampler and a MultiLine P4 (WTW) Universal Pocket Sized Meter (Lake Czaplino: conductivity – 327 µS/cm, pH – 7.0; Lake Iławskie: conductivity – 368 µS/cm, pH – 8.5; Lake Sosno: conductivity – 369 µS/cm, pH – 8.3). The collecting of snails and the aforementioned water measurements were carried out once a year – in September 2018, 2019 and 2020 – from a sandy bottom of the littoral zone. Using a core sampler with a diameter of 40 mm (POZNAŃSKA-KAKAREKO *et al*. 2017), 12 samples were taken at random during each sampling session. The contents of the core samplers were always placed in three containers (Fig. 2). In the laboratory, the snails were isolated and counted on white trays. Then, their densities per m^2 (D) were converted according to the following formula: $D = ((B_1 \times 10\,000 / S) + (B_2 \times 10\,000 / S) + (B_3 \times 10\,000 / S)) / 3$; B – number of snails in the box; S – the sum of the surface of the four-core sampler, i.e. 50.24 cm^2.

The preliminary identification of the collected snails as *P. antipodarum* species was verified based on morphological data (PIECHOCKI &

Table 1

General characteristics of the investigated lakes (CHOIŃSKI 2006; LANDSBERG-UCZCIWEK 2001; PTAK 2013)

	Lake Czaplino	Lake Iławskie	Lake Sosno
Lake type	Glacial lake	Ribbon lake	Ribbon lake
Surface area	108.3 ha	116-154.5 ha	188 ha
Average depth	12.3 m	1.1 m	5 m
Max. depth	23 m	2.5 m	12.6 m
Water volume	13344.7 m^3	1773.6 m^3	9390.3 m^3
Water quality	III* (1996)**	III* (2002)**	II* (2006)**
Level of overgrowth	Moderate	Moderate	Moderate
Type of sampling site substrate	Sandy	Sandy	Sandy
Sampling site use	Recreational beach	Recreational beach	Recreational beach

* Classes of water purity condition: I – very good, II – good, III – moderate, IV – bad, V– very bad
** Year of the data update

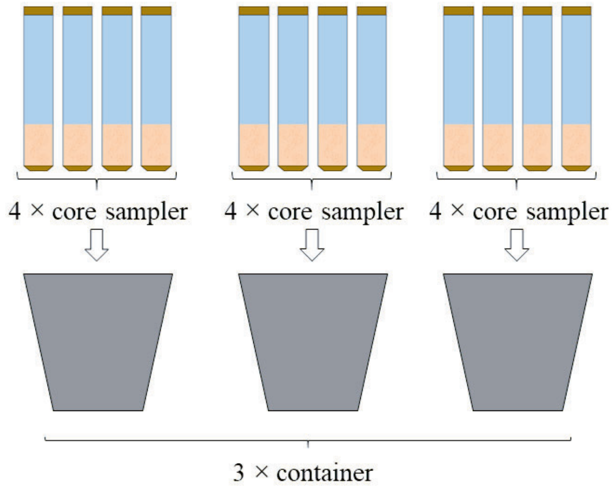

Fig. 2. Methodology of the sample collection.

Fig. 3. Morphotypes of Potamopyrgus antipodarum recorded in the presented study: A – *carinatus*, B – regular.

WAWRZYNIAK-WYDROWSKA 2016). Then, randomly selected individuals from each research area (in 2018) were subjected to a molecular species identification. Small pieces of tissue from each snail were stored in 1.5 ml tubes with 99.8% ethanol and were used for DNA extraction. According to the manufacturer's protocol, DNA was extracted from the collected tissue using the Sherlock AX Kit (A & A Biotechnology, Poland). To obtain DNA sequences of the 16S rRNA gene, PCR reactions were run using the S1-Universal (5'-CGGCCGCCTGTTTATCAAAAACAT-3') and S2-Potamo (5'-GTGGTCGAACAGACCAACCC-3') set of primers (STÄDLER *et al.* 2005). A PCR cocktail and profiles/conditions were used according to (STANICKA *et al.* 2020). To check the DNA quality, a 3 µl sample of the PCR product sample was run on a 1.5% agarose gel for 30 min at 100 V. The PCR products were cleaned up using a Clean-up Kit (A&A Biotechnology, Poland). A sequencing reaction was performed in 10 µl of the reaction mixture, according to the cocktail and profile conditions described in ZAJĄC *et al.* (2020). The sequencing products were cleaned up using ExTerminator (A&A Biotechnology, Poland) and were sequenced in one direction. The sequencing reactions were performed by Genomed (Warsaw, Poland). The sequences were deposited in GenBank with the following accession numbers: MK578225, MK578226 and MK578227.

Moreover, we observed the shell surfaces of the snails under a stereomicroscope (Motic K-700) to divide the collected individuals into carinatus and regular morphotypes (with and without a haired keel on the shell whorls, respectively), according to BUTKUS *et al.* (2012). Selected individuals of the carinatus morphotype were photographed using a digital camera, and we then used the images to measure their haired keel with the ImageJ program.

Results

All *P. antipodarum* 16S rRNA sequences belonged to the same haplotype and showed a 100% similarity to *P. antipodarum* (MG979469, SHARBROUGH *et al.* 2018), confirming the morphological identification.

The carinatus morphological form (Fig. 3) was detected only in the specimens from Lake Iławskie, and it was the only morphotype of *P. antipodarum* in this location. Their haired keel had an average length of 121 (SE 2) µm. At Lake Iławskie, where the carinatus morphotype was noted, individuals of *P. antipodarum* were recorded only in the first year of the study. By contrast, only their empty shells were detected in the following two years. In the other two research areas, where no haired keels on the shell whorls of *P. antipodarum* were recorded, the individuals of *P. antipodarum* were recorded during all three years (Table 2).

Table 2

The density of individuals of *Potamopyrgus antipodarum*

Study sites	Number of individuals per m^2 (± SE)		
	2018 year	2019 year	2020 year
Lake Czaplino	9222 (± 922)	10085 (± 518)	7696 (± 893)
Lake Iławskie	4246 (± 351)	0	0
Lake Sosno	2189 (± 230)	1460 (± 175)	929 (± 239)

Discussion

There are known cases where trematode larvae in the snail body can modify the shell morphology of their hosts (in terms of the shape, size or ornamentation) (LAGRUE *et al.* 2007). ŻBIKOWSKA & ŻBIKOWSKI (2005) emphasised that the morphology of snail shells can be affected by parasites only in cases where they play the role of the first intermediate host. Nevertheless, it is well known that outside their native range, infections of *P. antipodarum* with sporocysts, rediae or cercariae are extremely rare (EVANS *et al.* 1981; GÉRARD & LE LANNIC 2003; ŻBIKOWSKI & ŻBIKOWSKA 2009; STANICKA *et al.* 2020).

Shell polymorphisms may have a genetic basis (VERHAEGEN *et al.* 2018), and regular and carinatus morphotypes might result from two independent invasion events, as was suggested by BUTKUS *et al.* (2012). However, both morphotypes collected in the present study belonged to the one haplotype (STANICKA *et al.* 2020). This suggests that the presence of the two morphotypes might not be solely attributed to a genetic basis, but could also have been promoted by a plastic response to the environment. Accordingly, variations in the shell morphology could reflect the adaptive responses to abiotic and biotic factors, and may be the result of phenotypic plasticity in conjunction with evolutionary changes (HAASE 2003; KRISTNER & DYBDAHL 2013).

It is well known that the ability of organisms to form inducible defences in response to environmental changes is based on their phenotypic plasticity (SPITZE & SADLER 1996). We did not find a population exhibiting both morphotypes in the investigated research areas. It is most likely that the analysed physicochemical parameters of the habitats did not have a decisive influence on the morphotype formation, because these parameters seemed similar in the habitats with and without carinatus morphotypes (especially the conductivity, which is of particular importance for *P. antipodarum* (LARSON *et al*. 2020)). However, the impact of unfavourable environmental factors (other than the water quality, e.g. the type of substrate, water flow and composition of co-inhabitants) on creating the carinatus morphological form of these snails seems to be the most probable conclusion, because in the next two years of research at Lake Iławskie only the empty shells of *P. antipodarum* were recorded. This proves the constant presence of a stress factor for *P. antipodarum* in this environment. It is also unlikely that the destruction of the population would be caused by any short-term event, such as a spillover of pollutants or pesticides, because our qualitative observations indicated the presence of other species of snails. HOLOMUZKI & BIGGS (2006) suggested two reasons for the emergence of carinatus forms: (i) a higher probability of snail dislocation caused by flow; and (ii) for protection against being eaten by fish. Laboratory experiments have indicated that the spines collected seston, making the individuals of the carinatus morphotype more prone to flow-induced dislodgment than individuals of the regular morphotype (HOLOMUZKI & BIGGS 2006). This seems very beneficial for these snails, because *P. antipodarum* naturally avoids strong currents by wandering into safer habitats (HOLOMUZKI & BIGGS 2006). It is also well known that water movement may affect animal populations (HUBENDICK 1958). In addition to the movement of water, such as the action of waves or currents, issues related to changes in the water level should be mentioned (HUBENDICK 1958), which were most often observed at Lake Iławskie during seasonal observations among the examined lakes (personal observations). At the sampling site, individual representatives of other species of prosobranch snails with small shell sizes were found. Nevertheless, these snails belonging to the genus *Valvata* and *Bithynia* inhabited the neighbouring habitat that was heavily overgrown with elodeids, which may indicate their attempt to avoid unfavourable water movements. On the other hand, Iławskie Lake is abundantly inhabited by numerous species of fish, with the most abundant being bream, tench and pike (as reported by a local fish farm). Therefore, it can be expected that predation could have caused the appearance of carinatus forms here. Generally, it is challenging to link this with the destruction of the snail population, because if one individual of *P. antipodarum* remains in the setting that is enough for the population to recover (SCHREIBER *et al.* 1998). However, such a change probably requires a huge investment of energy, and in the event of an additional negative factor, such as the aforementioned water level fluctuations, we can assume that such a population would collapse. In conclusion, the presence of a haired keel on the shell whorls may be considered as a type of snail defensive reaction; however, long-term field and experimental research are necessary to identify the exact cause of its occurrence. Learning the exact purpose of the appearance of the carinatus morphotype may prove useful in developing tools to combat invasions of *P. antipodarum*.

Acknowledgements

This project was supported by the grant of the National Science Centre, Poland No. 2017/25/N/NZ8/01345.

Author Contributions

Research concept and design: A.S., E.Ż.; Collection and/or assembly of data: A.S., K.S.Z., D.L.-C., K.L., M.L., A.C., J.Ż.; Data analysis and interpretation: A.S., K.S.Z., D.L.-C., E.Ż.; Writing the article: A.S., K.S.Z., K.L., M.L.; Critical revision of the article: A.S., K.S.Z., D.L.-C., K.L., M.L., A.C., J.Ż., E.Ż.; Final approval of article: A.S., K.S.Z., D.L.-C., K.L., M.L., A.C., J.Ż., E.Ż.

Conflict of Interest

The authors declare no conflict of interest.

References

ALONSO A., CASTRO-DÍEZ P. 2008. What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? Hydrobiologia **614**: 107-116. https://doi.org/10.1007/s10750-008-9529-3

ALONSO A., CASTRO-DÍEZ P. 2012. The exotic aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca): state of the art of a worldwide invasion. Aquat. Sci. **74**: 375-383. https://doi.org/10.1007/s00027-012-0254-7

BOYCOTT A.E. 1936. The Habitats of Fresh-Water Mollusca in Britain. J. Anim. Ecol. **5**: 116-186. https://doi.org/10.2307/1096

BUTKUS R., ŠIDAGYTĖ E., ARBAČIAUSKAS K. 2012. Two morphotypes of the New Zealand mud snail *Potamopyrgus antipodarum* (J.E. Gray, 1843) (Mollusca: Hydrobiidae) invade Lithuanian lakes. Aquat. Invasions **7**: 211-218. https://doi.org/10.3391/ai.2012.7.2.007

CHOIŃSKI A. 2006. *Katalog jezior Polski*. Wydawnictwo Naukowe UAM, Poznań pp. 600.

EVANS N.A., WHITFIELD P.J., DOBSON A.P. 1981. Parasite utilization of a host community: the distribution and occurrence of metacercarial cysts of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) in seven species of mollusc at Harting Pond, Sussex. Parasitology **83**: 1-12. https://doi.org/10.1080/03014223.2003.9517743

GEIST J.A., MANCUSO J.L., MORIN M.M., BOMMARITO K.P., BOVEE E.N., WENDELL D., BURROUGHS B., LUTTENTON M.R., STRAYER D.L., TIEGS S.D. 2022. The New Zealand mud snail (*Potamopyrgus antipodarum*): autecology and management of a global invader. Biol. Invasions **24**: 905-938. https://doi.org/10.1007/s10530-021-02681-7

GÉRARD C., LE LANNIC J. 2006. Establishment of a new host-parasite association between the introduced invasive species *Potamopyrgus antipodarum* (Smith) (Gastropoda) and *Sanguinicola* sp. Plehn (Trematoda) in Europe. J. Zool. **261**: 213-216. https://doi.org/10.1017/S0952836903004084

HAASE M. 2003. Clinal variation in shell morphology of the freshwater gastropod *Potamopyrgus antipodarum* along two hill-country streams in New Zealand. J. R. Soc. N Z. **33**: 549-560. https://doi.org/10.1080/03014223.2003.9517743

HOLOMUZKI J.R., BIGGS B.J. 2006. Habitat-specific variation and performance trade-offs in shell armature of New Zealand mud snails. Ecol. **87**: 1038-1047. https://doi.org/10.1890/0012-9658(2006)87[1038:HVAPTI]2.0.CO;2

HUBENDICK B. 1958. Factors conditioning the habitat of freshwater snails. Bull. World Health Organ. **18**: 1072-1080.

KISTNER E.J., DYBDAHL M.F. 2013. Adaptive responses and invasion: the role of plasticity and evolution in snail shell morphology. Ecol. Evol. **3**: 424-436. https://doi.org/10.1002/ece3.471

LAGRUE C., MCEWAN J., POULIN R., KEENEY D.B. 2007. Co-occurrences of parasite clones and altered host phenotype in a snail-trematode system. Int. J. Parasitol. **37**: 1459-1467. https://doi.org/10.1016/j.ijpara.2007.04.022

LANDSBERG-UCZCIWEK M. 2001. Raport o stanie środowiska w województwie zachodniopomorskim w roku 2000. IOŚ Biblioteka Monitoringu Środowiska, Szczecin pp. 199.

LARSON M.D., DEWEY J.C., KRIST A.C. 2020. Invasive *Potamopyrgus antipodarum* (New Zealand mud snails) and native snails differ in sensitivity to specific electrical conductivity and cations. Aquat. Ecol. **54**: 103-117. https://doi.org/10.1007/s10452-019-09729-w

LEVRI E.P., KELLY A.A., LOVE E. 2007. The invasive New Zealand mud snail (*Potamopyrgus antipodarum*) in lake Erie. J. Great Lakes Res. **33**: 1-6. https://doi.org/10.3394/0380-1330(2007)33[1:TINZMS]2.0.CO;2

MARSZEWSKA A., CICHY A., BULANTOVÁ J., HORÁK P., ŻBIKOWSKA E. 2018 *Potamopyrgus antipodarum* as a potential defender against swimmer's itch in European recreational water bodies-experimental study. PeerJ **6**: e5045. https://doi.org/10.7717/peerj.5045

NENTWIG W., BACHER S., KUMSCHICK S., PYŠEK P., VILÀ M. 2018. More than "100 worst" alien species in Europe. Biol. Invasions **20**: 1611-1621. https://doi.org/10.1007/s10530-017-1651-6

PIECHOCKI A., WAWRZYNIAK-WYDROWSKA B. 2016. Guide to freshwater and marine Mollusca of Poland. Bogucki Wydawnictwo Naukowe, Poznań pp. 280.

POZNAŃSKA-KAKAREKO M., BUDKA M., ŻBIKOWSKI J., CZARNECKA M., KAKAREKO T., KOBAK J. 2017. Survival and vertical distribution of macroinvertebrates during emersion of sandy substratum in outdoor mesocosms. Fundam. Appl. Limnol. **190**: 29-47. https://doi.org/10.1127/fal/2017/1017

PTAK M. 2013. Zmiany powierzchni i batymetrii wybranych jezior pojezierza pomorskiego. Prace Geograficzne **133**: 61-76.

SCHREIBER E.S.G., GLAISTER A., QUINN G.P., LAKE P.S. 1998. Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in Lake Purrumbete, Victoria, Australia. Mar. Freshw. Res. **49**: 73-78. https://doi.org/10.1071/MF97113

SHARBROUGH J., LUSE M., BOORE J.L., LOGSDON Jr J.M., NEIMAN M. 2018. Radical amino acid mutations persist longer in the absence of sex. Evolution **72**: 808-824. https://doi.org/10.1111/evo.13465

SPITZE K., SADLER T.D. 1996. Evolution of a generalist genotype: multivariate analysis of the adaptiveness of phenotypic plasticity. Am. Nat. **148**: 108-123. https://doi.org/10.1086/285905

STANICKA A., ZAJĄC K.S., LACHOWSKA-CIERLIK D., CICHY A., ŻBIKOWSKI J., ŻBIKOWSKA E. 2020. *Potamopyrgus antipodarum* (Gray, 1843) in Polish waters – its mitochondrial haplotype and role as intermediate host for trematodes. Knowl. Manag. **421**: 48. https://doi.org/10.1051/kmae/2020040

STÄDLER T., FRYE M., NEIMAN M., LIVELY C.M. 2005. Mitochondrial haplotypes and the New Zealand origin of clonal European *Potamopyrgus*, an invasive aquatic snail. Mot. Ecol. **14**: 2465-73. https://doi.org/10.1071/MF97113

VERHAEGEN G., MCELROY K.E., BANKERS L., NEIMAN M., HAASE M. 2018. Adaptive phenotypic plasticity in a clonal invader. Ecol. Evol. **8**: 4465-4483. https://doi.org/10.1002/ece3.4009

ZAJĄC K. S., PROĆKÓW M., STEC D., LACHOWSKA-CIERLIK D. 2020. Phylogeography and potential glacial refugia of terrestrial gastropod *Faustina faustina* (Rossmässler, 1835)(Gastropoda: Eupulmonata: Helicidae) inferred from molecular data and species distribution models. Org. Divers. Evol. **20**: 747-762. https://doi.org/10.1007/s13127-020-00464-x

ŻBIKOWSKA E., ŻBIKOWSKI J. 2005. Differences in shell shape of naturally infected *Lymnaea stagnalis* (L.) individuals as the effect of the activity of digenetic trematode larvae. J. Parasitol. **91**: 1046-1051. https://doi.org/10.1645/GE-420R1.1

ŻBIKOWSKI J., ŻBIKOWSKA E. 2009. Invaders of an invader-Trematodes in *Potamopyrgus antipodarum* in Poland. J. Invertebr. Pathol. **101**: 67-70. https://doi.org/10.1016/j.jip.2009.02.005