FOLIA MALACOLOGICA
Folia Malacol. 26(2): 103–120
ISSN 1506-7629 (print) • ISSN 2300-7125 (on-line)
The Association of Polish Malacologists
Faculty of Biology, Adam Mickiewicz University
Bogucki Wydawnictwo Naukowe
Poznań, June 2018
https://doi.org/10.12657/folmal.026.009

DISTRIBUTION OF *MONACHA CLAUSTRALIS* (ROSSMÄSSLER, 1834) AND *M. CARTUSIANA* (O. F. MÜLLER, 1774) (EUPULMONATA: HYGROMIIDAE) IN CENTRAL EUROPEAN AND BALKAN COUNTRIES: NEW DATA

Joanna R. Pieńkowska[1], Małgorzata Proćków[2], Marcin Górka[3], Andrzej Lesicki[1]*

[1]Department of Cell Biology, Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland (e-mail: pienkowj@amu.edu.pl, alesicki@amu.edu.pl)
[2]Museum of Natural History, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland (e-mail: malgorzata.prockow@uwr.edu.pl)
[3]Faculty of Geology, University of Warsaw, Żwirki i Wigury 93, 02-089 Warszawa, Poland (e-mail: magurka@uw.edu.pl)
*corresponding author

Abstract: *Monacha claustralis* (Rossmässler) and *M. cartusiana* (O. F. Müller) are frequently confused due to their great shell similarity. The only reliable identification of these species can be based on the genital and molecular characters. Based on sequences of two mitochondrial gene (*COI* and *16SrDNA*) fragments we identified the distribution of these species. *M. claustralis* was recognised as a species spreading northwards from its typical Turkish and Greek localities since its occurrence was discovered in Montenegro and Bosnia-Herzegovina on the basis of anatomical and molecular evidence. Moreover, its several new localities in Poland are reported. The occurrence of *M. cartusiana* is for the first time molecularly confirmed in Germany, Slovakia, Austria, Croatia and Kosovo, and some new localities are also reported from Poland, Hungary, and Bosnia-Herzegovina.

Key words: nucleotide sequences, *COI*, *16SrDNA*, *Monacha claustralis*, *Monacha cartusiana*, European distribution

INTRODUCTION

The hygromiid genus *Monacha* Fitzinger, 1833 includes ca. 100 species occurring in a large area extending from Western Europe to North Africa, Asian part of Turkey, the Caucasus, Iran and Arabia (Hausdorf 2000a, b, Hausdorf & Pall-Gergely 2009, Neubert & Bariche 2013, Neiber & Hausdorf 2017). In Europe, according to the maps in Kerney et al. (1983) and Welter-Schultes (2012), almost all but three species of this genus occupy rather narrow areas. These three taxa, included in the subgenus *Monacha* s. str. (Neiber & Hausdorf 2017), namely: *Monacha cartusiana* (O. F. Müller, 1774), the type species of the genus, *M. cantiana* (Montagu, 1803) and *M. claustralis* (Rossmässler, 1834), spread northwards from southern Europe. *M. cartusiana* can be found almost in the whole of Europe excluding its north-eastern fringes (Scandinavia, Russia, Baltic States, Belarus, N. Ukraine). *M. cantiana* occurs on the one hand in southern and central Britain (to Scotland), northern parts of France and Germany including the Benelux countries, and, on the other, in northern Spain, southern France, Italy and some Tyrrhenian islands (Elba, Corsica, Sardinia), with a gap in central France and Germany (Pieńkowska et al. 2018). *M. claustralis*, originally distributed in the European and Anatolian parts of Turkey (Hausdorf

Ministry of Science and Higher Education
Republic of Poland
Folia Malacologica is funded by the Ministry of Science and Higher Education, Republic of Poland under agreement no. 534/P-DUN/2018 allocated to the activities of disseminating science.

2000a) and Greece (with Corfu/Kerkyra as type locality, Welter-Schultes 2012), is now rapidly spreading northwards, having been found in chronological order in Bulgaria (initially recognised as *M. dissimulans*: Pintér 1968, later as *M. claustralis*: Irikov 2008, Georgiev 2016), Albania (as *M. dissimulans*: Dhora & Welter-Schultes 1996, Welter-Schultes 1996, Dhora 2009), Crimea (Hausdorf 2000a), Macedonia (Welter-Schultes 2012), and very recently in the Czech Republic (Pieńkowska et al. 2015, Korábek & Juřičková pers. com.) and Poland (Pieńkowska et al. 2015, 2016). It cannot be excluded that other *Monacha* species are spreading west-northward from their Mediterranean centres of origin (Neiber & Hausdorf 2017). For example, *Monacha ocellata* (Roth, 1839), known so far from West-Anatolian and European parts of Turkey and southern Bulgaria (Hausdorf 2000a, Irikov 2008, Welter-Schultes 2012), was recently discovered in Great Britain (Anderson et al. 2018).

Analysis of selected gene nucleotide sequences is nowadays frequently used for species classification, delimitation and identification (Falniowski & Wilke 2001, Hebert et al. 2003a, b, 2013, Manganelli et al. 2005, Szarowska 2006, Fiorentino et al. 2008, 2010, 2016, Sauer & Hausdorf 2009, 2012, Szarowska et al. 2014, Osikowski et al. 2015, 2017, Osikowski 2017, Neiber & Hausdorf 2015, 2017, Rysiewska et al. 2016) and makes it possible to verify species ranges and their distribution. Nucleotide sequences of cytochrome oxidase subunit I gene (*COI*) were mainly used in the above cited papers. However, *COI* sequences were originally used for recognition of cryptic species among lepidopterans (Hebert et al. 2003b), and were later recognised as 'barcode' sequences (Hebert et al. 2003a, 2013, Packer et al. 2009, Goldstein & DeSalle 2010) to resolve several taxonomic problems among gastropods, including species of the family Hygromiidae. For example, *COI* was, among other genes, used for species differentiation within the genus *Trochulus* (Duda et al. 2011, Proćków et al. 2013, 2014), and also for the analysis of bio- and phylogeographic aspects of hygromiids (Neiber & Hausdorf 2015, 2017, Neiber et al. 2017). The use of *COI* sequences allowed to re-identify *M. cantiana* from the Czech Republic (Hlaváč & Peltanová 2010) as *M. claustralis* (Pieńkowska et al. 2015, Korábek & Juřičková pers. com.). In Poland, *M. cartusiana*, initially reported from several localities (Cholewa et al. 2003, Lesicki & Koralewska-Batura 2007, Stworzewicz & Górka 2012), turned out to be *M. claustralis* based on molecular analyses (Pieńkowska et al. 2015, 2016). As stressed before, to solve taxonomic problems, molecular features should be integrated with morphological and anatomical data (Pieńkowska et al. 2016). Nevertheless, any new data obtained as a result of verification of species identification on the basis of molecular analysis, are worth publication. These data allow to assess distribution ranges of particular species and to track their possible migration routes. Such new data, presented in this paper, reveal the occurrence of *M. claustralis* and *M. cartusiana* in some new localities in Poland, Germany, Slovakia, Austria, Hungary and a few Balkan countries.

MATERIAL AND METHODS

Forty-nine specimens of *M. claustralis* and 52 specimens of *M. cartusiana* collected in 2014–2017 were used for anatomical and molecular examination. Their identification was confirmed based on the genitalia (Pieńkowska et al. 2015). Data on population localities, GenBank accession numbers, and the classification of the specimens used in this study are listed in Table 1. All voucher specimens preserved in 75% ethanol are deposited in the Department of Cell Biology Collection (DCBC), Adam Mickiewicz University, Poznań, Poland, except a specimen from Plovdiv kept in A. Irikov's collection (AIC), University of Plovdiv, Bulgaria.

Total genomic DNA was extracted from 20 mg of foot tissue using Tissue Genomic DNA extraction MiniKit (Genoplast) following the manufacturer's instructions. Partial sequences of mitochondrial *COI* (often called 'barcode sequence') and *16SrDNA* were amplified by polymerase chain reaction (PCR) using two degenerated primers for *COI*: F01 5'-CATTTTCHACTAAYCATAARGATATTGG-3' and R04 5'-TATAAACYTCDGGATGNCCAAAAAA-3' (Dabert et al. 2010), and two non-degenerated primers for *16SrDNA*: 5'-CGATTTGAACTCAGATCA-3' (LR-J-12887, Simon et al. 1994) and 5'-GTGCAAAGG-TAGCATAATCA-3' (Gantenbein et al. 1999).

Amplification of 650 bp long 'barcode sequence' was performed according to the modified protocol prepared by the Biodiversity Institute of Ontario for the Consortium for the Barcode of Life (http://barcoding.si.edu/PDF/Protocols_for_High_Volume_DNA_Barcode_Analysis.pdf). Reactions were carried out in a volume of 10 μl under the following thermal profile: 1 min at 94 °C followed by 42 cycles of 40 s at 94 °C, 40 s at 53 °C, 1 min at 72 °C, and finally 5 min at 72 °C. Amplification of about 370 bp long fragment of *16SrDNA* was conducted in a volume of 10 μl according to a previously described procedure (Manganelli et al. 2005). The PCR products were verified by agarose gel electrophoresis (1% agarose).

Table 1. List of localities of *Monacha claustralis* and *M. cartusiana* populations analysed in this paper

Localities			*Monacha* species	*COI*			*16SrDNA*		
coordinates	short description	collector name, date (no. of specimens)		new sequence	no. of specimens	GenBank ##	new sequence	no. of specimens	GenBank ##
Poland									
54°43'31.8"N 18°05'22.8"E	**Opalino n. Żarnowiec,** N. Poland, Pomorskie Province, dry meadow on sandy soil; ca. 1,000 m from hydroelectric power plant	A. Sulikowska-Drozd, 30.07.2014 (6)	*M. claustralis*	COI 1 COI 2	2 4	MH203899 MH203900 MH203904 MH203905 MH203906 MH203907	16S 1 16S 2	2 4	MH203999 MH204000 MH204004 MH204005 MH204006 MH204007
52°14'02.9"N 17°04'57.2"E	**Bnin n. Kórnik,** W. Poland, Wielkopolska Province, home garden, vegetation	J. R. Pieńkowska, 13.08.2017 (2)	*M. claustralis*	COI 3	2	MH203908 MH203909	16S 3	2	MH204008 MH204009
50°56'29"N 20°18'00"E	**Snochowice,** Świętokrzyskie Province, S. Poland, in abandoned gravel-pit, used as illegal garbage dump	M. Górka, 09.09.2014 (5)	*M. claustralis*	COI 3	5	MH203910 MH203911 MH203912 MH203913 MH203914	16S 3	5	MH204010 MH204011 MH204012 MH204013 MH204014
50°52'32"N 20°22'13"E	**Jeżynów n. Bławatków,** Świętokrzyskie Province, S. Poland, ditch and vegetation near local road	M. Górka, 07.09.2014 (5)	*M. claustralis*	COI 3	5	MH203915 MH203916 MH203917 MH203918 MH203919	16S 3	5	MH204015 MH204016 MH204017 MH204018 MH204019
50°39'01"N 20°35'19"E	**"Ślichowice" Reserve** Świętokrzyskie Province, S. Poland, vegetation along newly constructed gravel paths around the reserve	M. Górka, 30.08.2014 (5)	*M. claustralis*	COI 3	5	MH203920 MH203921 MH203922 MH203923 MH203924	16S 3	5	MH204020 MH204021 MH204022 MH204023 MH204024
50°44'40"N 20°28'45"E	**Wolica,** Świętokrzyskie Province, S. Poland, along recently renovated access road to unused quarry	M. Górka, 17.08.2016 (5)	*M. claustralis*	COI 3	5	MH203925 MH203926 MH203927 MH203928 MH203929	16S 3	5	MH204025 MH204026 MH204027 MH204028 MH204029

Table 1 continued

50°54'40"N 20°14'52"E	**Czartoszowy n. Łopuszno,** Świętokrzyskie Province, S. Poland. ditch near newly renovated busy road	M. GÓRKA, 04.08.2016 (5)	*M. claustralis*	COI 3	5	MH203930 MH203931 MH203932 MH203933 MH203934	16S 3	5	MH204030 MH204031 MH204032 MH204033 MH204034
50°51'17.0"N 20°38'24.4"E	**Kielce-Wietrznia,** Świętokrzyskie Province, S. Poland, old quarry on Wietrznia hill	M. GÓRKA, 30.05.2012 (5)	*M. claustralis*	COI 3	2	MH203935 MH203936	16S 3	2	MH204035 MH204036
			or *M. cartusiana*	COI 8	3	MH203948 MH203949 MH203950	16S 6	3	MH204046 MH204047 MH204048
50°51'34"N 20°35'34"E	**Kielce-Lidl,** Świętokrzyskie Province, S. Poland, on vegetation near parking of „Lidl" supermarket, between Kielce-Kraków road and railway	M. GÓRKA, 30.08.2014 (7)	*M. claustralis*	COI 3	1	MH203937	16S 3	1	MH204037
			or *M. cartusiana*	COI 8	6	MH203951 MH203952 MH203953 MH203954 MH203955 MH203956	16S 6	6	MH204049 MH204050 MH204051 MH204052 MH204053 MH204054
Montenegro									
42°13'00.3"N 18°58'43.8"E	**Petrovac n. Budva**, road to Lake Skadar, 685 m a.s.l.	J.R. PIEŃKOWSKA, 24.08.2014 (8)	*M. claustralis*	COI 4 COI 5 COI 6	1 1 6	MH203938 MH203939 MH203940 MH203941 MH203942 MH203943 MH203944 MH203945	16S 4 16S 5	5 2	MH204038 MH204039 MH204040 MH204041 MH204042 MH204044 MH204045
Bulgaria									
42°07'59.8"N 24°46'57.9"E	**Plovdiv**, Trakiya residential district, open, ruderal, xeromesothermic grass habitat; vast, undeveloped, secondary spaces between roads and block buildings; secondary vegetation of various grass types and bushes, 147 m a.s.l.	A. IRIKOV, 19.9.2006 (1)	*M. claustralis*	COI 7	1	MH203947			

Table 1 continued

Bosnia and Herzegovina									
44°19'03.2"N 17°08'58.7"E	**Jezero – Šipovo**, roadside along road between towns, 435 m a.s.l.	M. Proćków, BH.17.92, 28.08.2017 (2)	*M. claustralis*	COI 1	2	MH203901 MH203902	16S 1	2	MH204001 MH204002
44°34'41.3"N 17°07'56.3"E	**Krupa na Vrbasu n. Banja Luka,** roadside along Vrbas river, 232 m a.s.l.	M. Proćków, BH.17.89a, 28.08.2017 (1)	*M. claustralis*	COI 6	1	MH203946	16S 4	1	MH204043
44°07'38.5"N 17°52'59.2"E	**Kaonik n. Zenica**, meadow along river 379 m a.s.l.	M. Proćków, BH.17.76, 23.08.2017 (2)	*M. claustralis* or *M. cartusiana*	COI 1 COI 9	1 1	MH203903 MH203957	16S 1 16S 7	1 1	MH204003 MH204063
44°29'37.9"N 16°08'33.4"E	**Martin Brod**, National Park, vegetation along roadside, 232 m a.s.l.	M. Proćków, 23.09.2017 (1)	*M. cartusiana*	COI 10	1	MH203958	16S 6	1	MH204055
44°12'26.9"N 17°20'01.3"E	**Babin Potok**, vegetation along Vrbas river, 480 m a.s.l.	M. Proćków, BH.17.86, 27.08.2017 (4)	*M. cartusiana*	COI 11 COI 12 COI 13	1 2 1	MH203959 MH203963 MH203964 MH203965	16S 7 16S 8 16S 9	1 2 1	MH204064 MH204082 MH204083 MH204084
44°04'46.0"N 17°27'42.9"E	**Boganovci**, vegetation along roadside edge of village, 531 m a.s.l.	M. Proćków, BH.17.93, 29.08.2017 (3)	*M. cartusiana*	COI 11	3	MH203960 MH203961 MH203962	16S 7	3	MH204065 MH204066 MH204067
44°16'46.7"N 17°16'10.4"E	**Vinac n. Jajce**, meadow, 392 m a.s.l.	M. Proćków, BH.17.87, 27.08.2017 (1)	*M. cartusiana*	COI 13	1	MH203966	16S 9	1	MH204085
44°18'40.0"N 17°34'26.2"E	**Babanovac,** N slope near old ski jump, 1,251 m a.s.l.	M. Proćków, BH.17.78, 24.08.2017 (2)	*M. cartusiana*	COI 14	2	MH203969 MH203970	16S 7	2	MH204068 MH204069
Kosovo									
42°44'00.1"N 20°05'39.1"E	**Košutane**, xerothermic grassland, under stones, 1479 m a.s.l.	M. Proćków, 07.08.2017 (4)	*M. cartusiana*	COI 15 COI 16	3 1	MH203971 MH203973 MH203972 MH203974	16S 10 16S 11	1 3	MH204086 MH204087 MH204088 MH204089
Croatia									
45°58'57.0"N 15°57'16.0"E	**Donja Stubica**, edge of shrubs, 201 m a.s.l.	M. Proćków, HR.17.93, 17.09.2017 (1)	*M. cartusiana*	COI 17	1	MH203975	16S 12	1	MH204090

Table 1 continued

Hungary									
46°37'37.9"N 19°30'05.3"E	**Bočsa**, vegetation near field road	J.R. PIEŃKOWSKA, 21.08.2015 (7)	*M. cartusiana*	COI 18	2	MH203976	16S 6	7	MH204056
						MH203977			MH204057
				COI 19	2	MH203978			MH204058
						MH203979			MH204059
				COI 20	1	MH203980			MH204060
				COI 21	1	MH203981			MH204061
									MH204062
Austria									
48°16'50.5"N 16°54'50.4"E	**Marchegg,** vegetation along March river, 131 m a.s.l.	M. PROĆKÓW, A.17.32, 24.06.2017 (5)	*M. cartusiana*	COI 22	2	MH203982	16S 13	3	MH204091
						MH203983			MH204092
				COI 23	2	MH203984			MH204093
						MH203985	16S 14	2	MH204094
				COI 24	1	MH203986			MH204095
Slovakia									
47°56'N 18°38'E	**Čata**, open grassland strip, 124 m, a.s.l.	P. W. WHITEHEAD, 2015 (3)	*M. cartusiana*	COI 25	3	MH203987	16S 15	3	MH204096
						MH203988			MH204097
						MH203989			MH204098
Germany									
50°53'49.2"N 07°00'36.0"E	**Cologne-Westhoven**, Rhine banks	H. KAPPES, 14.08.2016 (6)	*M. cartusiana*	COI 13	2	MH203967	16S 7	6	MH204070
						MH203968			MH204071
				COI 26	2	MH203990			MH204072
						MH203991			MH204073
				COI 27	1	MH203992			MH204074
									MH204075
50°42'50.4"N 07°07'26.4"E	**Bonn**, construction area opposite Bundeskunsthalle	H. KAPPES, 7.08.2016 (5)	*M. cartusiana*	COI 28	5	MH203993	16S 7	5	MH204076
						MH203994			MH204077
						MH203995			MH204078
						MH203996			MH204079
						MH203997			MH204080
52°19'28.4"N 09°57'51.3"E	**Sehnde**, Region Hannover, Lower Saxony; pedestrian subway under train line near cemetery, 65 m a.s.l.	M. NEIBER, 07.10.2017 (1)	*M. cartusiana*	COI 29	1	MH203998	16S 7	1	MH204081

All collected specimens are deposited in the Department of Cell Biology Collection (DCBC), Adam Mickiewicz University, Poznań, Poland (except a specimen from Plovdiv kept in A. IRIKOV collection (AIC), University of Plovdiv, Bulgaria).

Prior to sequencing, to improve its quality, the PCR products of *COI* were purified with thermosensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Scientific). Amplified fragments of *16SrDNA* were much shorter than the fragments of *COI* and sufficiently pure, therefore they did not require additional purification. Properly prepared PCR products were sequenced bidirectionally with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocols. All obtained sequences were deposited in GenBank (Table 1).

Sequences were aligned and edited by eye using the programme BioEdit, version 7.0.5. (Hall 1999). The alignments were performed using the Clustal W programme (Thompson et al. 1994) implemented in BioEdit. The *COI* sequences were aligned according to the translated amino acid sequences. The ends of all sequences were trimmed to 544 bp for *COI* and 288 positions for *16SrDNA*. The sequences were collapsed to haplotypes using the programme ALTER (Alignment Transformation EnviRonment) (Glez-Peña et al. 2010).

The following *COI* sequences from GenBank were used: KM247383–KM247388 (Pieńkowska et al. 2015), KX258308, KX258369, KX258374, KX258390 (Pieńkowska et al. 2016), KX507199 (Neiber & Hausdorf 2015) of *M. claustralis*, KF986836 (Dahirel et al. 2015), KM247376–KM247380, KM247382, KM247389 (Pieńkowska et al. 2015), KX258393 (Pieńkowska et al. 2016), KX258403, KX258407, KX258410–KX258411, KX258415, KX258417–KX258418 (Pieńkowska et al. 2016), KX507189, KX507235 (Neiber & Hausdorf 2015, 2017) of *M. cartusiana* and KM247375 (Pieńkowska et al. 2015) of *M. cantiana* (as outgroup). The following *16SrDNA* sequences from GenBank were used: KM247392–KM247396 (Pieńkowska et al. 2015), KX258234, KX258258, KX258260, KX258267, KX258275, KX258287 (Pieńkowska et al. 2016), KX495388 (Neiber & Hausdorf 2015) of *M. claustralis*, AY741416 (Manganelli et al. 2005), KJ458540 (Razkin et al. 2014), KM247391, KM247397 (Pieńkowska et al. 2015), KX258288, KX258292, KX258302, KX258304–KX258305, KX258307 (Pieńkowska et al. 2016), KX495378, KX495429 (Neiber & Hausdorf 2015) of *M. cartusiana* and KM247390 (Pieńkowska et al. 2015) of *M. cantiana* (as outgroup). The genetic distances between the above GenBank sequences and these obtained in this study (Table 1) were analysed by Neighbour-Joining method (Saitou & Nei 1987) included in MEGA7 (Kumar et al. 2016) using the Kimura two-parameter model (K2P) for pairwise distance calculations (Kimura 1980). An NJ tree credibility was tested by bootstrap analysis with 1,000 replicates (Felsenstein 1985).

The Bayesian analysis of combined *COI* and *16SrDNA* sequences (together 832 positions – 544 of *COI* + 288 of *16SrDNA*) was conducted with the programme MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). HKY substitution model (Hasegawa et al. 1985), assuming a gamma distributed rate variation among sites was found as the best-fit substitution model (ML) for our data set according to the Bayesian Information Criterion (BIC). Best-fit substitution model was calculated using algorithm implemented in MEGA 7. Four Monte Carlo Markov chains were run for 1 million generations, sampling every 100 generations (the first 250,000 trees were discarded as 'burn-in'). This gave us a 50% majority rule consensus tree. At the same time, Maximum Likelihood (ML) analysis was performed with MEGA7 and calculated bootstrap values were placed on the 50% majority rule consensus Bayesian tree.

RESULTS

Twenty nine haplotypes (COI 1 – COI 29) of *COI* and fifteen haplotypes (16S 1 – 16S 15) of *16SrDNA* mitochondrial gene fragments were found in molecular analysis of DNA extracted from 101 specimens (Table 1, Figs 1 & 2).

The sequence of haplotype COI 1, found in specimens from N. Poland (Opalino) and Bosnia-Herzegovina (Jezero-Šipovo and Kaonik), was exactly the same as sequences KX258390–KX258392 deposited in GenBank by Pieńkowska et al. (2016) for *M. claustralis* from Krokowa in N. Poland. Similarly, the COI 2 sequence, obtained from other specimens collected in Opalino, was the same as sequences KX258374–KX258389 isolated from *M. claustralis* from Jastrzębia Góra, Nadole, Chłapowo and Krokowa (all N. Poland) (Pieńkowska et al. 2016). The sequence of haplotype COI 3 found in several Polish populations (Bnin nr. Kórnik, Snochowice, Jeżynów, Ślichowice Reserve, Wolica, Czartoszowy, Kielce-Wietrznia, Kielce-Lidl) was the same as *COI* sequences KM247385 and KX258308–KX258350 deposited by Pieńkowska et al. (2015) and Pieńkowska et al. (2016), respectively, for *M. claustralis* from different populations of western and southern Poland. Finally, the sequence of haplotype COI 7 from Plovdiv (Bulgaria) was the same as KM247387 deposited in GenBank by Pieńkowska et al. (2015). Similar results were obtained for *16SrDNA* haplotypes. The sequence of haplotype 16S 1 (Opalino, Jezero-Šipovo and Kaonik) was exactly the same as

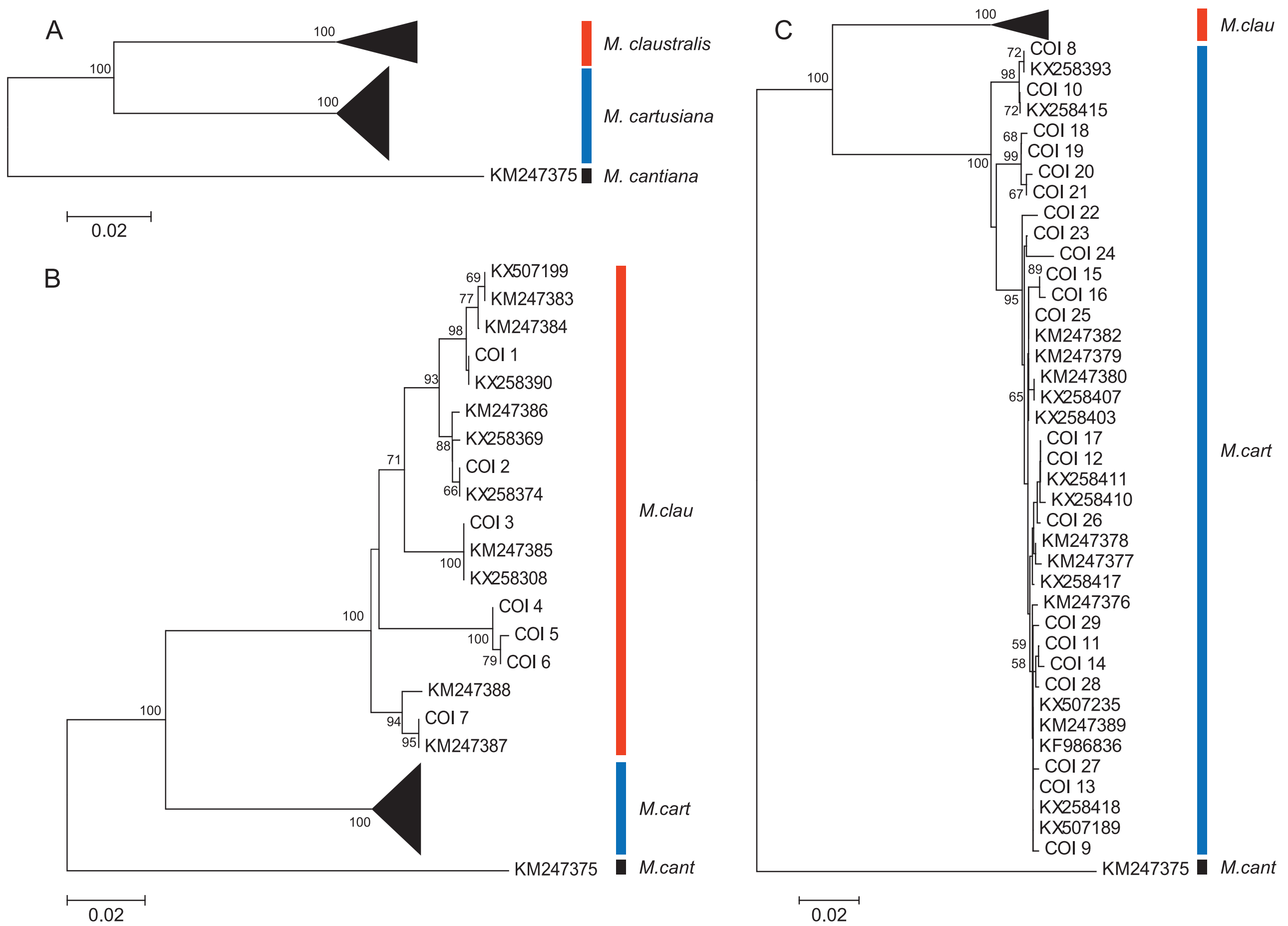


Fig. 1. Neighbour-Joining tree based on the 544-nt-long fragment of new *COI* sequences of *M. claustralis* and *M. cartusiana* (own data compared with GenBank sequences): A – general view, B – *M. claustralis* haplotypes, C – *M. cartusiana* haplotypes. The *COI* sequence of *M. cantiana* KM247375 was chosen as outgroup, and GenBank sequences of *M. claustralis* KM247383-KM247388, KX258308, KX258369, KX258374, KX258390, KX507199 and of *M. cartusiana* KF9868236, KM247376-KM247380, KM247382, KM247389, KX258393, KX258403, KX258407, KX258410, KX258411, KX258415, KX258417, KX258418, KX507189, KX507235 were used as references. Numbers on branches represent bootstrap support above 50%. The evolutionary distances were computed using the Kimura two-parameter method and are expressed as of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option)

sequences KM247393 and KX495388 deposited in GenBank for *M. claustralis* from Saguramo, Georgia by Pieńkowska et al. (2015) and Neiber & Hausdorf (2015, 2017), respectively. It was also the same as GenBank sequence KX258267 of *M. claustralis* from Jędrzejów, S. Poland (Pieńkowska et al. 2016). The haplotype 16S 2 (found in specimens from Opalino) did not differ from sequences KX258260–KX258266, KX258275-KX258287 deposited in GenBank by Pieńkowska et al. (2016) for *M. claustralis* from several Polish populations (N. Poland: Jastrzębia Góra, Nadole, Chłapowo, Krokowa and S. Poland: Małogoszcz and Jędrzejów). The haplotype 16S 3, found in several *M. claustralis* populations from W. (Bnin) and S. (Snochowice, Jeżynów, Ślichowice, Wolica, Czartoszowy, Kielce-Wietrznia) Poland, was the same as GenBank sequences KM247396 (from Poznań-Cybina and Poznań-Morasko, W. Poland) and KX258234–KX258257 (from Kielce-Na Ługach, Kielce-Białogon, Jaworznia, Morawka, Morawica, Sobków, Kielce-Grzybowa, all from S. Poland, and from Poznań-Wola, Rogalinek, Puszczykowo in W. Poland) deposited in GenBank by Pieńkowska et al. (2015, 2016). These results allow to identify unequivocally the populations with haplotypes COI 1 – COI 3, COI 7 and 16S 1 –16S 3 as populations of *M. claustralis*. Several haplotypes (COI 4 – COI 6, 16S 4 & 16S 5) originating from Petrovac in Montenegro were new, i.e. never found previously. However, in the NJ dendrograms constructed for *COI* (Fig. 1B) and *16SrDNA* (Fig. 2B) sequences they clustered together with the sequences characteristic of *M. claustralis*, both the sequences found in this study (COI 1 – COI 7 and 16S 1 – 16S 5) and those deposited in GenBank (Pieńkowska et al. 2015, 2016, Neiber & Hausdorf 2015, 2017 – *COI*: KM247383–KM247388, KX258308, KX258369, KX258374, KX258390, KX507199; *16SrDNA*: KM247392–

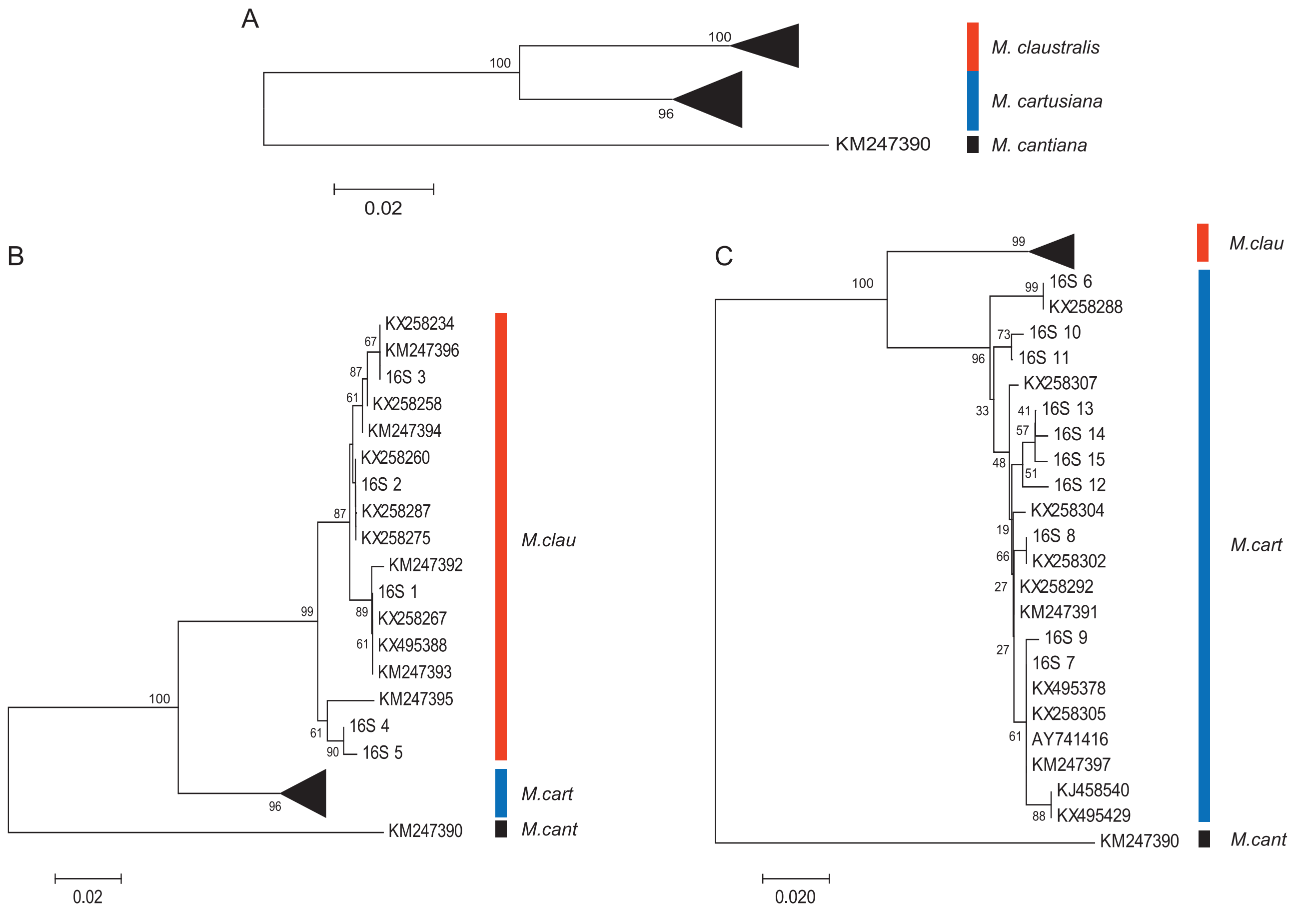


Fig. 2. Neighbour-Joining tree of new *16SrDNA* sequences of *M. claustralis* and *M. cartusiana* (own data compared with GenBank sequences): A – general view, B – *M. claustralis* haplotypes, C – *M. cartusiana* haplotypes. The *16SrDNA* sequence of *M. cantiana* KM247390 was chosen as outgroup. GenBank sequences of *M. claustralis* KM247392-KM247396, KX258234, KX258258, KX258260, KX258267, KX258275, KX258287, KX495388 and *M. cartusiana* AY741416, KJ458540, KM247391, KM247397, KX258288, KX258292, KX258302, KX258304, KX258305, KX258307, KX495378 and KX495429 were used as references. Calculation parameters were the same as for Fig. 1

KM247396, KX258234, KX258258, KX258260, KX258267, KX258275, KX495388). The attribution of the populations with haplotypes COI 1 – COI 7 and 16S 1 – 16S 5 to *M. claustralis* is also confirmed by K2P distances (Table 2).

All other haplotypes recognised in this study, COI 8 – COI 29 and 16S 6 – 16S 15, were identified as belonging to *M. cartusiana*. They form a well separated clade in particular NJ dendrograms, with strong support of bootstrap values of 100% (Figs 1C & 2C). K2P distances among sequences of these haplotypes are much smaller (*COI* 0.2–3.3%, *16SrDNA* 0.0–3.4%) than between them and the sequences for *M. claustralis* (*COI* 12.5–15.7%, *16SrDNA* 8.8–11.4%) and *M. cantiana* (*COI* 19.3–20.5%, *16SrDNA* 19.2–21.6%) (Table 2). Several *COI* and *16SrDNA* sequences deposited in GenBank for *M. cartusiana* by Manganelli et al. (2005 – *16SrDNA*: AY741416), Dahirel et al. (2015 – *COI*: KF986836), Pieńkowska et al. (2015 – *COI*: KM247376–KM247380, KM247382, KM247389, *16SrDNA*: KM247391, KM247397; 2016 – *COI*: KX258393, KX258403, KX258407, KX258410, KX258411, KX258415, KX258417, KX258418, *16SrDNA*: KX258288, KX258292, KX258302,

Table 2. Ranges of K2P genetic distances for analysed *COI* and *16SrDNA* sequences

Comparison	*COI* (%)	*16SrDNA* (%)
Within *M. claustralis*	0.0–5.7	0.0–3.9
Within *M. cartusiana*	0.2–3.3	0.0–3.4
Between *M. claustralis* and *M. cartusiana*	12.5–15.7	8.8–11.4
Between *M. claustralis* and *M. cantiana*	19.2–23.1	21.2–23.5
Between *M. cartusiana* and *M. cantiana*	19.3–20.5	19.2–21.6

K2P distances were calculated for the new sequences obtained in this paper (Table 1) and those from GenBank (see: Material and methods). K2P distances within *M. cantiana* were not counted because singular *M. cantiana* sequences were used for the calculations.

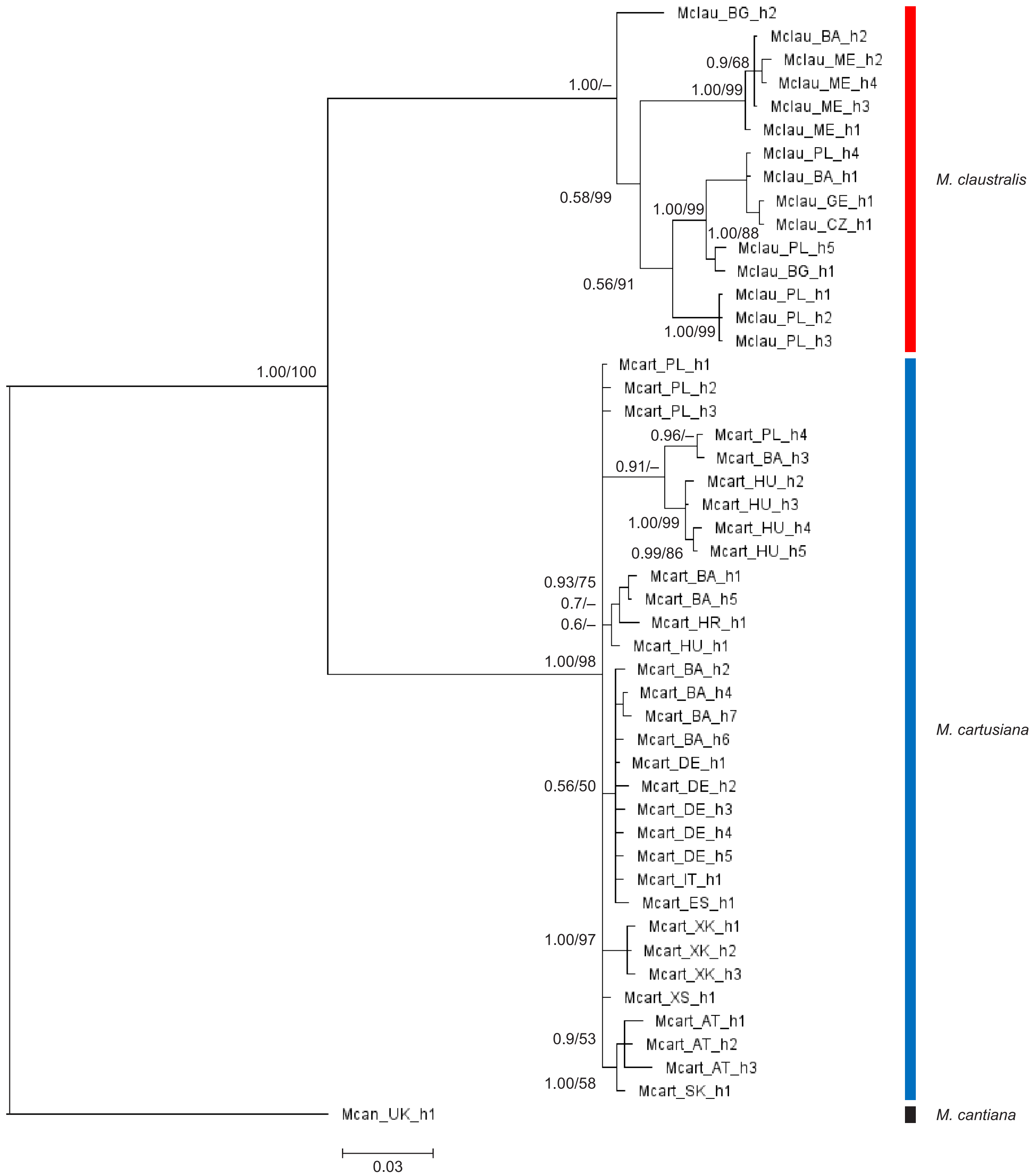
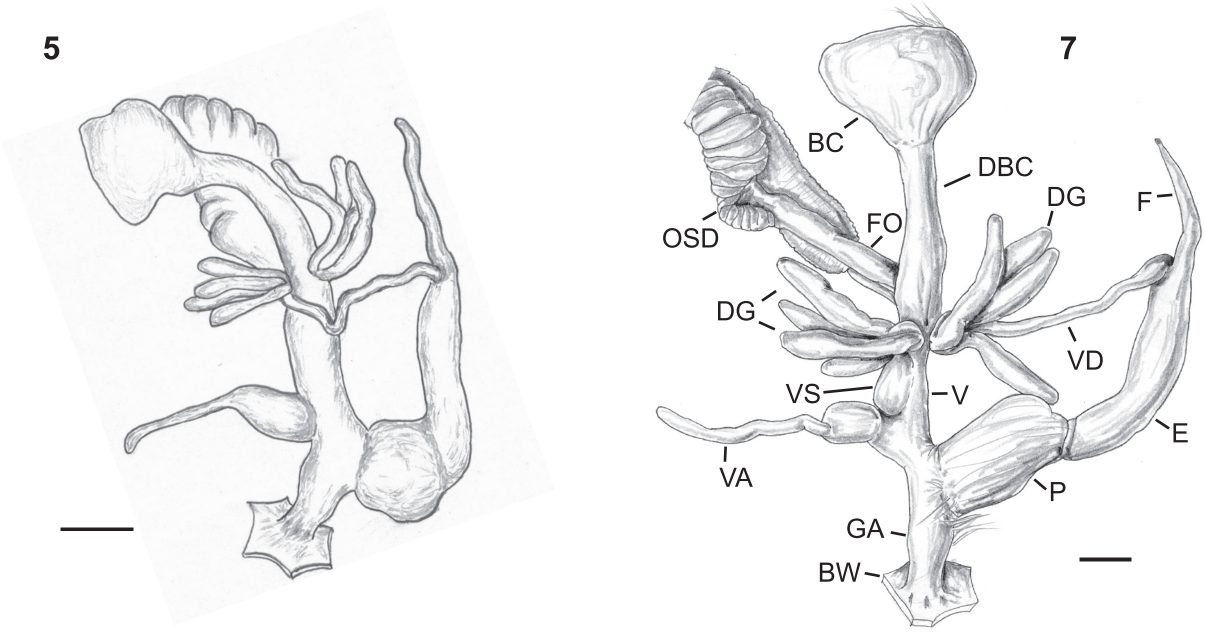


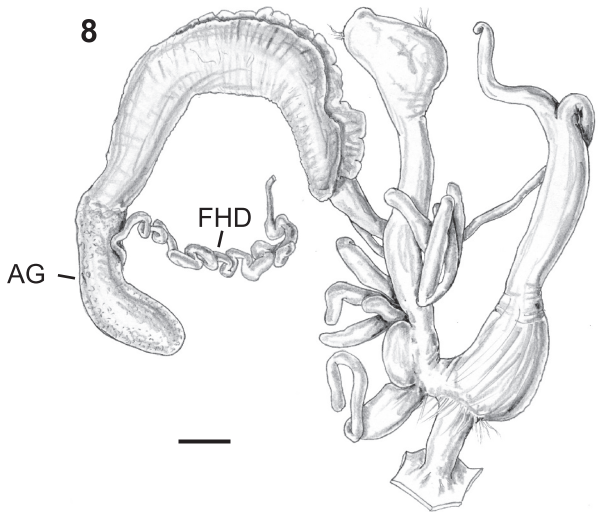
Fig. 3. Majority-rule consensus tree obtained from Bayesian inference analysis of the combined data set of *COI* and *16SrDNA* sequences of *M. claustralis* and *M. cartusiana* (Table 3). Posterior probabilities (left) and bootstrap support above 50% from maximum likelihood analysis (right) are marked at the nodes. Bootstrap analysis was run with 1,000 replicates (Felsenstein 1985). The tree was rooted with *M. cantiana*

KX258304, KX258305, KX258307), Razkin et al. (2015 – *16SrDNA*: KJ458540), Neiber & Hausdorf (2017 – *COI*: KX507189, KX507235, *16SrDNA*: KX495378, KX495429) are clustered within the same clade. Moreover, the sequence of COI 8 from some Kielce populations is exactly the same as sequences KX258393–KX258397 deposited in GenBank for *M. cartusiana* from a different population in Kielce (Pieńkowska et al. 2016). It is noteworthy that there are no differences between the haplotype sequences found in this paper and those deposited in GenBank for *M. cartusiana* by Pieńkowska et al. (2016): COI 10 from Martin Brod (Bosnia-Herzegovina) and KX258415 from Belgrade (Serbia), COI 12 from Babin Potok (Bosnia-Herzegovina) and KX258411–KX258414 from Gorni Vakuf-Uskoplje

Table 3. Combined *COI* and *16SrDNA* datasets for the analysed *Monacha* species

Combined haplotypes	*COI*	*16SrDNA*	Locality (reference)
Monacha claustralis			
Mclau-PL-h1	KM247385	KM247396	Poland: Poznań, Wietrznia (Pieńkowska et al. 2015)
Mclau-PL-h2	KX258308	KX258234	Poland: Kielce, Jaworznia, Morawka, Morawica, Sobków, Rogalinek, Puszczykowo (Pieńkowska et al. 2016)
Mclau-PL-h3	COI 3	16S 3	Poland: Snochowice, Jeżynów, Slichowice, Wolica, Czartoszowy, Kielce, Bnin (this paper)
Mclau-PL-h4	COI 1	16S 1	Poland: Opalino (this paper)
Mclau-PL-h5	COI 2	16S 2	Poland: Opalino (this paper)
Mclau-BA-h1	COI 1	16S 1	Bosnia-Herzegovina: Šipovo, Kaonik (this paper)
Mclau-BA-h2	COI 6	16S 4	Bosnia-Herzegovina: Vrbas (this paper)
Mclau-ME-h1	COI 4	16S 4	Montenegro: Petrovac (this paper)
Mclau-ME-h2	COI 5	16S 5	Montenegro: Petrovac (this paper)
Mclau-ME-h3	COI 6	16S 4	Montenegro: Petrovac (this paper)
Mclau-ME-h4	COI 6	16S 5	Montenegro: Petrovac (this paper)
Mclau-GE-h1	KX507199	KX495388	Georgia: Saguramo (Neiber & Hausdorf 2015)
Mclau-CZ-h1	KM247383	KM247393	Czech Republic: Prague (Pieńkowska et al. 2015)
Mclau-BG-h1	KM247386	KM247394	Bulgaria: Plovdiv (Pieńkowska et al. 2015)
Mclau-BG-h2	KM247387	KM247395	Bulgaria: Plovdiv (Pieńkowska et al. 2015)
Monacha cartusiana			
Mcart-PL-h1	KM247379	KM247391	Poland: Wrocław (Pieńkowska et al. 2015)
Mcart-PL-h2	KX258398	KX258292	Poland: Ostrowiec (Pieńkowska et al. 2016)
Mcart-PL-h3	KX258407	KX258297	Poland: Wrocław (Pieńkowska et al. 2016)
Mcart-PL-h4	COI 7	16S 6	Poland: Kielce (this paper)
Mcart-BA-h1	KX258410	KX258302	Bosnia-Herzegovina: Gornji Vakuf-Uskoplje (Pieńkowska et al. 2016)
Mcart-BA-h2	COI 8	16S 7	Bosnia-Herzegovina: Kaonik (this paper)
Mcart-BA-h3	COI 9	16S 6	Bosnia-Herzegovina: Martin-Brod (this paper)
Mcart-BA-h4	COI 10	16S 7	Bosnia-Herzegovina: Martin Potok, Boganovci (this paper)
Mcart-BA-h5	COI 11	16S 8	Bosnia-Herzegovina: Martin Potok (this paper)
Mcart-BA-h6	COI 12	16S 9	Bosnia-Herzegovina: Martin Potok, Jajce (this paper)
Mcart-BA-h7	COI 13	16S 7	Bosnia-Herzegovina: Babanovac (this paper)
Mcart-XK-h1	COI 14	16S 10	Kosovo: Košutane (this paper)
Mcart-XK-h2	COI 14	16S 11	Kosovo: Košutane (this paper)
Mcart-XK-h3	COI 15	16S 11	Kosovo: Košutane (this paper)
Mcart-HR-h1	COI 16	16S 12	Croatia: Donja Stubica (this paper)
Mcart-XS-h1	KX258405	KX258304	Serbia: Belgrade (Pieńkowska et al. 2016)
Mcart-HU-h1	KM247377	KM247391	Hungary: Kis Balaton (Pieńkowska et al. 2015)
Mcart-HU-h2	COI 17	16S 6	Hungary: Bočsa (this paper)
Mcart-HU-h3	COI 18	16S 6	Hungary: Bočsa (this paper)
Mcart-HU-h4	COI 19	16S 6	Hungary: Bočsa (this paper)
Mcart-HU-h5	COI 20	16S 6	Hungary: Bočsa (this paper)
Mcart-AT-h1	COI 21	16S 13	Austria: Marchegg (this paper)
Mcart-AT-h2	COI 22	16S 14	Austria: Marchegg (this paper)
Mcart-AT-h3	COI 23	16S 13	Austria: Marchegg (this paper)
Mcart-SK-h1	COI 24	16S 15	Slovakia: Čata (this paper)
Mcart-DE-h1	COI 12	16S 7	Germany: Cologne-Westhoven (this paper)
Mcart-DE-h2	COI 25	16S 7	Germany: Cologne-Westhoven (this paper)
Mcart-DE-h3	COI 26	16S 7	Germany: Cologne-Westhoven (this paper)
Mcart-DE-h4	COI 27	16S 7	Germany: Bonn (this paper)
Mcart-DE-h5	COI 28	16S 7	Germany: Sehnde (this paper)
Mcart-IT-h1	KM247389	KM247397	Italy: Brescia (Pieńkowska et al. 2015)
Mcart-ES-h1	KX507235	KX495429	Spain: Castilla-La Mancha (Neiber & Hausdorf 2015)
Monacha cantiana (as outgroup)			
Mcan-UK-h1	KM247375	KM247390	England: East Acton & Barrow (Pieńkowska et al. 2015)





Figs 4–8. Distal genitalia of: 4 – *M. claustralis* from Petrovac near Budva (Montenegro), leg. J. PIEŃKOWSKA, 24.08.2014; 5 – *M. claustralis* from Snochowice (Poland), leg. M. GÓRKA, 09.09.2014 (drawing by A. LESICKI); 6 – *M. cartusiana* from Bonn (Germany), leg. H. KAPPES, 7.08.2016; 7–8 – *M. cartusiana* from Košutane (Kosovo), leg. M. PROĆKOW, 07.08.2017 (drawings by F. GIUSTI & G. MANGANELLI). Abbreviations: AG – albumen gland, BC – bursa copulatrix, BW – body wall, DBC – duct of bursa copulatrix, DG – digitiform glands, E – epiphallus (from base of flagellum to beginning of penial sheath), F – flagellum, FHD – first hermaphrodite duct, FO – free oviduct, GA – genital atrium, OSD – ovispermiduct, P – penis, V – vagina, VA – vaginal appendix (appendiculum), VD – vas deferens, VS – vaginal sac. The diagnostic characters are shown: long vagina without vaginal sac in *M. claustralis* (4) and short vagina with vaginal sac in *M. cartusiana* (6). Scale bar – 2 mm

(Bosnia-Herzegovina), COI 13 from Babin Potok and Vinac near Jajce (Bosnia-Herzegovina) as well as from Cologne (Germany) and KX258418 from Belgrade (Serbia). Finally, the sequence of haplotype COI 25 from Čata (Slovakia) is the same as sequences KM247379 and KX258403–KX258406 deposited in GenBank for *M. cartusiana* from Wrocław (Poland) and Belgrade (Serbia) (Pieńkowska et al. 2015, 2016). The haplotype sequence of 16S 6 from Kielce-Wietrznia and Kielce-Lidl (S. Poland), Bočsa (Hungary) and Martin-Brod (Bosnia-Herzegovina) is exactly the same as GenBank sequences KX258288–KX258291 of *M. cartusiana* from Kielce (S. Poland) and Belgrade (Serbia) (Pieńkowska et al. 2016). The sequence of 16S 7 from Kaonik, Babin Potok, Boganovci, Babanovac (Bosnia-Herzegovina), and from Cologne-Westhoven, Bonn and Sehnde (Germany) is identical to GenBank *M. cartusiana* sequences AY741416 from Siena, Italy (Manganelli et al. 2005), KM247397 and KX495378 from Brescia, Italy (Pieńkowska et al. 2015 and Neiber & Hausdorf 2017) as well as KX258305-KX258306 from Belgrade, Serbia (Pieńkowska et al. 2016). The sequence 16S 8 from Babin Potok (Bosnia-Herzegovina) is the same as sequences KX258302–KX258303 of *M. cartusiana* from Gornji Vakuf-Uskoplje (Bosnia-Herzegovina) (Pieńkowska et al. 2016). All these results indicate that all haplotypes of the second clade in Figs 1 & 2 represent *M. cartusiana*.

The following *M. cartusiana COI* haplotypes were not found earlier: COI 9 from Kaonik (Bosnia-Herzegovina), COI 11, COI 14 from several populations in Bosnia-Herzegovina (Babin Potok, Boganovci, Babanovac), COI 15 and COI 16 from Košutane (Kosovo), COI 17 from Donja Stubica (Croatia), COI 18 – COI 21 from Bočsa (Hungary), COI 22 – COI 24 from Marchegg (Austria), COI 26 – COI 27 from Cologne (Germany), COI 28 from Bonn (Germany) and COI 29 from Sehnde (Germany). Likewise, several *16SrDNA* haplotypes were never found before: 16S 9 from Vinac near Jajce and Babin Potok (Bosnia-Herzegovina), 16S 10 and 16S 11 from Košutane (Kosovo), 16S 12 from Donja Stubica (Croatia), 16S 13 and 16S 14 from Marchegg (Austria) and 16S 15 from Čata (Slovakia).

The above identification of *M. claustralis* and *M. cartusiana* was confirmed in the Bayesian phylogenetic tree (Fig. 3) based on combined haplotypes of *COI* and *16SrDNA* gene sequences (Table 3).

Specimens from each population were also identified on the basis of their genital structure. Examples of the genitalia of representative specimens of *M. claustralis* and *M. cartusiana* are shown in Figs 4–5 and 6–8, respectively.

DISCUSSION

Our analyses of *COI* and *16SrDNA* gene nucleotide sequences support the hypothesis (Pieńkowska et al. 2016) that *M. claustralis* is rapidly spreading northward (Fig. 9). Originally it was reported from Turkey, Greece, Albania, Macedonia and Bulgaria (Hausdorf 2000a, Irikov 2008, Dhora & Welter-Schultes 1996, Welter-Schultes 1996, Dhora 2009, Georgiev 2016, see also distribution map in Welter-Schultes 2012), later its occurrence was confirmed anatomically and molecularly in the Czech Republic and Poland (Pieńkowska et al. 2015, 2016). Now *M. claustralis* is for the first time confirmed molecularly from Montenegro (one locality) as well as Bosnia-Herzegovina (three localities) (Table 1, Fig. 9). Moreover, nine new populations were found in Poland, i.e. in Świętokrzyskie Province, S. Poland (Snochowice, Jeżynów, Ślichowice, Wolica, Czartoszowy, Kielce-Wietrznia, Kielce-Lidl), in Wielkopolska Province, W. Poland (Bnin near Kórnik) and in Pomorskie Province, N. Poland (Opalino near Żarnowiec) (Table 1, Fig. 9). These data show that *M. claustralis* is now widely distributed in Poland.

The genetic distances within *M. claustralis* are relatively large (Table 2: *COI* 0.0–5.7%, *16SrDNA* 0.0–3.9%), however, it is noteworthy that extreme distances refer only to a few specific populations. One is the population from Plovdiv, Bulgaria – only the *COI* sequence KM247386 (Pieńkowska et al. 2015) is the same as *COI* sequences KX258308–KX258350 (Pieńkowska et al. 2016) and COI 3 (this paper) from several populations from W. and S. Poland. Three other *COI* sequences: COI 7 and KM247387–KM247388 (Pieńkowska et al. 2015), found in the Plovdiv population, are either the same (COI 7 and KM247387) or very similar (COI 7 and KM247388 – K2P 1.0%) but differ at the level of 3.3–5.2% from all other populations. Similar results refer to the *16SrDNA* sequence KM247395 (Pieńkowska et al. 2015) and the combined sequence Mclau-BG-h2 which are different from the other studied sequences (Figs 2C & 3). Because several *Monacha* species inhabiting Bulgaria (Irikov 2008, Georgiev 2016) are difficult to identify based only on conchology, molecular research is necessary to resolve their relationships. Such investigations are under way (Georgiev pers. comm.).

Besides, the COI 4 – COI 6 sequences, obtained in this study for specimens from Montenegro, also differ from all other analysed sequences of *M. claustralis* at the level of 4.6–5.7%. They are also different (4.6–5.2%) from *COI* sequences KM247387–KM247388 from Bulgaria. Moreover, *16SrDNA*

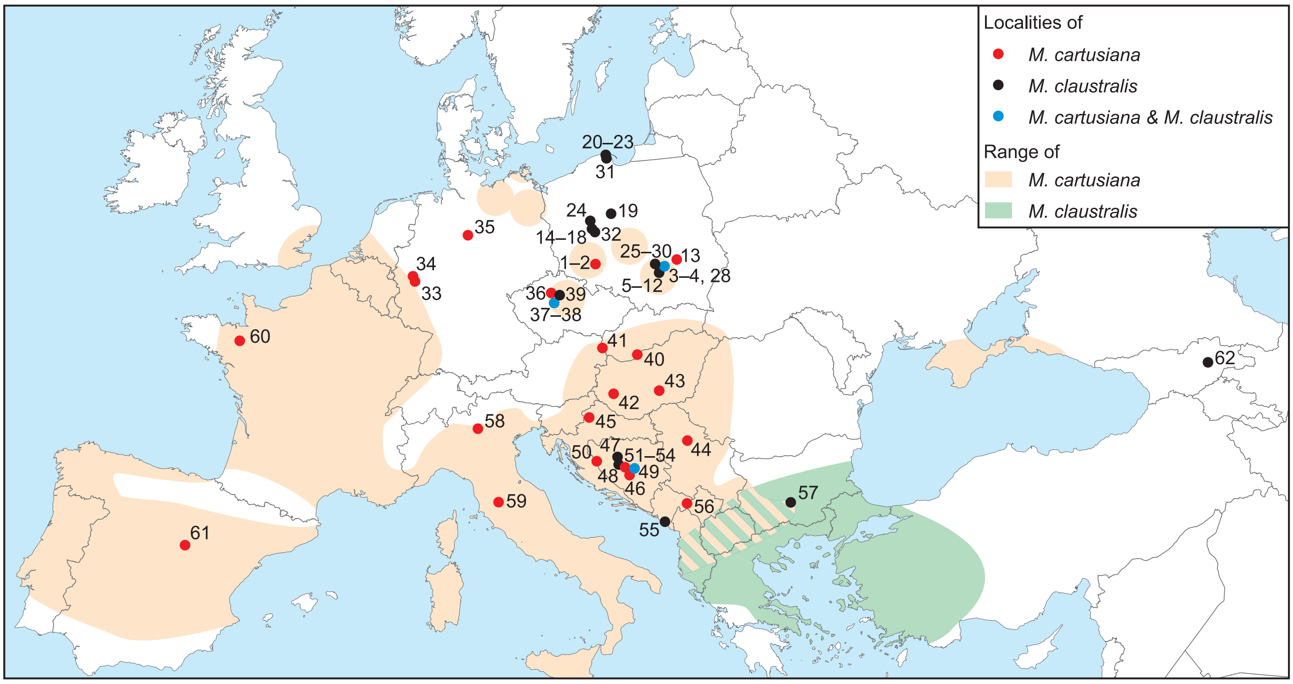


Fig. 9. European distribution of *M. claustralis* and *M. cartusiana* verified molecularly: Poland (1 – Wrocław-Pilczyce; 2 – Wrocław-Legnicka; 3 – Kielce-Wietrznia; 4 – Kielce-Grzybowa; 5 – Kielce-Na Ługach; 6 – Kielce-Białogon; 7 – Jaworznia near Kielce; 8 – Morawka near Kielce; 9 – Morawica near Kielce; 10 – Sobków near Jędrzejów; 11 – Jędrzejów; 12 – Małogoszcz; 13 – Ostrowiec Świętokrzyski; 14 – Poznań-Cybina; 15 – Poznań-Morasko; 16 – Poznań-Wola; 17 – Puszczykowo near Poznań; 18 – Rogalinek near Poznań; 19 – Janikowo; 20 – Nadole; 21 – Krokowa, 22 – Chłapowo; 23 – Jastrzębia Góra; 24 – Murowana Goślina; 25 – Snochowice; 26 – Jeżynów near Bławatków; 27 – "Ślichowice" Reserve; 28 – Kielce-Lidl; 29 – Wolica; 30 – Czartoszowy near Łopuszno; 31 – Opalino near Żarnowiec; 32 – Bnin near Kórnik); Germany (33 – Bonn; 34 – Cologne-Westhoven; 35 – Sehnde near Hannover); Czech Republic (36–39 – Prague); Slovakia (40 – Čata); Austria (41 – Marchegg); Hungary (42 – Kis-Balaton; 43 – Bočsa); Serbia (44 – Belgrade); Croatia (45 – Donja Stubica); Bosnia and Herzegovina (46 – Gornji Vakuf-Uskoplje; 47 – Jezero-Šipovo; 48 – Krupa na Vrbasu near Banja Luka; 49 – Kaonik near Zenica; 50 – Martin Brod; 51 – Babin Potok; 52 – Boganovci; 53 – Vinac near Jajce; 54 – Babanovac); Montenegro (55 – Petrovac near Budva); Kosovo (56 – Košutane); Bulgaria (57 – Plovdiv, Trakiya); Italy (58 – Brescia, Anfo towards Ponte Caffaro; 59 – Villa Medane, Siena); France (60 – Rennes); Spain (61 – Castilla-La Mancha, Cañon del Río Dulce); Georgia (62 – Mtskheta-Mtianeti, SE of Saguramo). For short descriptions of localities see: Pieńkowska et al. (2015: localities nos. 2, 3, 14, 15, 19, 36–39, 42, 58, 62), Pieńkowska et al. (2016: nos. 1, 3–13, 16–18, 20–24, 44, 46), Manganelli et al. (2005: no. 59); Dahirel et al. (2015: no. 60); Razkin et al. (2015: no. 61), Neiber & Hausdorf (2017: nos. 58, 61, 62), this paper (nos. 25–33, 39, 42, 57). Shaded areas – ranges of *M. claustralis* and *M. cartusiana* according to Welter-Schultes (2012). Map drawn by J. Bogucki

sequences (16S 4 & 16S 5) and combined sequences (Mclau-ME-h1 – Mclau-ME-h4 of specimens from Montenegro and Mclau-BA-h2 from Bosnia-Herzegovina) form separate subclades in Figs 2B & 3, respectively. This confirms the previously suggested need (Pieńkowska et al. 2015) of comparative studies including topotypical material of *M. subobstructa* (Bourguignat, 1855), a species whose occurrence was reported from nearby Albania (Fehér & Erőss 2009), although the name is currently listed among synonyms of *M. claustralis* (Welter-Schultes 2012). It should be added that the geological history of the region may explain the reasons for species diversity of snails (e.g., Ketmaier et al. 2006, Fiorentino et al. 2010, 2016). The geological events in the Balkan Peninsula history caused separation of some truncatelloid species into Bulgarian, Montenegrin and Greek populations (Osikowski et al. 2015, Osikowski 2017).

The presented results show that *M. cartusiana* is widespread in Europe. Fig. 9 shows its localities confirmed molecularly, both in earlier papers by various authors and in the present study: in Spain (Razkin et al. 2015, Neiber & Hausdorf 2017), north-west France (Dahirel et al. 2015), Germany (this paper), Italy (Manganelli et al. 2005, Pieńkowska et al. 2015, Neiber & Hausdorf 2017), Poland (Pieńkowska et al. 2015, 2016, this paper), the Czech Republic (Pieńkowska et al. 2015), Slovakia (this paper), Austria (this paper), Hungary (Pieńkowska et al. 2015, this paper), Serbia (Pieńkowska et al. 2016), Croatia (this paper), Bosnia-Herzegovina (Pieńkowska et al. 2016, this paper) and Kosovo (this paper).

The genetic distances within *M. cartusiana* are not large (Table 2: *COI* 0.2–3.3%, *16SrDNA* 0.0–3.4%), however, two subclades can be distinguished in Figs 1C & 3. The first one comprises sequences COI 8, COI 10, KX258393 and KX258415 (Fig. 1C) and Mcart-PL-h4 and Mcart-BA-h3 (Fig. 3) from Kielce (Poland) and Belgrade (Serbia). The second subclade consists of sequences COI 18 – COI 21 (Fig. 1C) and Mcart-HU-h2 – Mcart-HU-h5 (Fig. 3) from Bočsa (Hungary). In contrast, they do not differ in *16SrDNA* sequences (haplotype 16S 6 is common for these populations, see: Table 1). Therefore, together with the similarity of the genital structure, it suggests that this is only inter-population variation in *COI* gene.

Unique *COI* (COI 15, COI 16) and *16SrDNA* (16S 10, 16S 11) sequences as well as combined sequences (Mcart-XK-h1 – Mcart-XK-h3) were obtained from Košutane (Kosovo) specimens. The genetic distances between them and other *M. cartusiana* sequences are small (0.3–3.2% – slightly larger 2.1–3.2% in comparison with the above mentioned sequences from Kielce, Belgrade and Bočsa specimens, but only 0.3–1.6% regarding all other studied *M. cartusiana* sequences). However, it is noteworthy that shells of these specimens were small, without umbilicus. They were collected in a xerothermic sward at high altitude. Nevertheless, the structure of their genitalia confirms the molecular results (Figs 7–8) and we report the Košutane population as representing *M. cartusiana*.

Taking into account the ranges of these two species shown in WELTER-SCHULTES (2012) and this study (Fig. 9), there are still many localities which should be revised molecularly. Since the shells of these species are very similar (HAUSDORF 2000a) their conchological identification is unreliable. Moreover, genital anatomy requires very careful examination due to the overall similarities (HAUSDORF 2000a). Interestingly, *M. claustralis* and *M. cartusiana* co-occur in some Polish (Kielce-Wietrznia) and Czech (Prague) populations (PIEŃKOWSKA et al. 2015, 2016). Now we add new Polish (Kielce-Lidl) and Bosnian (Kaonik) populations with such a co-occurrence (Table 1, Fig. 9). This further emphasises the need of cautious identification confirmed by molecular and anatomical features.

We think and hope that the molecularly checked localities presented on the map (Fig. 9) will soon be supplemented by new data.

Finally, we can state that the results of this paper confirm the earlier observations that two species of *Monacha* (*M. claustralis* and *M. cartusiana*), aliens in the Polish malacofauna, invaded Poland during the last ca. 40 years (STWORZEWICZ & GÓRKA 2012, PIEŃKOWSKA et al. 2016), similarly to other pairs of molluscan species, for example slugs *Arion vulgaris* (Moquin-Tandon, 1855) and *A. rufus* (Linnaeus, 1758) (SOROKA et al. 2009, KOZŁOWSKI & KOZŁOWSKI 2011) and bivalves *Corbicula fluminea* (O. F. Müller, 1774) and *C. fluminalis* (O. F. Müller, 1774) (DOMAGAŁA et al. 2004, ŁABĘCKA et al. 2005, MAĆKIEWICZ 2013, PIECHOCKI & SZLAUER-ŁUKASZEWSKA 2013), as well as *Dreissena polymorpha* (Pallas, 1771) and *D. rostriformis bugensis* (Andrusov, 1897) (KOŁODZIEJCZYK et al. 2011, WOŹNICZKA et al. 2016). They are expanding their range in Poland.

ACKNOWLEDGEMENTS

We are very grateful to Prof. FOLCO GIUSTI and Prof. GIUSEPPE MANGANELLI (Siena University, Italy) for their help in the study of *M. claustralis* and *M. cartusiana* genital structures, drawings of *M. cartusiana* genitalia from Kosovo as well as for comments on the manuscript. We thank Mr. PIOTR BOCIANOWSKI for technical support in laboratory, Dr. KATERINA VARDINOYANNIS (University of Crete, Herakleion, Greece) for comments on the manuscript and Dr. HEIKE KAPPES (Department of Ecology, University of Cologne, Germany), Dr. MARCO NEIBER (Zoological Museum, University of Hamburg, Germany), Prof. ATANAS IRIKOV (University of Plovdiv, Bulgaria) and PAUL W. WHITEHEAD (Pershore, UK) for providing specimens. We also thank Mr. JAROSŁAW BOGUCKI (Poznań, Poland) for drawing the map (Fig. 9).

REFERENCES

ANDERSON R., GIUSTI F., TELFER M. G., MANGANELLI G., PIEŃKOWSKA J. R., LESICKI A. 2018. *Monacha ocellata* (Roth, 1839) (Gastropoda: Hygromiidae) established in Essex, an addition to the fauna of Britain and Ireland. J. Conchol. (submitted).

CHOLEWA S., KORALEWSKA-BATURA E., BATURA M. 2003. A new locality of *Monacha cartusiana* (O. F. Müller, 1774) (Gastropoda: Pulmonta: Helicidae) in Poland. Folia Malacol. 11: 59–61. https://doi.org/10.12657/folmal.011.007

DABERT M., WITALINSKI W., KAZMIERSKI A., OLSZANOWSKI Z., DABERT J. 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): Strong conflict between phylogenetic signal and long-branch attraction artifacts. Mol. Phylogenet. Evol. 56: 222–241. https://doi.org/10.1016/j.ympev.2009.12.020

DAHIREL M., OLIVIER E., GUILLER A., MARTIN M.-C., MADEC L., ANSART A. 2015. Movement propensity and ability correlate with ecological specialization in European land snails: comparative analysis of a dispersal syndrome. J. Animal. Ecol. 84: 228–238. https://doi.org/10.1111/1365-2656.12276

Dhora D. 2009. Mollusks of Albania. Arch. Biol. Sci. Belgrade 61: 537–553. https://doi.org/10.2298/ABS0903537D

Dhora D., Welter-Schultes F. W. 1996. List of species and atlas of the non-marine mollusks of Albania. Schr. Malakozool. Haus. Nat. Cismar 9: 90–197.

Domagała J., Łabęcka A. M., Pilecka-Rapacz M., Migdalska B. 2004. *Corbicula fluminea* (O. F. Müller, 1774) (Bivalvia: Corbiculidae) – a species new to the Polish malacofauna. Folia Malacol. 12: 145–148. https://doi.org/10.12657/folmal.012.011

Duda M., Sattmann H., Haring E., Bartel D., Winkler H., Harl J., Kruckenhauser L. 2011. Genetic differentiation and shell morphology of *Trochulus oreinos* (Wagner, 1915) and *T. hispidus* (Linnaeus, 1758) (Pulmonata: Hygromiidae) in the northeastern Alps. J. Mollus. Stud. 77: 30–40. https://doi.org/10.1093/mollus/eyq037

Falniowski A., Wilke T. 2001. The genus *Marstoniopsis* (Gastropoda: Rissoidea): intra- and intergeneric phylogenetic relationships. J. Mollus. Stud. 67: 483–488. https://doi.org/10.1093/mollus/67.4.483

Fehér Z., Erőss Z. P. 2009. Checklist of the Albanian mollusc fauna. Schr. Malakozool. Haus. Nat. Cismar 25: 22–38.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fiorentino V., Manganelli G., Giusti F., Ketmaier V. 2016. Recent expansion and relic survival: Phylogeography of the land snail genus *Helix* (Mollusca, Gastropoda) from south to north Europe. Mol. Phylogenet. Evol. 98: 358–372. https://doi.org/10.1016/j.ympev.2016.02.017

Fiorentino V., Salomone N., Manganelli G., Giusti F. 2008. Phylogeography and morphological variability in land snails: the Sicilian *Marmorana* (Pulmonata, Helicidae). Biol. J. Linn. Soc. 94: 809–823. https://doi.org/10.1111/j.1095-8312.2008.01023.x

Fiorentino V., Salomone N., Manganelli G., Giusti F. 2010. Historical biogeography of Tyrrhenian land snails: *Marmorana* - *Tyrrheniberus* radiation (Pulmonata, Helicidae). Mol. Phylogenet. Evol. 55: 26–37. https://doi.org/10.1016/j.ympev.2009.11.024

Gantenbein B., Fet V., Largiadèr C. R., Scholl A. 1999. First DNA phylogeny of *Euscorpius* Thorrell, 1876 (Scorpiones: Euscorpiidae) and its bearing on taxonomy and biogeography of this genus. Biogeographica (Paris) 75: 49–65.

Georgiev D. 2016. The 46 years overlooked species *M. oshanovae* Pintér & Pintér, 1970. Ecol. Mont. 6: 46–55.

Glez-Peña D., Gómez-Blanco D., Reboiro-Jato M., Fdez-Riverola F., Posada D. 2010. ALTER: program-oriented format conversion of DNA and protein alignments. Nucleic Acids Res. 38 (Web Server issue): W14–W18.

Goldstein P. Z., DeSalle R. 2010. Integrating DNA barcode data and taxonomic practise: determination, discovery, and description. Bioessays 33: 135–147. https://doi.org/10.1002/bies.201000036

Hall T. A. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

Hasegawa M., Kishino H., Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22: 160–174. https://doi.org/10.1007/BF02101694

Hausdorf B. 2000a. The genus *Monacha* in Turkey (Gastropoda: Pulmonata: Hygromiidae). Arch. Molluskenkd. 128: 61–151. https://doi.org/10.1127/arch.moll/128/2000/61

Hausdorf B. 2000b. The genus *Monacha* in the Western Caucasus (Gastropoda: Hygromiidae). J. Nat. Hist. 34: 1575–1594. https://doi.org/10.1080/00222930050117495

Hausdorf B., Páll-Gergely B. 2009. *Monacha oecali* new species from Southern Turkey (Gastropoda: Hygromiidae). J. Conchol. 40: 15–17.

Hebert P. D. N., Cywinska A., Ball S. L., deWaard J. R. 2003a. Biological identifications through DNA bardcodes. Proc. R. Soc. Lond. B 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert P. D. N., deWaard J. R., Zakharov E. V., Prosser S. W. J., Sones J. E., McKeown J. A. T., Mantle B., La Salle J. 2013. DNA 'barcode blitz': rapid digitization and sequencing of a natural history collection. PLoS ONE 8: e68535. https://doi.org/10.1371/journal.pone.0068535

Hebert P. D. N., Ratnasingham S., deWaard J. R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. B 270: 596–599. https://doi.org/10.1098/rsbl.2003.0025

Hlaváč J. Č., Peltanová A. 2010. First occurrence of the Kentish Snail *Monacha cantiana* (Mollusca: Gastropoda: Hygromiidae) in the Czech Republic. Malacol. Bohemosl. 9: 11–15. https://core.ac.uk/download/pdf/26016007.pdf

Irikov A. 2008. Genus *Monacha* Fitzinger 1833 in Bulgaria (Gastropoda, Pulmonata, Hygromiidae). Linzer biol. Beitr. 40: 785–811.

Ketmaier V., Giusti F., Caccone A. 2006. Molecular phylogeny and historical biogeography of the land snail genus *Solatopupa* (Pulmonata) in the peri-Tyrrhenian area. Mol. Phylogenet. Evol. 39: 439–451. https://doi.org/10.1016/j.ympev.2005.12.008

Kerney M. P., Cameron R. A. D., Jungbluth J. H. 1983. Die Landschnecken Nord- und Mitteleuropas. Paul Parey Verlag, Hamburg & Berlin.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120. https://doi.org/10.1007/BF01731581

Kołodziejczyk A., Łabęcka A. M., Stańczykowska A. 2011. *Corbicula* i *Dreissena* – niewyjaśnione problemy inwazji. In: Głowaciński Z. H. (ed.). Gatunki obce w faunie Polski. II. Zagadnienia problemowe i syntezy. Instytut Ochrony Przyrody PAN, Kraków, pp. 551–563.

KOZŁOWSKI J., KOZŁOWSKI R. J. 2011. Expansion of the invasive slug species *Arion lusitanicus* Mabille, 1868 (Gastropoda: Pulmonata: Stylommatophora) and dangers to garden crops. Folia Malacol. 19: 249–258. https://doi.org/10.2478/v10125-011-0005-8

KUMAR S., STECHER G., TAMURA K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

LESICKI A., KORALEWSKA-BATURA E. 2007. *Monacha cartusiana* (O. F. Müller, 1774) (Gastropoda: Pulmonata: Hygromiidae) becomes more frequent in Poland. Folia Malacol. 15: 181–184. https://doi.org/10.12657/folmal.015.016

ŁABĘCKA A. M., DOMAGAŁA J., PILECKA-RAPACZ M. 2005. First record of *Corbicula fluminalis* (O.F. Müller, 1774) (Bivalvia: Corbiculidae) – in Poland. Folia Malacol. 13: 25–27. https://doi.org/10.12657/folmal.013.003

MAĆKIEWICZ J. 2013. The first record of the Asian clam *Corbicula fluminea* (Bivalvia: Veneroida: Corbiculidae) in the upper Vistula (south Poland). Folia Malacol. 21: 87–90. https://doi.org/10.12657/folmal.021.009

MANGANELLI G., SALOMONE N., GIUSTI F. 2005. A molecular approach to the phylogenetic relationships of the western palaearctic Helicoidea (Gastropoda: Stylommatophora). Biol. J. Linn. Soc. Lond. 85: 501–512. https://doi.org/10.1111/j.1095-8312.2005.00514.x

NEIBER M. T., HAUSDORF B. 2015. Phylogeography of the land snail genus *Circassina* (Gastropoda: Hygromiidae) implies multiple Pleistocene refugia in the western Caucasus region. Mol. Phylogenet. Evol. 93: 129–142. https://doi.org/10.1016/j.ympev.2015.07.012

NEIBER M. T., HAUSDORF B. 2017. Molecular phylogeny and biogeography of the land snail genus *Monacha* (Gastropoda, Hygromiidae). Zool. Scr. 46: 308–321. https://doi.org/10.1111/zsc.12218

NEIBER M. T., RAZKIN O., HAUSDORF B. 2017. Molecular phylogeny and biogeography of the land snail family Hygromiidae (Gastropoda: Helicoidea). Mol. Phylogenet. Evol. 111: 169–184. https://doi.org/10.1016/j.ympev.2017.04.002

NEUBERT E., BARICHE M. 2013. On the *Monacha* species of Lebanon (Gastropoda, Hygromiidae). ZooKeys 311: 1–18. https://doi.org/10.3897/zookeys.311.5408

OSIKOWSKI A. 2017. Isolation as a phylogeny-shaping factor: historical geology and cave habitats in the Mediterranean Truncatelloidea Gray, 1840 (Caenogastropoda). Folia Malacol. 25: 213–229. https://doi.org/10.12657/folmal.025.015

OSIKOWSKI A., GEORGIEV D., HOFMAN S., FALNIOWSKI A. 2015. Does the genetic structure of spring snail *Bythinella* (Caenogastropoda, Truncatelloidea) in Bulgaria reflect geological history? ZooKeys 518: 67–86. https://doi.org/10.3897/zookeys.518.10035

OSIKOWSKI A., HOFMAN S., GEORGIEV D., RYSIEWSKA A., FALNIOWSKI A. 2017. Unique, ancient stygobiont clade of Hydrobiidae (Truncatelloidea) in Bulgaria: the origin of cave Fauna. Folia Biol. (Kraków) 65: 79–93. https://doi.org/10.3409/fb65_2.79

PACKER L., GRIXTI J. C., ROUGHLEY R. E., HANNER R. 2009. The status of taxonomy in Canada and the impact of DNA barcoding. Can J. Zool. 87: 1097–1110. https://doi.org/10.1139/Z09-100

PIECHOCKI A., SZLAUER-ŁUKASZEWSKA A. 2013. Molluscs of the middle and lower Odra: the role of the river in the expansion of alien species in Poland. Folia Malacol. 21: 73–86. https://doi.org/10.12657/folmal.021.008

PIEŃKOWSKA J., GIUSTI F., MANGANELLI G., LESICKI A. 2015. *Monacha claustralis* (Rossmässler 1834) new to Polish and Czech malacofauna (Gastropoda: Pulmonata: Hygromiidae). J. Conchol. 42: 79–93. http://docplayer.net/56112556-Monacha-claustralis-rossmassler-1834-new-to-polish-and-czech-malacofauna-gastropoda-pulmonata-hygromiidae.html

PIEŃKOWSKA J. R., GÓRKA M., MATUSZAK M., BOCIANOWSKI P., GWARDJAN M., LESICKI A. 2016. New data on the distribution and molecular diagnostics of *Monacha claustralis* (Rossmässler 1834) and *M. cartusiana* (O. F. Müller, 1774) (Gastropoda: Eupulmonata: Hygromiidae) in Poland, Bosnia and Serbia. Folia Malacol. 24: 223–237. https://doi.org/10.12657/folmal.024.019

PIEŃKOWSKA J., MANGANELLI G., GIUSTI F., HALLGASS A., LESICKI A. 2018. Exploring *Monacha cantiana* (Montagu, 1803) phylogeny: cryptic lineages and new insights into the origin of the English populations (Eupulmonata, Stylommatophora, Hygromiidae). ZooKeys 765: 1–41. https://doi.org/10.3897/zookeys.765.24386

PINTÉR L. 1968. Über bulgarischen Mollusken. Malakol. Abh. 2: 209–230.

PROĆKÓW M., MACKIEWICZ P., PIEŃKOWSKA J. R. 2013. Genetic and morphological studies of species status for poorly known endemic *Trochulus phorochaetius* (Bourguignat, 1864) (Gastropoda: Pulmonata: Hygromiidae), and its comparison with closely related taxa. Zool. J. Linn. Soc. 169: 124–143. https://doi.org/10.1111/zoj.12048

PROĆKÓW M., STRZAŁA T., KUŹNIK-KOWALSKA E., MACKIEWICZ P. 2014. Morphological similarity and molecular divergence of *Trochulus striolatus* and *T. montanus*, and their relationship to sympatric congeners (Gastropoda: Pulmonata: Hygromiidae). Syst. Biodivers. 12: 366–384. https://doi.org/10.1080/14772000.2014.925986

RAZKIN O., GOMEZ-MOLINER B. J., PRIETO C. E., MARTINEZ-ORTI A., ARREBOLA J. R., MUNOZ B., CHUECA L. J., MADEIRA M. J. 2015. Molecular phylogeny of the western Palaearctic Helicoidea (Gastropoda, Stylommatophora). Mol. Phylogenet. Evol. 83: 99–117. https://doi.org/10.1016/j.ympev.2014.11.014

RONQUIST F., HUELSENBECK J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

RYSIEWSKA A., GEORGIEV D., OSIKOWSKI A., HOFMAN S., FALNIOWSKI A. 2016. *Pontobelgrandiella* Radoman, 1973 (Caenogastropoda: Hydrobiidae): a recent invader of subterranean waters? J. Conchol. 42: 193–203.

SAITOU N., NEI M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

SAUER J., HAUSDORF B. 2009. Sexual selection is involved in speciation in a land snail radiation on Crete. Evolution 63: 2535–2546. https://doi.org/10.1111/j.1558-5646.2009.00751.x

SAUER J., HAUSDORF B. 2012. A comparison of DNA-based methods for delimiting species in a Cretan land snail radiation reveals shortcomings of exclusively molecular taxonomy. Cladistics 28: 300–316. https://doi.org/10.1111/j.1096-0031.2011.00382.x

SIMON C., FRATI F., BECKENBACH A. T., CRESPI B., LIU H., FLOOK P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701. https://doi.org/10.1093/aesa/87.6.651

SOROKA M., KOZŁOWSKI J., WIKTOR A., KAŁUSKI T. 2009. Distribution and genetic diversity of the terrestrial slugs *Arion lusitanicus* Mabille, 1868 and *Arion rufus* (Linnaeus, 1758) in Poland based on mitochondrial DNA. Folia Biol. (Kraków) 57: 71–81. https://doi.org/10.3409/fb57_1-2.71-81

STWORZEWICZ E., GÓRKA M. 2012. Ślimak kartuzek *Monacha cartusiana* (O. F. Müller, 1774). In: GŁOWACIŃSKI Z., OKARMA H., PAWŁOWSKI J., SOLARZ W. (eds). Księga gatunków inwazyjnych obcych fauny Polski. Instytut Ochrony Przyrody PAN, Cracow: http://www.iop.krakow.pl/gatunkiobce/

SZAROWSKA M. 2006. Molecular phylogeny, systematics and morphological character evolution in the Balkan Rissooidea (Caenogastropoda). Folia Malacol. 14: 99–168. https://doi.org/10.12657/folmal.014.014

SZAROWSKA M., HOFMAN S., OSIKOWSKI A., FALNIOWSKI A. 2014. *Pseudorientalia* Radoman, 1973 (Caenogastropoda: Rissooidea) on Samos Island, Aegean Sea. Folia Malacol. 22: 11–20.

THOMPSON J. D., HIGGINS D. G., GIBSON T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680. https://doi.org/10.1093/nar/22.22.4673

WELTER-SCHULTES F. W. 1996. Non-marine molluscs recently collected in Albania. Schr. Malakozool. Haus. Nat. Cismar 9: 21–31.

WELTER-SCHULTES F. W. 2012. European non-marine molluscs, a guide for species identification. Planet Poster Editions, Göttingen.

WOŹNICZKA A., WAWRZYNIAK-WYDROWSKA B., RADZIEJEWSKA T., SKRZYPACZ A. 2016. The quagga mussel (*Dreissena rostriformis bugensis* Andrusov, 1897) – another Ponto-Caspian dreissenid bivalve in the southern Baltic catchment: the first record from the Szczecin Lagoon. Oceanologia 58: 154–159. https://doi.org/10.1016/j.oceano.2015.12.002

Received: April 18th, 2018
Revised: May 1st , 2018
Accepted: May 11th, 2018
Published on-line: June 5th, 2018