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Monacha samsunensis (Pfeiffer, 1868): another Anatolian species introduced to Western Europe, where it is known as *Monacha atacis* Gittenberger & de Winter, 1985 (Gastropoda: Eupulmonata: Hygromiidae)

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Abstract
Populations of *Monacha atacis* from southern Occitania in France and of *M. samsunensis* from northern Anatolia in Turkey (Atakum/Samsun and Kastamonu) were investigated by an integrative approach based on morphological (shell and genitalia) and molecular (mitochondrial and nuclear gene sequences) features. Morphological examination revealed a complex pattern of variation within and between geographically separated populations, while molecular analysis showed strong similarity between the two species, confirming earlier suggestions that the species are conspecific. Pfeiffer's name *Helix samsunensis* introduced in 1868 has priority over the name *M. atacis* given by Gittenberger & de Winter in 1985.

Keywords: *Genital anatomy*, *molecular features*, *shell*, *species delimitation*, *synonymy*

Introduction

Monacha Fitzinger, 1833 is a speciose hygromiid genus with species occurring from Britain and north-western France to the Caucasus, Middle East and north African coast (Hausdorf 2000a, 2000b; Welter-Schultes 2012; Neiber & Hausdorf 2017 and other references therein). After Hesse (1914), Hausdorf (2000a) recognised three subgenera within *Monacha* on the basis of presence/absence of penial retractor muscle and vaginal appendix: *Monacha* s.s. Fitzinger, 1833) (type species: *Helix cartusiana* Müller, 1774) for species without penis retractor but with appendix, *Metatheba* Hesse, 1914 (type species: *Helix samsunensis* Pfeiffer, 1868) for species with penial retractor but without appendix, and *Paratheba* Hesse, 1914 (type species: *Helix fruticola* Krynicki, 1833) for species with both penial retractor and appendix. In an excellent integrative phylogenetic and biogeographic analysis of *Monacha* based on anatomical features of the reproductive system and molecular data (mitochondrial and nuclear gene sequences), Neiber and Hausdorf (2017) established four new subgenera: *Pontotheba* (type species: *Monacha* (*Paratheba*) *bithynica* Hausdorf, 2000a), *Aegaeotheba* (type species: *Monacha* (*Paratheba*) *cretica* Hausdorf, 2003), *Trichotheba* (type species: *Monacha* (*Monacha*) *comata* Hausdorf, 2000a), *Rhytidotheba* (type species: *Helix* (*Trichia*) *densecostulata* Retowski, 1887). They also resurrected the subgenus *Platytheba* Pilsbry, 1895 (type species: *Caracolla nummus* Ehrenberg, 1831) but left the status of *Eutheba* Nordsieck, 1993 unresolved.

Most *Monacha* species have limited ranges of distribution, restricted to their type localities, or if

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wider, to the southern Balkans and Anatolia (especially the Pontic region along the Black Sea coast). The exceptions include three species from the subgenus *Monacha* s.s., namely *M. cartusiana* (Müller, 1774), *M. claustralis* (Rossmässler, 1834) and *M. cantiana* (Montagu, 1803). *M. cartusiana* is widespread throughout Europe except in the north-east (Scandinavia, Russia, Baltic states, Belarus, northern Ukraine) (Welter-Schultes 2012). *M. claustralis* is now spreading quickly northward (Pinter 1968; Hlaváč & Peltanová 2010; Pieńkowska et al. 2015, 2016, 2018a; Hutchinson et al. 2019; Čejka et al. 2020; Gural-Sverlova & Gural 2022) from its native range in European and Anatolian Turkey (Hausdorf 2000a) and Greece (with Corfu/Kerkyra as type locality, Welter-Schultes 2012). *M. cantiana* is found in Great Britain, northern France and Germany, in the Benelux countries as well as in Spain, where it was probably introduced in Roman times from its native area in central Italy (Kerney et al. 1964; Kerney 1970; Evans 1972; Pieńkowska et al. 2018b). *Monacha* (*Platytheba*) *ocellata* (Roth 1839), known from the vicinity of Istanbul in Turkey, was recently discovered in a single locality in Britain, probably resulting from passive introduction in unknown circumstances (Anderson et al. 2018).

All but one species of subgenus *Metatheba* occur in northern Anatolia, mainly along the Black Sea coast. The only exception is *M.* (*Metatheba*) *atacis* Gittenberger & de Winter, 1985, known from southern Occitania, France (Hausdorf 2000a; Falkner et al. 2002; Gargominy et al. 2011; Neiber & Hausdorf 2017) and a site in Catalonia, north-eastern Spain (Bertrand 2003). However, when describing the new species, Gittenberger and de Winter (1985) already drew attention to its close relationship with *M. samsunensis* (Pfeiffer, 1868). Considering the great similarity between *M. atacis* and *M. samsunensis*, Hausdorf (2000a) and Neiber and Hausdorf (2017) suggested that despite their disjunct ranges these two taxa could be conspecific and that the French populations of *M. atacis* might be the result of introduction of *M. samsunensis* in historical times. One of us (MP) collected *M. atacis* in several sites in the foothills of the Pyrenees, southern France, while another member of our team (GG) found *M. samsunensis* in two localities in northern Anatolia, Turkey, one in the vicinity of her university in Kastamonu and the other near Atakum/Samsun, i.e. in the type locality of the species. This enabled us to undertake the task of verifying the hypothesis of Hausdorf (2000a) and Neiber and Hausdorf (2017, also see Cadevall et al. 2020: p. 155). The results of our study are reported in this paper.

Material and methods

Taxonomic sampling

Specimens for research were collected on the basis of the literature data on the occurrence of *M. atacis* in France (Gittenberger & de Winter 1985) and *M. samsunensis* in Turkey (Hausdorf 2000a; Welter-Schultes 2012). Species identification was based on morphological and molecular research. Thus, the specimens were obtained from 18 populations of *Monacha atacis* from southern France and three populations of *M. samsunensis* from northern Anatolia in Turkey. They were considered in our analysis of molecular and genital structure (Table I and Figure 1). A new French population of *M. cartusiana* (Table I) as well as literature data on several *Monacha* species and lineages were used in the analysis. Several sequences deposited in GenBank (Table II) were also used for molecular analysis. Sequences of *Trochulus hispidus* (Linnaeus, 1758) from GenBank were used as an outgroup to construct phylogenetic trees.

Material examined

New material examined is listed as follows, when possible: geographic coordinates of locality, locality (country, region, municipality and province, site), collector(s), date, number of specimens, with the collection where the material is kept in parenthesis (Table I). The material is kept in the collection of the Department of Cell Biology, Adam Mickiewicz University, Poland (DCBC), the Małgorzata Proćków collection (MNHW; Museum of Natural History, University of Wrocław, Poland) and the Folco Giusti collection (FGC; Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente, Università di Siena, Italy). The material used for comparison has already been described (see Pieńkowska et al. 2018b: table 1, 2019a: table 1, 2020: table 1).

Morphological study

Twenty-four specimens from 15 sites in France and ten specimens from two sites in Turkey were analysed for shell and anatomy (see Table I). Snail bodies were dissected under a light microscope (Wild M5A, or Zeiss SteREO Lumar. V12). Anatomical details were drawn using a Wild camera lucida. Adult specimens from Turkey were obtained in sufficient number to describe their genital structure, however the scarcity of specimens per population (where several were juveniles or subadults) meant that no quantitative analysis was done on the morphological characters.

Table I. List of localities of the French and Turkish populations of *Monacha atacis*, *M. samsunensis* and *M. cartusiana* used for molecular and morphological research.

Localities						COI		16SrDNA		H3		sITS2 (5.8S rDNA + ITS2)		lgITS2 (5.8S rDNA + ITS2 + 28S rDNA)			
No.	coordinates	country and site	collector / date / no. of specimens (collection)	Revised taxonomy	Designation of voucher sps	new haplotype (no. spec.)	GenBank ##	new haplotype (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	AA	Figs
1	42°44'27.8"N 01°28'34.7"E	France, Occitania, Ariège, Arties near Auzat, edge of riparian forest, 984 m a.s.l.	M. Proćków / 3.07.2018 / 2 / (DCBC & MNHW-F.18.38)	*M. atacis* = *M. samsunensis*	Art1	COI 1 (1)	ON332579	16S 1* (1)	ON350884	H3 1 (1)	ON325311			lgITS2 1 (1)	ON332736	+	
					Art2	COI 2 (1)	ON332580	16S 2 (1)	ON350885	H3 2 (1)	ON325312						
2	42°52'41.6"N 02°01'51.9"E	France, Occitania, Aude, Le Chandelier, clearing, 826 m a.s.l.	M. Proćków / 27.06.2018 / 1 / (DCBC & MNHW-F.18.35)	*M. atacis* = *M. samsunensis*	Cha1	COI 2 (1)	ON332581	16S 3* (1)	ON350886	H3 1 (1)	ON325313			lgITS2 1 (1)	ON332737		
3	42°48'35.5"N 02°15'04.0"E	France, Occitania, Aude, near Axat, vegetation along road, 460 m a.s.l.	M. Proćków / 26.06.2018 / 5 / (DCBC & MNHW-F.18.26)	*M. atacis* = *M. samsunensis*	Axa1	COI 3 (2)	ON332582	16S 4* (1)	ON350887	H3 2 (3)	ON325314			lgITS2 1 (1)	ON332738	+	
					Axa2		ON332583	16S 5 (1)	ON350888		ON325315	sITS2 1 (1)	ON332739				
					Axa5	COI 4 (1)	ON332584	16S 6 (1)	ON350889		ON325316	sITS2 2 (1)	ON332740				
4	42°48'40.2"N 02°17'12.2"E	France, Occitania, Aude, near Lapradelle, roadside, 497 m a.s.l.	M. Proćków / 25.06.2018 / 5 / (DCBC & MNHW-F.18.27; FGC 51247)	*M. atacis* = *M. samsunensis*	Lap1	COI 4 (1)	ON332585	16S 7* (1)	ON350890	H3 2 (5)	ON325317			lgITS2 2 (1)	ON332741	+	12-14
					Lap2	COI 2 (2)	ON332586	16S 2 (1)	ON350891		ON325318						
					Lap3			16S 8 (1)	ON350892		ON325319						
					Lap4		ON332587	16S 9 (2)	ON350893		ON325320						
					Lap5				ON350894		ON325321						
5	42°43'39.2"N 02°01'22.0"E	France, Occitania, Ariège, Mijanès, vegetation beneath rocks, 1518 m a.s.l.	M. Proćków / 4.07.2018 / 4 / (DCBC & MNHW-F.18.50)	*M. atacis* = *M. samsunensis*	Mij1	COI 4 (3)	ON332588	16S 7* (1)	ON350895	H3 2 (1)	ON325322			lgITS2 3 (2)	ON332742	+	
					Mij2		ON332589	16S 5 (1)	ON350896	H3 1 (1)	ON325323				ON332743		
					Mij3		ON332590	16S 6 (2)	ON350897	H3 4 (1)	ON325324	sITS2 3 (2)	ON332744				
					Mij4	COI 1 (1)	ON332591		ON350898	H3 2 (1)	ON325325		ON332745				
6	42°44'19.3"N 02°13'13.6"E	France, Occitania, Aude, near Grotte de Majestier, vegetation beneath rocks, 729 m a.s.l.	M. Proćków / 29.06.2018 / 5 / (DCBC & MNHW-F.18.41; FGC 51099)	*M. atacis* = *M. samsunensis*	Maj1	COI 4 (1)	ON332592	16S 7* (1)	ON350899	H3 2 (5)	ON325326			lgITS2 4 (1)	ON332746	+	
					Maj2	COI 1 (1)	ON332593	16S 5 (1)	ON350900		ON325327					+	
					Maj3	COI 4 (3)	ON332594	16S 6 (3)	ON350901		ON325328						2
					Maj4		ON332595		ON350902		ON325329	sITS2 4 (1)	ON332747				
					Maj5		ON332596		ON350903		ON325330	sITS2 3 (1)	ON332748				
7	42°45'25.1"N, 02°03'27.6"E	France, Occitania, Aude, Campagna-de-Sault, vegetation along the road, 1024 m a.s.l.	M. Proćków / 4.07.2018 / 5 / (DCBC & MNHW-F.18.51)	*M. atacis* = *M. samsunensis*	Des1	COI 5 (1)	ON332597	16S 10 (1)	ON350904	H3 2 (3)	ON325331					+	
					Des2	COI 4 (1)	ON332598	16S 11* (1)	ON350905		ON325332			lgITS2 5 (1)	ON332749		
					Des3	COI 6 (1)	ON332599	16S 12* (1)	ON350906		ON325333						
					Des4	COI 4 (1)	ON332600	16S 11* (1)	ON350907	H3 1 (1)	ON325334	sITS2 5 (2)	ON332750				
					Des5	COI 6 (1)	ON332601	16S 12* (1)	ON350908	H3 2 (1)	ON325335		ON332751				

(*Continued*)

Table I. (Continued).

Localities						COI		16SrDNA		H3		sITS2 (5.8S rDNA + ITS2)		lgITS2 (5.8S rDNA + ITS2 + 28S rDNA)			
No.	coordinates	country and site	collector / date / no. of specimens (collection)	Revised taxonomy	Designation of voucher sps	new haplotype (no. spec.)	GenBank ##	new haplotype (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	AA	Figs
8	42°53'29.8"N 02°13'32.5"E	France, Occitania, Aude, Saint-Ferriol 1, vegetation, 454 m a.s.l.	M. Proćków / 29.06.2018 / 5 / (DCBC & MNHW-F.18.39)	*M. atacis* = *M. samsunensis*	Fer1-1	COI 1 (1)	ON332602	16S 13* (1)	ON350909	H3 2 (4)	ON325336			lgITS2 6 (1)	ON332752	+	
					Fer1-2	COI 4 (2)	ON332603	16S 5 (2)	ON350910		ON325337			lgITS2 2 (1)	ON332753	+	
					Fer1-3		ON332604		ON350911		ON325338						
					Fer1-4	COI 7 (1)	ON332605	16S 6 (2)	ON350912		ON325339	sITS2 3 (2)	ON332754				
					Fer1-5	COI 4 (1)	ON332606		ON350913	H3 3 (1)	ON325340		ON332755				
9	42°53'16.4"N 02°12'39.4"E	France, Occitania, Aude, Saint-Ferriol 2, garrigue shrubs, 338 m a.s.l.	M. Proćków / 29.06.2018 / 5 / (DCBC & MNHW-F.18.39[a]; FGC 51097)	*M. atacis* = *M. samsunensis*	Fer2-1	COI 6 (1)	ON332607	16S 12* (1)	ON350914	H3 2 (1)	ON325341			lgITS2 2 (1)	ON332756	+	
					Fer2-2	COI 8 (2)	ON332608	16S 5 (1)	ON350915	H3 1 (2)	ON325342					+	
					Fer2-3		ON332609	16S 6 (3)	ON350916								3
					Fer2-4	COI 1 (2)	ON332610		ON350917		ON325343	sITS2 6 (1)	ON332757				
					Fer2-5		ON332611		ON350918	H3 2 (1)	ON325344	sITS2 7 (1)	ON332758				
10	42°49'34.2"N 02°13'38.8"E	France, Occitania, Aude, Saint-Martin-Lys, vegetation on rocky wall, 333 m a.s.l.	M. Proćków / 5.07.2018 / 5 / (DCBC & MNHW-F.18.52)	*M. atacis* = *M. samsunensis*	Lys1	COI 8 (1)	ON332612	16S 14* (1)	ON350919					lgITS2 1 (1)	ON332759	+	
					Lys2	COI 3 (1)	ON332613	16S 5 (1)	ON350920	H3 2 (4)	ON325345					+	
					Lys3	COI 8 (1)	ON332614	16S 6 (1)	ON350921		ON325346	sITS2 8 (1)	ON332760				
					Lys4	COI 6 (1)	ON332615	16S 15 (1)	ON350922		ON325347	sITS2 9 (1)	ON332761				
					Lys5	COI 4 (1)	ON332616	16S 6 (1)	ON350923		ON325348	sITS2 8 (1)	ON332762				
11	42°49'55.8"N 02°03'23.4"E	France, Occitania, Aude, Belfort-sur-Rebenty 1, roadside, vegetation under trees, 704 m a.s.l.	M. Proćków / 26.06.2018 / 4 / (DCBC & MNHW-F.18.33)	*M. atacis* = *M. samsunensis*	Reb1-1	COI 1 (1)	ON332617	16S 16* (1)	ON350924	H3 2 (2)	ON325349	sITS2 10 (1)	ON332763			+	
					Reb1-2	COI 4 (1)	ON332618	16S 5 (1)	ON350925		ON325350			lgITS2 1 (1)	ON332764	+	
					Reb1-3	COI 1 (1)	ON332619	16S 6 (1)	ON350926	H3 1(1)	ON325351						
					Reb1-4	COI 8 (1)	ON332620	16S 17 (1)	ON350927	H3 2 (1)	ON325352	sITS2 11 (1)	ON332765				
12	42°46'47.2"N 02°18'32.6"E	France, Occitania, Aude, Salvezines, roadside, 547 m a.s.l.	M. Proćków / 25.06.2018 / 5 / (DCBC & MNHW-F.18.28)	*M. atacis* = *M. samsunensis*	Sal1	COI 2 (1)	ON332621	16S 2 (1)	ON350928	H3 2 (1)	ON325353	sITS2 12 (1)	ON332766			+	
					Sal2	COI 8 (1)	ON332622	16S 14* (1)	ON350929	H3 1 (1)	ON325354			lgITS2 1 (1)	ON332767	+	
					Sal3	COI 6 (1)	ON332623	16S 15 (1)	ON350930	H3 2 (3)	ON325355						
					Sal4	COI 3 (1)	ON332624	16S 6 (2)	ON350931		ON325356						
					Sal5	COI 8 (1)	ON332625		ON350932		ON325357	sITS2 3 (1)	ON332768				
13	42°46'41.0"N 02°13'06.7"E	France, Occitania, Aude, Gorges de Saint-Georges, vegetation along road, 440 m a.s.l.	M. Proćków / 29.06.2018 / 5 / (DCBC & MNHW-F.18.40)	*M. atacis* = *M. samsunensis*	Gor1	COI 5 (1)	ON332626	16S 18* (1)	ON350933	H3 2 (2)	ON325358			lgITS2 7 (1)	ON332769	+	
					Gor2	COI 4 (1)	ON332627	16S 5 (1)	ON350934					lgITS2 8 (1)	ON332770	+	
					Gor3	COI 9 (1)	ON332628	16S 15 (1)	ON350935		ON325359						
					Gor4	COI 1 (2)	ON332629	16S 6 (2)	ON350936	H3 3 (1)	ON325360						
					Gor5		ON332630		ON350937	H3 2 (3)	ON325361						
14	42°48'38.2"N 02°02'04.3"E	France, Occitania, Aude, Belfort-sur-Rebenty 2, meadow near riparian forest, 750 m a.s.l.	M. Proćków / 26.06.2018 / 4 / (DCBC & MNHW-F.18.34)	*M. atacis* = *M. samsunensis*	Reb2-1	COI 8 (1)	ON332631	16S 14* (1)	ON350938	H3 2 (2)	ON325362			lgITS2 3 (2)	ON332771	+	
					Reb2-2	COI 10 (2)	ON332632	16S 5 (1)	ON350939						ON332772	+	
					Reb2-3		ON332633	16S 6 (1)	ON350940			sITS2 1 (1)	ON332773				
					Reb2-4	COI 6 (1)	ON332634				ON325363						

(*Continued*)

Table I. (Continued).

Localities						COI		16SrDNA		H3		sITS2 (5.8S rDNA + ITS2)		lgITS2 (5.8S rDNA + ITS2 + 28S rDNA)			
No.	coordinates	country and site	collector / date / no. of specimens (collection)	Revised taxonomy	Designation of voucher sps	new haplotype (no. spec.)	GenBank ##	new haplotype (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	AA	Figs
15	42°44'10.2"N 02° 12'36.7"E	France, Occitania, Aude, Roquefort-de-Sault, vegetation beneath rocks, 838 m a.s.l.	M. Proćków / 29.06.2018 / 5 / (DCBC & MNHW-F.18.42)	*M. atacis* = *M. samsunensis*	Roq1	COI 4 (1)	ON332635	16S 7* (1)	ON350941	H3 1 (1)	ON325364			lgITS2 9 (1)	ON332774	+	
					Roq2	COI 1 (1)	ON332636	16S 5 (1)	ON350942	H3 2 (3)	ON325365			lgITS2 1 (1)	ON332775	+	
					Roq3	COI 4 (1)	ON332637	16S 6 (2)	ON350943		ON325366						
					Roq4	COI 3 (1)	ON332638		ON350944		ON325367						
					Roq5	COI 11 (1)	ON332639	16S 9 (1)	ON350945	H3 5 (1)	ON325368						
16	ca. 43°12'N, 02° 20'E	France, Occitania, Aude, Carcassonne	G. Manganelli / 15.07.2004 / 1 / FGC35773	*M. atacis* = *M. samsunensis*	FGC 35773											+	4, 8-11
17	42°48'35"N 02° 16'32"E	France, Occitania, (Languedoc-Roussillon-Midi-Pyrénées), Aude, 2.5 km W of Lapradelle, edge of woodland, 550 m a.s.l.	unknown (Neiber & Hausdorf 2017: ZMH 119337/ 2637)	*M. atacis* = *M. samsunensis*	ZMH 119337/ 2637		KX507213		KX495402						KX495455		
18	42°33'00"N 02° 23'00"E	France, Occitania, (Languedoc-Roussillon-Midi-Pyrénées), Pyrénées-Orientales, Vernet les Bains	unknown (Neiber & Hausdorf 2017: SP295)	*M. atacis* = *M. samsunensis*	SP295		KX507236		KX495430						KX495480		
19	41°22'45.6"N - 41° 26'14.9"N 33°45'38.5"E - 33° 45'44.7"E	Turkey, Kuzeykent neighbourhood, Kastamonu, near Kastamonu University Central Research Laboratory Application and Research Centre	G. Gürelli / 27.11.2019 / 5 (DCBC & FGC 51095)	*M. samsunensis*	Sam2			16S 19* (3)	ON350946	H3 1 (7)	ON325369	sITS2 13 (3)	ON332776			+	
					Sam3				ON350947		ON325370		ON332777			+	21-22
					Sam6	COI 12 (1)	ON332640		ON350948		ON325371		ON332778			+	
					Sam7	COI 13 (1)	ON332641	16S 20* (1)	ON350949		ON325372			lgITS2 10 (4)	ON332779		
					Sam8	COI 14 (1)	ON332642	16S 21* (1)	ON350950		ON325373				ON332780		
			G. Gürelli / 30.09.2020 / 6 (DCBC & FGC 51094)	*M. samsunensis*	Kas1	COI 13 (1)	ON332643	16S 22* (1)	ON350951		ON325374				ON332781	+	
					Kas2	COI 15 (1)	ON332644	16S 23* (1)	ON350952		ON325375				ON332782	+	5
					Kas3	COI 12 (1)	ON332645	16S 24 (1)	ON350953	H3 6 (1)	ON325376			lgITS2 11 (1)	ON332783	+	7, 20
					Kas4	COI 16 (1)	ON332646	16S 25 (1)	ON350954	H3 1 (2)	ON325377			lgITS2 12 (1)	ON332784	+	
					Kas5	COI 15 (1)	ON332647	16S 26 (1)	ON350955		ON325378			lgITS2 10 (1)	ON332785	+	
					Kas6											+	6
20	41°18'34.9"N - 41° 18'35.6"N 36°16'00.5"E - 36° 16'01.2"E	Turkey, Balaç neighbourhood, Atakum, Samsun	G. Gürelli / 8.09.2021 / 4 (DCBC & FGC 51175)	*M. samsunensis*	Bey1	COI 17 (1)	ON332648	16S 27 (1)	ON350956	H3 2 (4)	ON325379			lgITS2 13 (1)	ON332786		
					Bey2	COI 18 (1)	ON332649	16S 28 (1)	ON350957		ON325380			lgITS2 14 (3)	ON332787		16-19
					Bey3	COI 19 (1)	ON332650	16S 29 (1)	ON350958		ON325381				ON332788		15
					Bey4	COI 20 (1)	ON332651	16S 30 (1)	ON350959		ON325382				ON332789		

(*Continued*)

Table I. (Continued).

Localities						COI		16SrDNA		H3		sITS2 (5.8S rDNA + ITS2)		lgITS2 (5.8S rDNA + ITS2 + 28S rDNA)			
No.	coordinates	country and site	collector / date / no. of specimens (collection)	Revised taxonomy	Designation of voucher sps	new haplotype (no. spec.)	GenBank ##	new haplotype (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	new common sequence (no. spec.)	GenBank ##	AA	Figs
21	40°42'23"N 39° 04'09"E	Turkey, Gümüshane, Kürtün (towards Tirebolu, 0.1-0.3 km along the road from junction towards) Taslica kyü (Harsit river valley)	unknown (Neiber & Hausdorf 2017: ZMH 96199/ 2241)	*M. samsunensis*	ZMH 96199/ 2241		KX507202		KX495391						KX495444		
22	42°52'09.7"N 02° 29'06.0"E	France, Occitania, Aude, Cubières-sur-Cinoble, roadside, 419 m a.s.l.	M. Proćków / 28.06.2018 / 5 / (DCBC & MNHW-F.18.38)	*M. cartusiana*	Cur1	COI 21 (1)	ON332652	16S 31 (1)	ON350960	H3 7 (1)	ON325383						
					Cur2	COI 22 (1)	ON332653	16S 32* (1)	ON350961	H3 9 (1)	ON325384			lgITS2 15 (1)	ON332790		
					Cur3	COI 23 (1)	ON332654	16S 33 (1)	ON350962	H3 8 (1)	ON325385						
					Cur4	COI 24 (1)	ON332655	16S 34* (1)	ON350963	H3 9 (1)	ON325386	sITS2 14 (1)	ON332791				
					Cur5			16S 35* (1)	ON350964	H3 10 (1)	ON325387	sITS2 15 (1)	ON332792				

Particular gene sequences were trimmed and then deposited in GenBank with the following lengths:
Sequences COI were 684 bp long, except those for Kas1 - Kas5 which were 678 bp;
Sequences 16SrDNA were 308–317 bp long, except those marked by asterisk which were 814–821 bp;
Sequences H3 were 303 bp long;
Sequences sITS2 (ITS2 flanked by 5.8S rDNA fragment) were of 558–581 bp (71 bp 5.8S + 487–510 bp ITS2);
Sequences lgITS2 (complete ITS2 flanked by 5.8S and 28S rDNA fragments) were of 835–856 bp long (89 bp 5.8S + 489–510 bp ITS2 + 257 bp 28S).
AA – specimens used in anatomical studies marked with +.

Abbreviations: BC bursa copulatrix (also known as gametolytic gland), BW body wall, DBC duct of bursa copulatrix (also known as pedunculus), DG digitiform glands (also known as mucous glands or glandulae mucosae), DV distal vagina (from digitiform glands to genital atrium), E epiphallus (from base of flagellum to beginning of penial sheath), F flagellum, FO free oviduct, GA genital atrium, GAR genital atrium retractor, OSD ovispermiduct (also known as spermoviduct), P penis (from beginning of penial sheath to genital atrium), PP penial papilla (also known as glans), PR penial retractor, PV proximal vagina (from confluence of free oviduct and duct of bursa copulatrix to digitiform glands), VD vas deferens.

Molecular study

Sixty-three specimens of *M. atacis* and 14 of *M. samsunensis* were used in the molecular analysis (Table I). Total genomic DNA was extracted from 20 mg of foot tissue using Tissue Genomic DNA extraction MiniKit (Genoplast) following the manufacturer's instructions. Purified total DNA was used as template for amplification by polymerase chain reaction (PCR) of partial sequences of the following gene fragments: mitochondrial 5'-end of cytochrome c oxidase subunit I (COI) and large subunit ribosomal DNA gene (16S rDNA), as well as nuclear internal transcribed spacer 2 (ITS2) in ribosomal DNA flanked with 5.8S and 28S ribosomal DNA fragments (5.8S rDNA and 28S rDNA, respectively) and histone 3 (H3). Partial sequences of these gene fragments were obtained by PCR with the primer sets listed in Table III.

All PCRs were carried out with total volumes of 10 µl. The following thermal profile was used for COI amplification: 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 1 min at 50°C, 1 min at 72°C, and finally 5 min at 72°C using Type-it Microsatellite PCR kit (Qiagen) or 5 min at 95°C followed by 40 cycles of 30 s at 94°C, 30 s at 50°C, 1 min at 72°C, and finally 7 min at 72°C using tiTaq Polymerase (EUR_x). Amplifications of fragments of 16S rDNA (short fragment, see Table III), H3 and ITS2 (flanked with 5.8S rDNA and short fragment of 28S rDNA, see Table III) were performed according to procedures previously described by Manganelli et al. (2005), Colgan et al. (1998) and Almeyda-Artigas et al. (2000), respectively. Amplifications of 16S rDNA (longer fragment, see Table III) and ITS2 (flanked with 5.8S rDNA and longer fragment of 28S rDNA, see Table III) were performed with the same thermal profile as for COI amplification with tiTaq Polymerase (EUR_x), however for this ITS2 sequence, two rounds of amplifications were performed: the first with the purified total DNA as template and the second with 1 µl of the 10× diluted product from the first round as template. Lengths of amplification products were as follows: COI – 710 bp; 16S rDNA – 371–382 (short fragments) or 873–882 bp (long fragments); ITS2 (flanked with 5.8S rDNA and short fragment of 28S rDNA) – 655–681 bp; ITS2 (flanked with 5.8S rDNA and longer fragment of 28S rDNA) – 911–932 bp; H3 – 375 bp.

The PCR products were verified by agarose gel electrophoresis (1% agarose) and purified for sequencing with thermosensitive Exonuclease I and FastAP alkaline phosphatase (Fermentas, Thermo Scientific). Finally, the amplified products were sequenced in both directions using the BigDye Terminator v3.1 sequencing kit on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocols.

Sequences were edited with BioEdit version 7.0.6 (Hall 1999; BioEdit 2017). Alignments were performed with ClustalW, implemented in BioEdit (Thompson et al. 1994). The COI and H3 sequences were aligned according to the translated amino acid sequences to correct errors that could arise from the presence of ambiguous nucleotides after sequencing. The ends of all sequences were trimmed and deposited in GenBank (Table I). Sequences obtained by PCR with NEWS2 and ITS2-RIXO, and LSU1 and LSU3 primer pairs (Table III) were joined in longer sequences marked as lgITS2 (see Table I). After trimming, the lengths of sequences were 684 and 678 bp for COI, 309–317 bp for 16S rDNA short fragment, 814–821 for 16S rDNA long fragment, 558–581 for short ITS2 (marked sITS2, including 71 bp 5.8S rDNA + 487–510 bp ITS2), 835–856 bp for long ITS2 (marked lgITS2, including 89 bp 5.8S rDNA + 489–510 bp ITS2 + 257 bp 28S rDNA) and 303 bp for H3 (see also Table I). For phylogenetic analysis, the following alignments were made: 600 positions long for COI, 332 or 869 positions long for 16S rDNA, 279 position long for H3. Sequences of ITS2 alone and sequences of ITS2 flanked by fragments of 5.8S rDNA at the 3'-end and 28S rDNA at the 5'-end, for comparison with sequences obtained from GenBank, were 546 (or 564) and 856 positions of alignment length, respectively. The sequences were collapsed to haplotypes (COI, 16S rDNA) and to common sequences (H3, ITS2 flanked with 5.8S rDNA, ITS2 flanked with 5.8S rDNA and 28S rDNA) using the programme ALTER (Alignment Transformation EnviRonment)

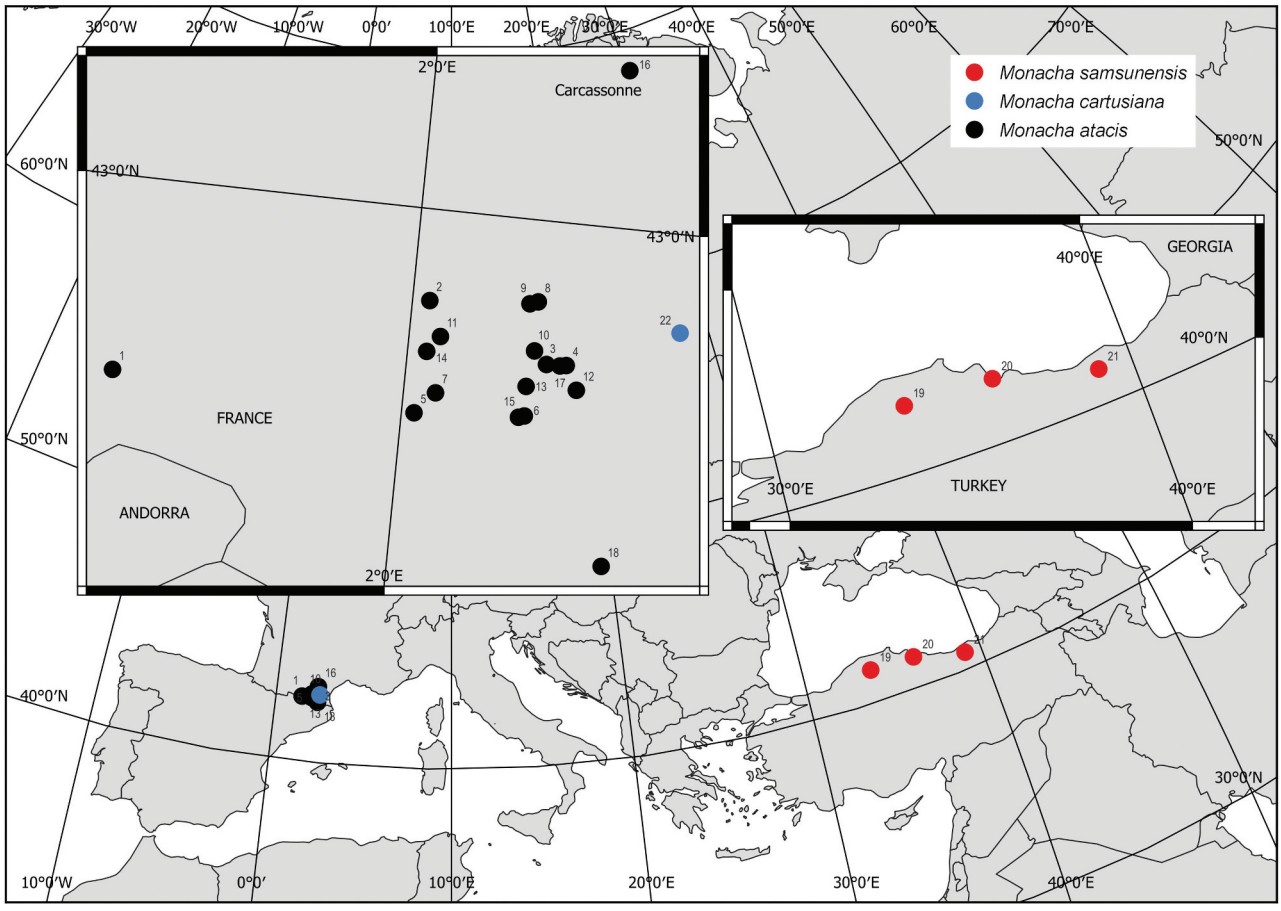


Figure 1. Map of localities of the populations of *Monacha atacis*, *M. samsunensis* and *M. cartusiana* analysed (see Table I for details).

(Glez-Peña et al. 2010). Finally COI and 16S rDNA haplotypes were joined into concatenated sequences COI+16S rDNA, 932 or 1469 positions (600 COI + 332 16S rDNA or 600 COI + 869 16S rDNA) in length, ITS2 and H3 common sequences were joined into concatenated sequences ITS2 + H3, 825 positions (546 ITS2 + 279 H3) in length and COI and 16S rDNA haplotypes were joined with ITS2 (flanked with 5.8S and 28S rDNA fragments) common sequences into concatenated sequences of COI + 16S rDNA + ITS2, 2325 positions in length (600 COI + 869 16S rDNA + 42 5.8S rDNA + 557 ITS2 + 257 28S rDNA).

For each alignment file, best nucleotide substitution models were specified according to the Bayesian Information Criterion (BIC): for COI (600 bp) – HKY+G+I, for concatenated sequences COI + short 16S rDNA (932 positions) – T92+G+I, for COI + 16S rDNA + ITS2 (flanked with 5.8S and 28S rDNA) – GTR+G + I, for COI + long 16S rDNA (1469 positions) – GTR+G, for ITS2 (564 bp) and for concatenated sequences ITS2 + H3 (825 positions) – K2 + G (Kimura 1980; Hasegawa et al. 1985; Tamura 1992; Nei & Kumar 2000; Kumar et al. 2016). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). Neighbour Joining (NJ) analysis (Saitou & Nei 1987) and Maximum Likelihood (ML) analysis were performed with MEGA7 (Kumar et al. 2016). Calculated bootstrap values obtained by ML and NJ analysis were mapped on the ML trees. In addition, Bayesian Inference (BI) was conducted for concatenated COI + 16S rDNA + ITS2 (flanked with 5.8S and 28S rDNA) sequences with the use of the programme MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003; Ronquist et al. 2012). Four Monte Carlo Markov chains were run for 1 million generations, sampling every 100 generations (the first 25% of trees were discarded as "burn-in"). Posterior probability (PP) values obtained on the 50% majority rule consensus Bayesian tree of concatenated sequences were mapped together with bootstrap values obtained by ML and NJ analysis on the ML tree.

Table II. GenBank sequences used for molecular analysis comparisons.

Species	COI	16S rDNA	H3	(5.8S rDNA) + ITS2 * (5.8S rDNA) + ITS2 + (28S rDNA)	References
Monacha atacis	KX507213, KX507236	KX495402, KX495430		KX495455, KX495480	Neiber and Hausdorf (2017)
Monacha cantiana	KX507234	KX495428		KX495478	Neiber and Hausdorf (2017)
Monacha cantiana CAN-1	MG208884, MG208905	MG208966	MG209038, MG209048	MH137963*, MH137971*, MH137972*, MH137978*	Pieńkowska et al. (2018b)
Monacha cantiana CAN-2	MG208925, MG208931	MG208996	MG209050, MG209052	MH137981*	Pieńkowska et al. (2018b)
				MK067000*	Pieńkowska et al. (2019a)
Monacha cantiana CAN-3	MG208933, MG208936	MG209005	MG209040	MH137982*, MH137983*	Pieńkowska et al. (2018b)
				MK067001*	Pieńkowska et al. (2019a)
	MN107010, MN107015				Pieńkowska et al. (2019b)
Monacha cantiana CAN-4 = *Monacha cemenelea*	MG208939, MG208940	MG209011	MG209058, MG209059	MH137984*	Pieńkowska et al. (2018b)
				MK067003*	Pieńkowska et al. (2019a)
	MT947641	MT952445			Čejka et al. (2020)
Monacha sp. CAN-5	MK066934, MK066938	MK066952	MK066970, MK066976	MK066985*, MK066991*	Pieńkowska et al. (2019a)
Monacha sp. CAN-6	MK066942, MK066943	MK066960	MK066980	MK066995*, MK066996*, MK066999*	Pieńkowska et al. (2019a)
Monacha cartusiana	KX507189, KX507235	KX495378, KX495429		KX495431, KX495479	Neiber and Hausdorf (2017)
			MG209072	MH137993*	Pieńkowska et al. (2018b)
	MT947646	MT952354			Čejka et al. (2020)
Monacha claustralis	KX507199	KX495388		KX495441	Neiber and Hausdorf (2017)
Monacha cretica	KX507190	KX495379			Neiber and Hausdorf (2017)
Monacha devrekensis				KX495470	Neiber and Hausdorf (2017)
Monacha laxa	KX507200, KX507201	KX495389, KX495390		KX495442, KX495443	Neiber and Hausdorf (2017)
Monacha ocellata	KX507220	KX495409		KX495462	Neiber and Hausdorf (2017)
	MG918127	MG918128		MG918129	Anderson et al. (2018)
Monacha pantanellii	MT380013, MT380014, MT380038, MT380063	MT376033	MT385778, MT385781, MT385808	MT376088*, MT376090*, MT376110*	Pieńkowska et al. (2020)
Monacha samsunensis	KX507202	KX495391		KX495444	Neiber and Hausdorf (2017)
Monacha parumcinta	MG208949, MG208955	MG209023	MG209066, MG209067	MH137985*, MH137987*	Pieńkowska et al. (2018b)
Monacha perfrequens	KX507191	KX495380		KX495433	Neiber and Hausdorf (2017)
Monacha tibarenica	KX507227	KX495421		KX495472	Neiber and Hausdorf (2017)

(*Continued*)

Table II. (Continued).

Species	COI	16S rDNA	H3	(5.8S rDNA) + ITS2 * (5.8S rDNA) + ITS2 + (28S rDNA)	References
Trochulus hispidus	KY818415	KY818541		KY818647	Neiber et al. (2017)
				KX495451	Neiber and Hausdorf (2017)
				MG585474	Caro et al. (2019)
			MT758614	MT755395*	Proćków et al. (2021)

Table III. Primers used in molecular analysis.

Name	Sequence 5' – 3'	References
COI		
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HC02198	TAAACTTCAGGGTGACCAAAAAATCA	
F01	CATTTTCHACTAAYCATAARGATATTGG	Dabert et al. (2010)
R04	TATAAACYTCDGGATGNCCAAAAAA	
16S rRNA (shorter fragment)		
LR-J-12887	CGATTTGAACTCAGATCA	Simon et al. (1994)
(reverse)	GTGCAAAGGTAGCATAATCA	Gantenbein et al. (1999)
16S rRNA (longer fragment)		
16Scs1	AAACATACCTTTTGCATAATGG	Chiba (1999)
16Scs2	AGAAACTGACCTGGCTTACG	
ITS2 (flanked with 5.8S rDNA and short fragment of 28S rDNA)		
NEWS2	TGTGTCGATGAAGAACGCAG	Almeyda-Artigas et al. (2000)
ITS2-RIXO	TTCTATGCTTAAATTCAGGGG	
ITS2 (flanked with for 5.8S rDNA and longer fragment of 28S rDNA)		
LSU1	CTAGCTGCGAGAATTAATGTGA	Wade and Mordan (2000)
LSU3	ACTTTCCCTCACGGTACTTG	
H3		
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)
H3R	ATATCCTTRGGCATRATRGTGAC	

Results

Morphological study

Monacha atacis and *M. samsunensis* have globose to subglobose shell (Figures 2–7), sometimes variably depressed, variable in size (in *M. atacis*: diam. 7.5–15.2 mm; Gittenberger & de Winter 1985; in *M. samsunensis*: diam. 11.0–19.1 mm; Hausdorf 2000a), pale yellowish, pale ochre or whitish in colour (creamy-white, sometimes with sparse darker stripes in *M. samsunensis*), sometimes with one whitish peripheral band (only in some light horn-coloured specimens of *M. samsunensis* according to Hausdorf 2000a), surface with fine irregular growth-ridges, very fine spiral striae and evident hair scars on the first whorls. Aperture roundish to oval, slightly descending, with a variably thick white internal rib (only very evident in *M. samsunensis*). Peristome interrupted, its columellar margin reflexed to more or less cover the umbilicus, which may be very small to almost closed or rather open (for *M. atacis*, see also Gittenberger & de Winter 1985: fig. 2; Welter-Schultes 2012: fig. at p. 503; for *M. samsunensis*, see also Hudec & Ležava 1969: pl.

10 fig. 25; Schileyko 1978: pl. 16 fig. 159; Hausdorf 2000a: pl. 12 figs 56–57; Schileyko 2005: fig. 2534a; Welter-Schultes 2012: fig. at p. 512).

Monacha atacis and *M. samsunensis* show distal genitalia of the *Metatheba* type, i.e. with penial retractor muscle but without vaginal appendix. The literature reports evidence of a long vagina, bursa copulatrix duct and flagellum in *M. atacis* (Gittenberger & de Winter 1985) and a variably long vagina, bursa copulatrix duct and flagellum in *M. samsunensis* (Hesse 1931; Hudec & Ležava 1969; Hudec 1973; Schileyko 1978; Hausdorf 2000a). This picture was confirmed by our anatomical study. Both species showed vagina with very short proximal section and variably long distal section. In *M. atacis*, the distal vagina is long and variably slender, sometimes widening slightly in its subterminal portion where the internal surface shows a series of small swollen pleats (Figures 8 and 12). In *M. samsunensis*, the distal vagina is variably long and wide: sometimes long and slender (Figures 15 and 16; see also Hesse 1931: pl. 6 figs 55a-b; Hudec & Ležava 1969: fig. 22; Hudec 1973: fig. 6; Hausdorf 2000a: fig. 50) and at other times rather or very short (Figures 20 and 21). When short or very short, the vagina is also wider distally (Figure 21) or very wide with an internal ring of variably raised pleats (Figure 22), a detail never mentioned before. The duct of the bursa copulatrix is very long in *M. atacis* (Figures 8 and 12; see also Gittenberger & de Winter 1985: figs. 7–8) and variably long in *M. samsunensis*, ranging from very short or short (Hesse 1931: pl. 6 fig. 55a; Hudec & Ležava 1969: fig. 22) to long or very long (Figures 15, 16, 20 and 21; see also Hudec 1973: fig. 6; Hausdorf 2000a: fig. 50). Finally the flagellum is rather long in *M. atacis* (Figures 8, 12; see also Gittenberger & de Winter 1985: figs. 7–8) and variably long in *M. samsunensis*, ranging from short (Figures 20 and 21; see also Hesse 1931: pl. 6 fig. 55a; Hudec & Ležava 1969: fig. 22; Hudec 1973: fig. 6) to medium or rather long (Figures 15 and 16; see also Schileyko 1978: fig. 377; Hausdorf 2000a: fig. 50).

Molecular study

Two hundred and ninety-two new sequences were obtained and deposited in GenBank: 221 for *M. atacis* (61 COI, 62 16S rDNA, 58 H3, 20 sITS2 and 20 lgITS2), 54 for *M. samsunensis* (12 COI, 14 16S rDNA, 14 H3, 3 sITS2 and 11 lgITS2) and 17 for *M. cartusiana* (4 COI, 5 16S rDNA, 5 H3, 2 sITS2 and 1 lgITS2) (for details on their lengths and GenBank accession numbers, see Table I). We identified 24 COI haplotypes (eleven COI 1 – COI 11 for *M. atacis*, nine COI 12 – COI 20 for *M. samsunensis*, four COI 17 – COI 20 for *M. cartusiana*) and 35 16S rDNA haplotypes (18 16S 1–16S 18 for *M. atacis*; 12 16S 19–16S 30 for *M. samsunensis*; 5 16S 31–16S 35 for *M. cartusiana*). Among sequences of the H3 gene, 10 common sequences were identified (6 H3 1 – H3 6 for *M. atacis* and *M. samsunensis*; 4 H3 7 – H3 10 for *M. cartusiana*). We established 15 short ITS2 (sITS2: 5.8S rDNA + ITS2) and 15 long ITS2 (lgITS2: 5.8S rDNA + ITS2 + 28S rDNA) common sequences (for *M. atacis* – 12 sITS2: sITS2 1 – sITS2 12 and 9 lnITS2: lnITS2 1 – lnITS2 9; for *M. samsunensis* – one sITS2: sITS2 13 and 4 lnITS2: lgITS2 10 – lgITS2 14; for *M. cartusiana*: two sITS2: sITS2 14 – sITS2 15 and one lgITS2: lgITS2 13). Haplotypes and common sequences were used for phylogenetic analysis.

In the case of mitochondrial gene fragments (COI and 16S rDNA), sequences obtained from *M. atacis* and *M. samsunensis* were analysed separately as single locus data sets (not shown, except COI sequence analysis, see Supplementary Material Figure S1) or as concatenated COI + short 16S rDNA sequences (Figure 23 and Supplementary Material Table S1). A tree of similar topology was obtained from analysis of concatenated COI + long 16S rDNA sequences (Figure S2 and Supplementary Material Table S1). The *M. atacis* and *M. samsunensis* sequences clustered together in these trees, although two groups, one for *M. atacis* and another for *M. samsunensis* sequences, can be seen. In the case of new sequences obtained from the population of *M. cartusiana* from Cubières-sur-Cinoble, southern France, in the concatenated COI + 16S rDNA tree (Figure 23, see also Supplementary Material Table S1), they clustered quite separately from *M. atacis* and *M. samsunensis*, as well as from other representatives of *Metatheba*, but together with other COI and 16S rDNA sequences of *M. cartusiana* obtained from GenBank.

K2P distances for COI sequences characteristic of intraspecies differentiation were very small: mean 0.6% (range 0.0–1.4%) for *M. atacis* and mean 3.5% (range 0.0–7.6%) for *M. samsunensis* (Table IV). K2P distances between COI sequences of *M. atacis* and *M. samsunensis* were also small (mean 3.5%, range 1.2–6.9%, Table IV). They were an order of magnitude smaller than the distances that distinguished these two taxa from the other species of the

Figure 2–7. Shells of *Monacha atacis* from France: Grotte de Majestier [Maj3] (DCBC & MNHW-F.18.41; FGC 51099) (2), Saint-Ferriol [Fer2-1] (DCBC & MNHW-F.18.39; FGC 51097) (3) and Carcassonne (FGC 35773) (4) and *Monacha samsunensis* from Turkey: Kastamonu [Kas2: 5; Kas6: 6; Kas3: 7] (DCBC; FGC 51094) (5–7).

subgenus *Metatheba* i.e. *M. perfrequens, M. laxa* and *M. tibarenica* (3.5% vs. 13.0–18.6%) analysed here. The K2P distances separating the COI sequences of *M. atacis* and *M. samsunensis* from species of other *Monacha* subgenera were even greater (19.1–22.9%). K2P distances within

the southern French *M. cartusiana* populations, as well as between them and other *Monacha* species (0.4% intraspecies, 14.5% and 19.9% *M. cartusiana* vs. *M. claustralis* and *M. cantiana*, respectively; Table IV) were similar to those reported in our previous papers (Pieńkowska et al. 2015, 2016, 2018a).

Analysis of nuclear genes was hindered by the fact that only the 5.8S rDNA + ITS2 + 28S rDNA gene sequences (lgITS2) were deposited in great number in GenBank by Neiber and Hausdorf (2017), including *Metatheba* subgenus representatives. For the H3 gene, GenBank only contains sequences of the *M. cantiana* s.l. complex, deposited in connection with our previous papers (Pieńkowska et al. 2018b, 2019a, 2020). We therefore present two trees, based on various analyses of single or multiple locus data sets, one consisting of the ITS2 gene sequences cut off from flanking fragments (Figure 24) and the other built from concatenated ITS2 + H3 sequences (Figure 25, Supplementary Material Table S2). They confirm the results obtained with mitochondrial genes. The sequences obtained from *M. atacis* and *M. samsunensis* specimens are grouped into a common clade, and because they are mixed with each other, no separate subgroups can be distinguished (Figures 24 and 25). It is noteworthy that the sequence KX495444 deposited in GenBank by Neiber and Hausdorf (2017) for ITS2 of *M. samsunensis* is identical to the sequences lgITS2 1, found in some specimens of *M. atacis* from different French populations (Arties, Le Chandelier, Axat, Saint-Martin-Lys, Belfort-sur-Rebenty, Salvezines and Roquefort-de-Sault; Table I). Moreover, the H3 1 sequence was found in 9 out of the 10 specimens of *M. samsunensis* from Kastamonu as well as in nine specimens of *M. atacis* from eight French populations (Arties, Le Chandelier, Mijanès, Campagna-de-Sault, Saint-Ferriol 2, Belfort-sur-Rebenty 1, Salvezines and Roquefort-de-Sault; Table I) and the H3 2 sequence was found in four *M. samsunensis* specimens from Atakum/Samsun as well as in 44 specimens from 14 French populations (i.e. all but one: Le Chandelier, however only one specimen was available from this population; Table I).

Finally, we present an analysis of concatenated mitochondrial and nuclear gene sequences: COI + 16S rDNA + 5.8S rDNA + ITS2 + 28S rDNA conducted by three different methods (ML, NJ and BI) (Figure 26, Supplementary Material, Table S3). Again, sequences from *M. atacis* and *M. samsunensis* clustered in two slightly separate subgroups, but as a common clade they were clearly separate from sequences of other *Monacha* (*Metatheba*) and *Monacha* s.s. species.

Discussion

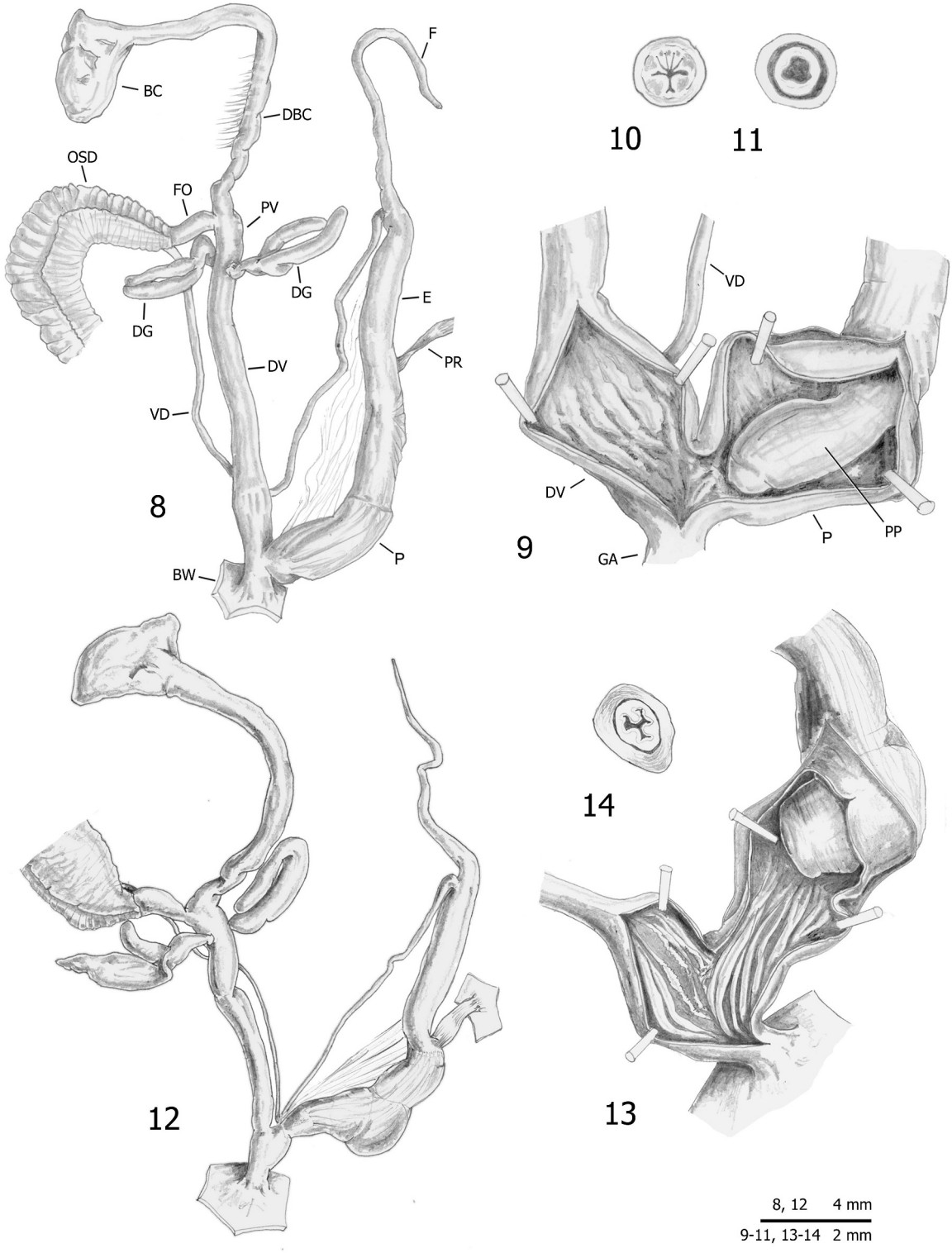
The shells of *M. atacis* from southern France and *M. samsunensis* from Turkey (Atakum/Samsun and Kastamonu) are very similar and do not differ from the lectotype of *M. samsunensis* deposited in the Naturhistorisches Museum Wien (Figure 27, see also Hausdorf 2000a: pl. 11, fig. 54).

The distal genitalia of *M. atacis* specimens from Carcassonne (Figures 8–11) and Lapradelle (Figures 12–14), characterised by a long vagina, a long bursa copulatrix duct and a long flagellum, exactly match those of the original description of this species (Gittenberger & de Winter 1985: figs 7–8). Specimens of *M. samsunensis* from the type locality (Figures 15–19) and the literature (Hesse 1931: pl. 6 fig. 55a; Hudec & Ležava 1969: fig. 22; Hudec 1973: fig. 6; Schileyko 1978: fig. 377; Hausdorf 2000a: fig. 50; Schileyko 2005: fig. 2534b) are usually characterised by a shorter flagellum, vagina and bursa copulatrix duct. Specimens of *M. samsunensis* from Kastamonu also seem to have a much shorter vagina than the others (Figures 20 and 21). However, vagina length is variable in *M. samsunensis* populations and possibly depends on sexual maturation (Hausdorf 2000a: tables 10 & 11, vagina total length 1.7–7.2 mm, measured in 35 specimens from different populations). In contrast to *M. samsunensis*, the features of the distal genitalia of *M. atacis* seem to vary little. In the absence of a more integrative approach to the study of the Turkish *Metatheba*, it is difficult to explain the significance of this pattern. For example, Gittenberger and de Winter (1985) wondered if the various figures of *M. samsunensis* reported in the literature really belong to a single species. However, high intra- and interpopulation variability is well known among the *Monacha* species so much so that the species of this genus can only occasionally be recognised morphologically (Pieńkowska et al. 2018b, 2019a, 2020).

Some sequences of nuclear genes (H3 and lgITS2) obtained from specimens of *M. atacis* and *M. samsunensis* are exactly the same. The sequences of nuclear genes from these two species mixed and grouped together in a common clade on phylogenetic trees (Figures 24 and 25). The sequences of their mitochondrial genes also cluster together (Figure 23, also Supplementary Material Figure S1) as found also in those of the concatenated mitochondrial and nuclear genes (Figure 26). Although there are two separate subgroups for sequences of *M. atacis* and *M. samsunensis* in these analyses, they create a single strongly supported clade on both phylogenetic trees (Figures 23 and

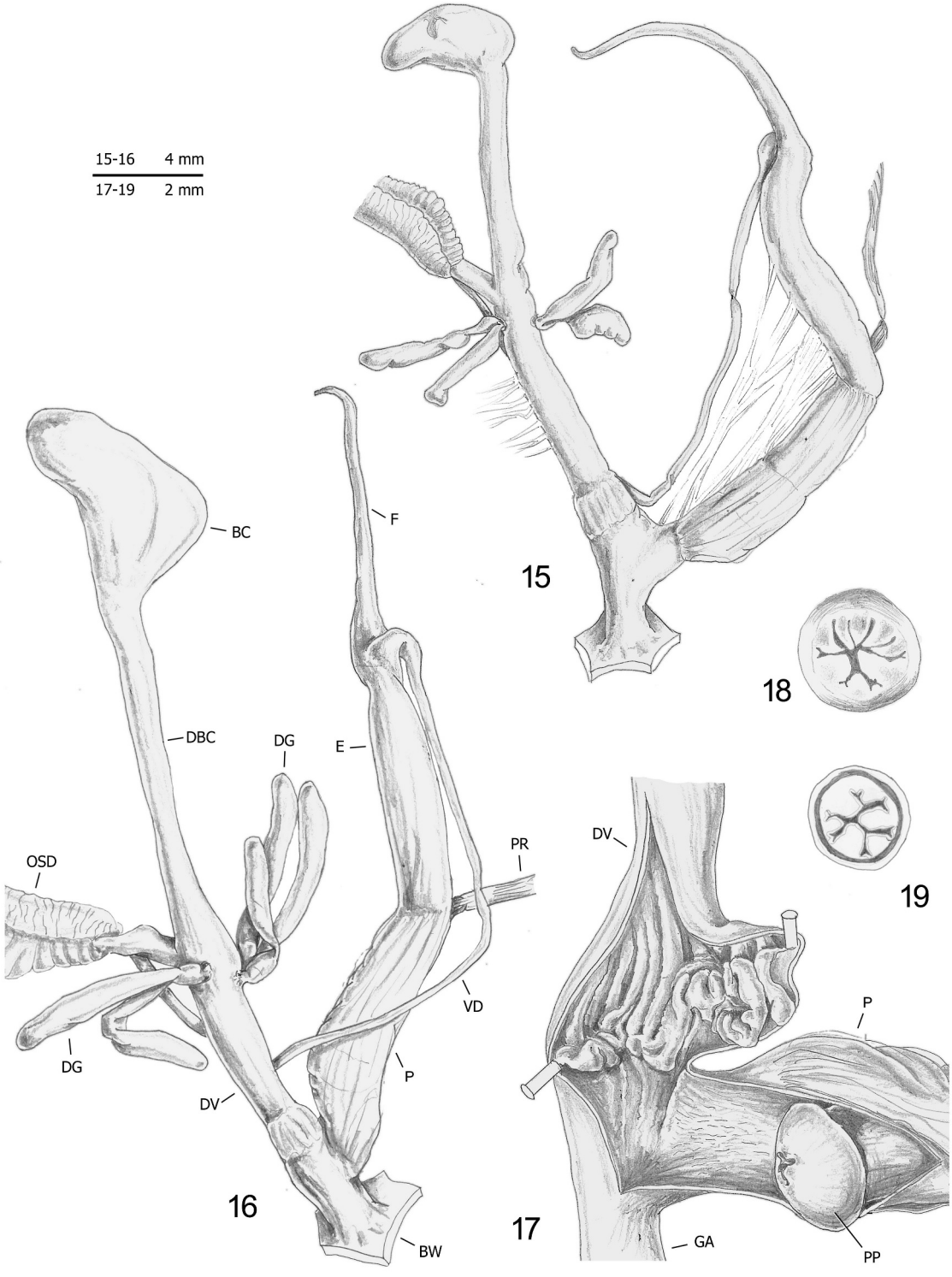
26). The mean K2P distance for COI sequences between *M. atacis* and *M. samsunensis* is small, reaching 3.5% (Table IV) which is almost at the 3% threshold of the "barcode method" based on COI sequences (Hebert et al. 2003a, 2003b; Pentinsaari et al. 2020).

It is noteworthy that the mean K2P distance between the French *M. atacis* and the topotypical



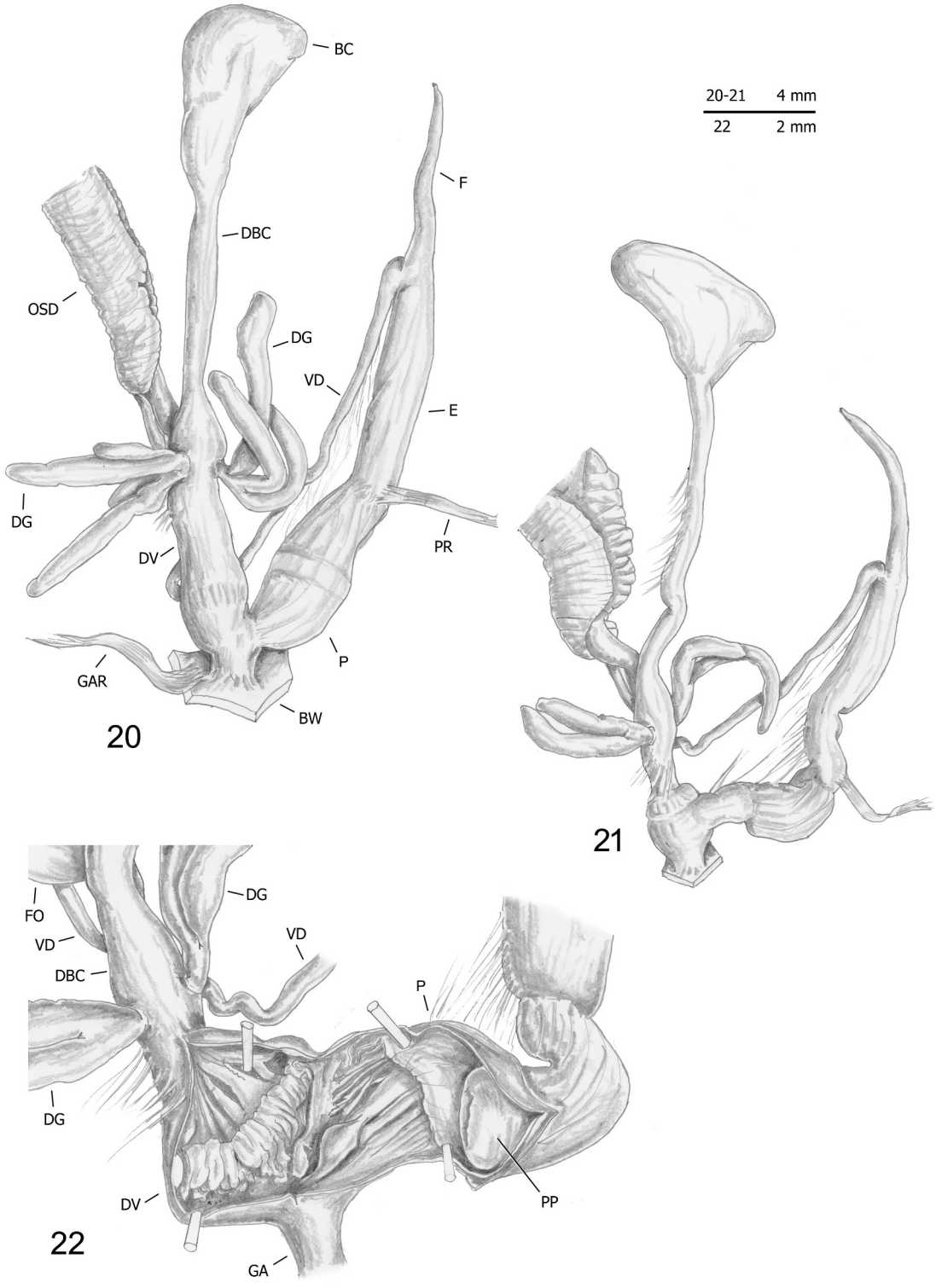
Figures 8–14. Distal genitalia (8, 12), internal structure of distal genitalia (9, 13), transverse sections of medial epiphallus (10) and apical penial papilla (11, 14) of *Monacha atacis* from France: Carcassonne (FGC 35773) (8–11) and Lapradelle (DCBC & MNHW-F.18.27; FGC 51247) (12–14).

M. samsunensis from Atakum/Samsun is smallest (2.8%) when the Turkish populations are analysed separately (Table V). The mean K2P distances between *M. atacis* and *M. samsunensis* from the Kastamonu population as well as between *M. atacis* and *M. samsunensis* from Kürtün (sequence KX507202 from Neiber & Hausdorf 2017) were 3.8% and 4.4%, respectively (Table V).



Figures 15–19. Distal genitalia (15–16), internal structure of distal genitalia (17), transverse sections of medial epiphallus (18) and apical penial papilla (19) of *Monacha samsunensis* from Turkey: Atakum/Samsun [Bey3: 15; Bey2: 16–19] (DCBC; FGC 51175).

Nevertheless it must be stressed that Turkish populations vary in K2P distances between COI sequences (see K2P distances between three Turkish populations as well as the ranges of K2P distances, Table V), which may suggest that they are somewhat genetically differentiated. We are aware of limits of the barcode method in the analysis of taxonomic relations of stylommatophoran snails



Figures 20–22. Distal genitalia (20–21) and internal structure of distal genitalia (22) of *Monacha samsunensis* from Turkey: Kastamonu [Kas3: 20; Sam3: 21–22] (DCBC; FGC 51094, 51095).

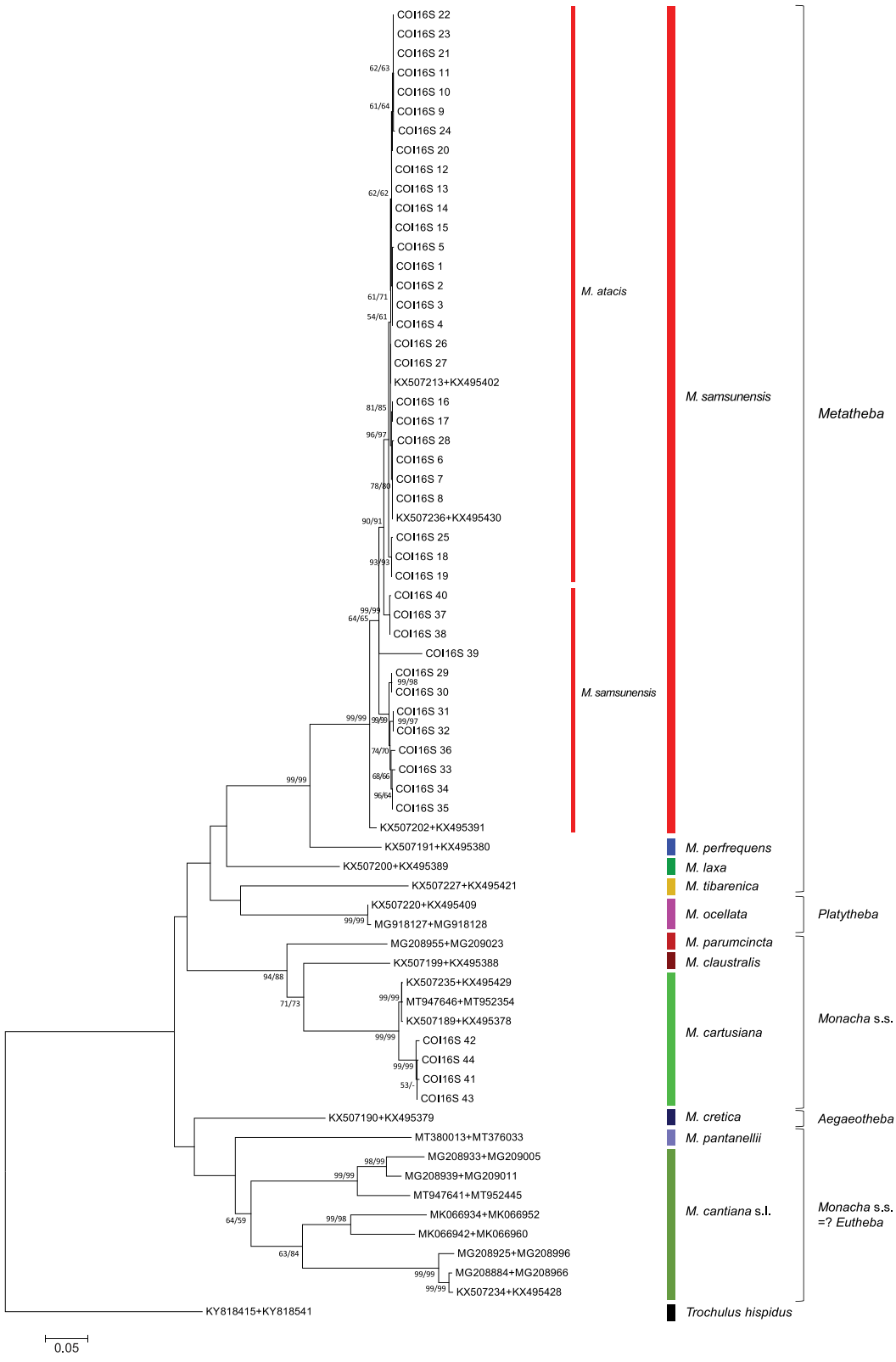


Figure 23. Maximum Likelihood (ML) tree of concatenated COI + (short) 16S rDNA haplotypes obtained from specimens of *Monacha atacis* and *Monacha samsunensis* compared with sequences obtained from GenBank for representatives of the other *Monacha* species. Concatenated COI + 16S rDNA sequences (Table S1) were cut to 932 positions (600 bp COI + 332 bp 16S) in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates from ML (left) and NJ (right) analysis (Felsenstein 1985). The tree was rooted with *Trochulus hispidus* concatenated sequences KY818415 + KY818541 deposited in GenBank by Neiber et al. (2017).

Table IV. Ranges of K2P genetic distances between analysed COI sequences (mean value in parenthesis).

Comparison	COI (%)
Within *M. atacis*	0.0–1.4 (0.6)
Within *M. samsunensis*	0.0–7.6 (3.5)
Within *M. cartusiana*	0.0–0.7 (0.4)
Between *M. atacis* and *M. samsunensis*	1.2–6.9 (3.5)
Between *M. atacis* and *M. perfrequens*	12.6–13.6 (13.0)
Between *M. atacis* and *M. laxa*	17.3–18.6 (17.9)
Between *M. atacis* and *M. tibarenica*	17.5–18.1 (17.8)
Between *M. atacis* and *M. parumcincta*	17.9–20.1 (19.1)
Between *M. atacis* and *M. claustralis*	18.7–20.5 (19.5)
Between *M. atacis* and *M. cartusiana*	19.6–21.7 (20.6)
Between *M. atacis* and *M. cantiana*	22.2–23.4 (22.9)
Between *M. samsunensis* and *M. perfrequens*	12.6–15.9 (13.4)
Between *M. samsunensis* and *M. laxa*	17.7–21.9 (18.6)
Between *M. samsunensis* and *M. tibarenica*	16.4–21.0 (17.4)
Between *M. samsunensis* and *M. parumcincta*	18.8–22.5 (20.3)
Between *M. samsunensis* and *M. claustralis*	19.6–23.0 (20.6)
Between *M. samsunensis* and *M. cartusiana*	20.1–23.2 (21.4)
Between *M. samsunensis* and *M. cantiana*	21.7–25.8 (22.5)
Between *M. perfrequens* and *M. laxa*	16.9
Between *M. perfrequens* and *M. tibarenica*	18.1
Between *M. perfrequens* and *M. parumcincta*	19.7–20.6 (20.2)
Between *M. perfrequens* and *M. claustralis*	19.2
Between *M. perfrequens* and *M. cartusiana*	20.7–21.6 (21.1)
Between *M. perfrequens* and *M. cantiana*	23.1–23.3 (23.2)
Between *M. laxa* and *M. tibarenica*	17.2
Between *M. laxa* and *M. parumcincta*	17.2
Between *M. laxa* and *M. claustralis*	17.5
Between *M. laxa* and *M. cartusiana*	18.2–18.7 (18.4)
Between *M. laxa* and *M. cantiana*	18.5
Between *M. tibarenica* and *M. parumcincta*	18.8–19.2 (19.0)
Between *M. tibarenica* and *M. claustralis*	18.0
Between *M. tibarenica* and *M. cartusiana*	20.4–20.9 (20.7)
Between *M. tibarenica* and *M. cantiana*	20.7–20.9 (20.8)
Between *M. claustralis* and *M. cartusiana*	14.2–14.6 (14.5)
Between *M. cartusiana* and *M. cantiana*	19.4–20.3 (19.9)

(Davison et al. 2009; Sauer & Hausdorf 2010, 2012; Köhler & Johnson 2012; see also discussions in our previous papers Pieńkowska et al. 2018b, 2019a, 2020). Nevertheless in this study we used Hebert's method to confirm conspecificity and not to support the conclusion about species distinctness.

Incidentally, we used molecular analysis to confirm the occurrence of *M. cartusiana* in southern France (population from Cubières-sur-Cinoble in Aude, Table I), where it may co-occur with *M. atacis*. This confirms previous molecular reports of *M. cartusiana* in France (Dahirel et al. 2015: north-western France 48°07′51″N, 01°41′34″W, near Rennes; Čejka et al. 2020: fig. 3 – in Provence, southern France: 43°31′34.7"N, 05°04′30.7"E, L'Etang de Berre; 43°49′13.1"N, 05°18′29.5"E, Bonnieux; 43°37′03.7"N, 05°18′37.8"E, St. Cannat; 43°37′58.4"N, 05°38′37.3"E, Jouques; 43°39′33.5"N, 05°20′43.1"E, Rognes).

In conclusion, our morphological (shell and genitalia, Figures 2–22 and 27) and molecular (mitochondrial and nuclear gene sequences, Figures 23–26) findings corroborate that *M. atacis* and *M. samsunensis* are conspecific and that the former should be named *M. samsunensis* because the name introduced by Pfeiffer in 1868 has priority over that established by

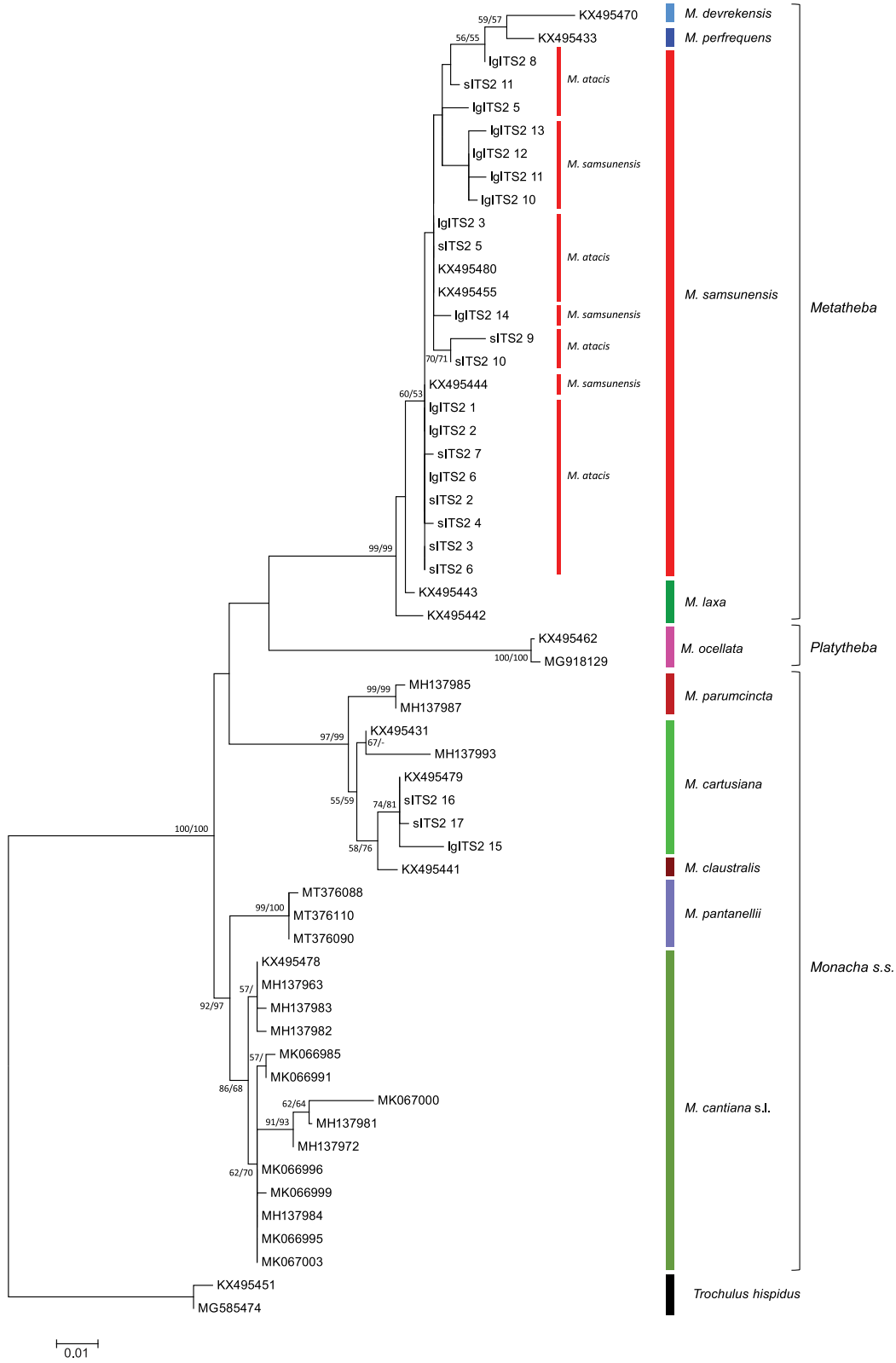


Figure 24. Maximum Likelihood (ML) tree of ITS2 common sequences obtained from specimens of *Monacha atacis* and *Monacha samsunensis* compared with sequences obtained from GenBank for representatives of the other *Monacha* species. ITS2 sequences were cut to 564 positions in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates from ML (left) and NJ (right) analysis (Felsenstein 1985). The tree was rooted with *Trochulus hispidus* ITS2 sequences KX495451 and MG585474 deposited in GenBank by Neiber and Hausdorf (2017) and Caro et al. (2019), respectively.

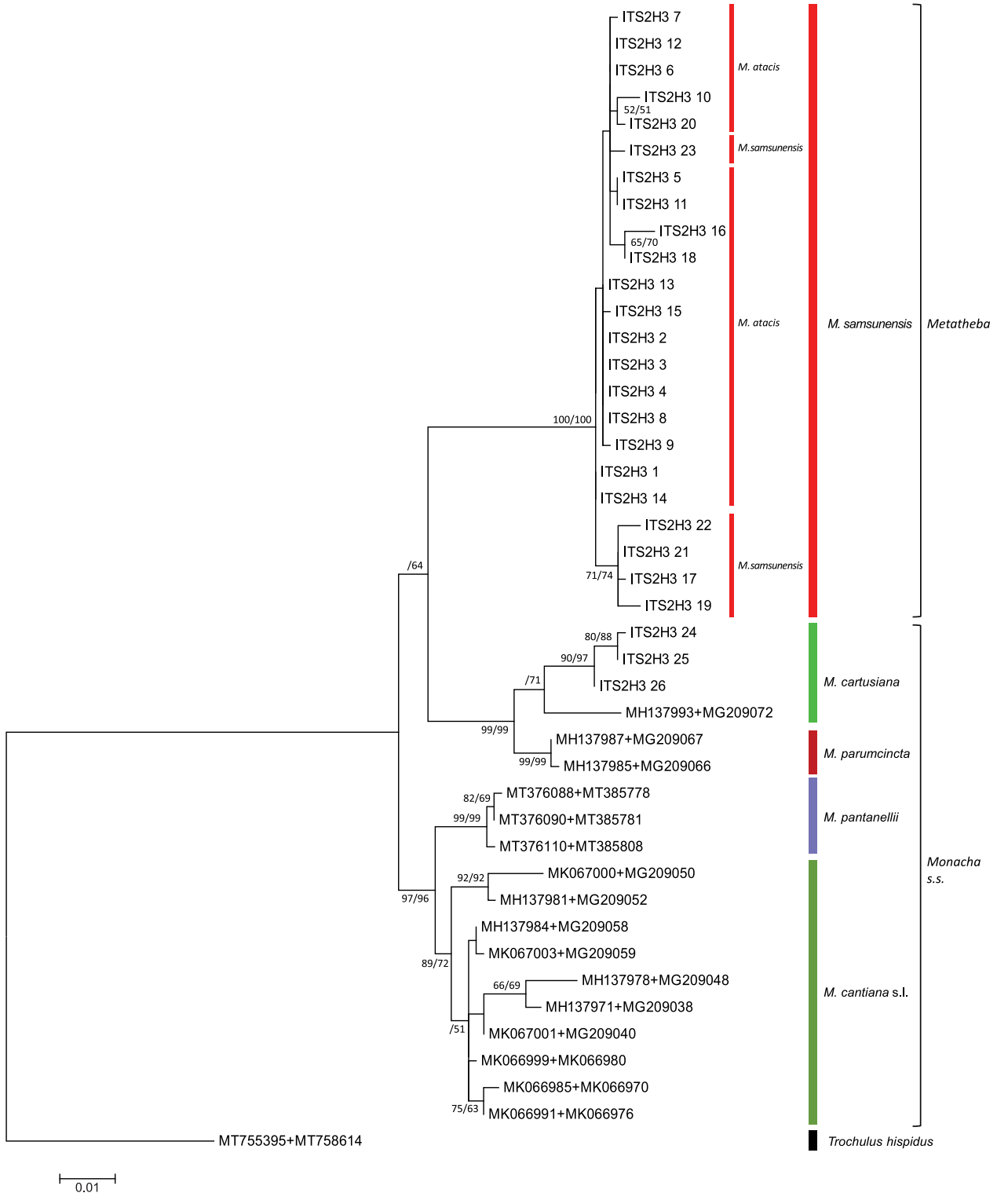


Figure 25. Maximum Likelihood (ML) tree of concatenated ITS2 + H3 common sequences obtained from specimens of *Monacha atacis* and *Monacha samsunensis* compared with sequences obtained from GenBank for representatives of the other *Monacha* species. Concatenated ITS2 + H3 sequences (Table S2) were cut to 825 positions (546 positions ITS2 and 279 positions H3) in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates from ML (left) and NJ (right) analysis (Felsenstein 1985). The tree was rooted with *Trochulus hispidus* ITS2 + H3 concatenated sequences MT755395 and MT758614 deposited in GenBank by Proćków et al. (2021).

Gittenberger & de Winter in 1985. According to Hausdorf (2000a) the occurrence of *M. atacis* in France is the result of an introduction of *M. samsunensis* in historic times. This possibility is supported by the fact that *M. atacis* occurs in a rather small area of France. However, the diversity of rapidly evolving mitochondrial genes may indicate that the French populations differentiated since their introduction. They may represent a distinct lineage that originated in France after their introduction (according to Falkner et al. 2002, the species has been reported from France at least since the 19th century) by the founder effect or by selection. This hypothesis cannot be verified without further research on a greater number of French populations of *M. samsunensis* (possibly also those from Catalonia, Spain).

On the other hand, it seems that there is greater genetic differentiation between Turkish populations from Atakum/Samsun, Kastamonu and Kürtün. It is noteworthy that there is more genetic similarity between specimens of *M. atacis* from France and the topotypical *M. samsunensis* from Atakum/Samsun than between Atakum/Samsun and Kastamonu populations. *M. samsunensis* has a wider distribution in Turkey than in France, and has probably existed there for much longer. A reason for the lower variability observed within French populations may be their smaller range and shorter evolution. The variability in populations of *M. samsunensis* occurring in northern Anatolia and along the Black Sea coast is worthy of further study.

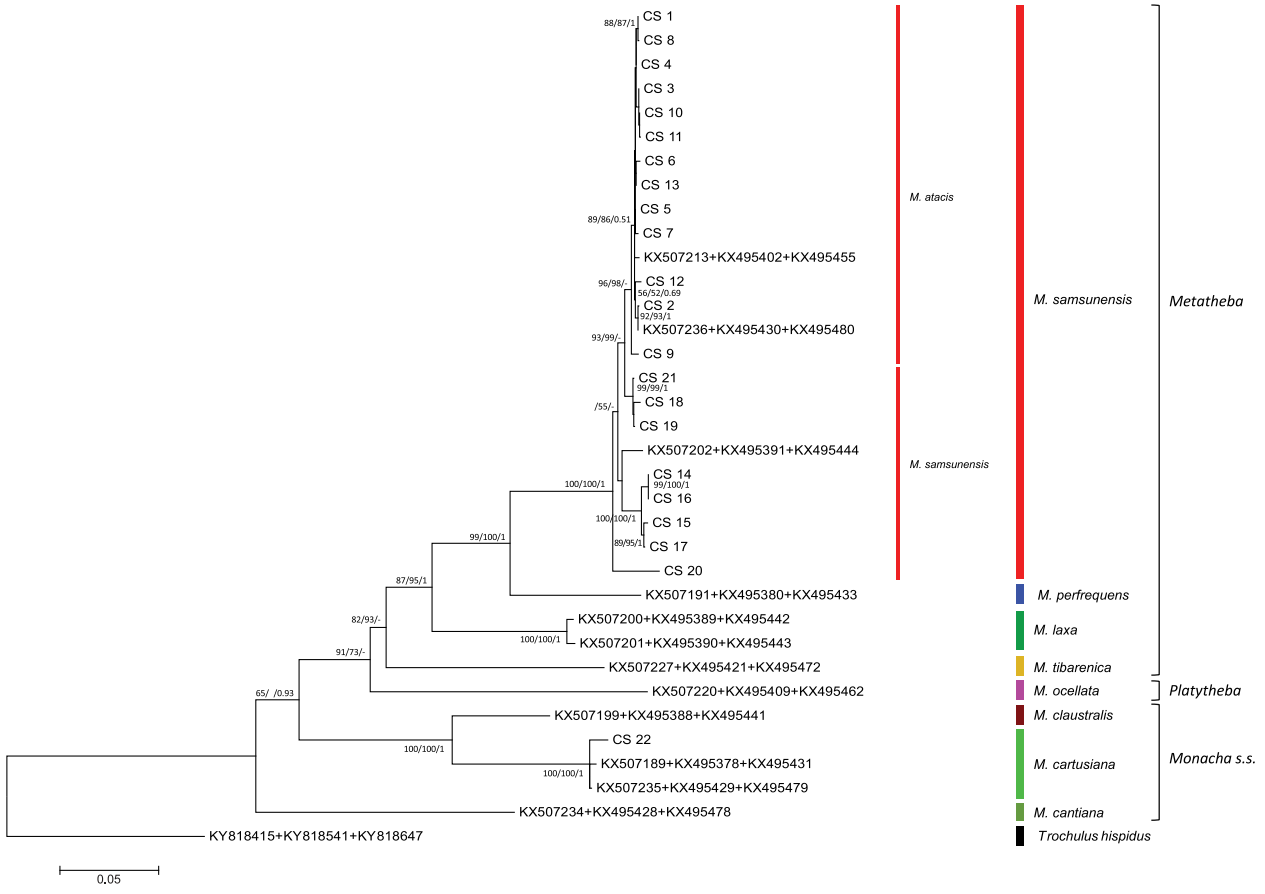


Figure 26. Maximum Likelihood (ML) tree of concatenated COI + 16S rDNA + (5.8S rDNA + ITS2 + 28S rDNA) sequences of *Monacha atacis* and *Monacha samsunensis* compared with sequences obtained from GenBank for representatives of the other *Monacha* species. Concatenated sequences (Table S3) were 2325 positions in length (600 COI + 869 16S rDNA + 42 5.8S rDNA + 557 ITS2 + 257 28S rDNA). Bootstrap support above 50% from ML (left) and NJ (middle) analysis as well as posterior probabilities (right) from Bayesian inference analysis are marked at the nodes. Bootstrap analysis was run with 1000 replicates (Felsenstein 1985). The tree was rooted with *Trochulus hispidus* COI + 16S rDNA + (5.8S rDNA + ITS2 + 28S rDNA) concatenated sequences KY818415 + KY818541 + KY818647 deposited in GenBank by Neiber et al. (2017).

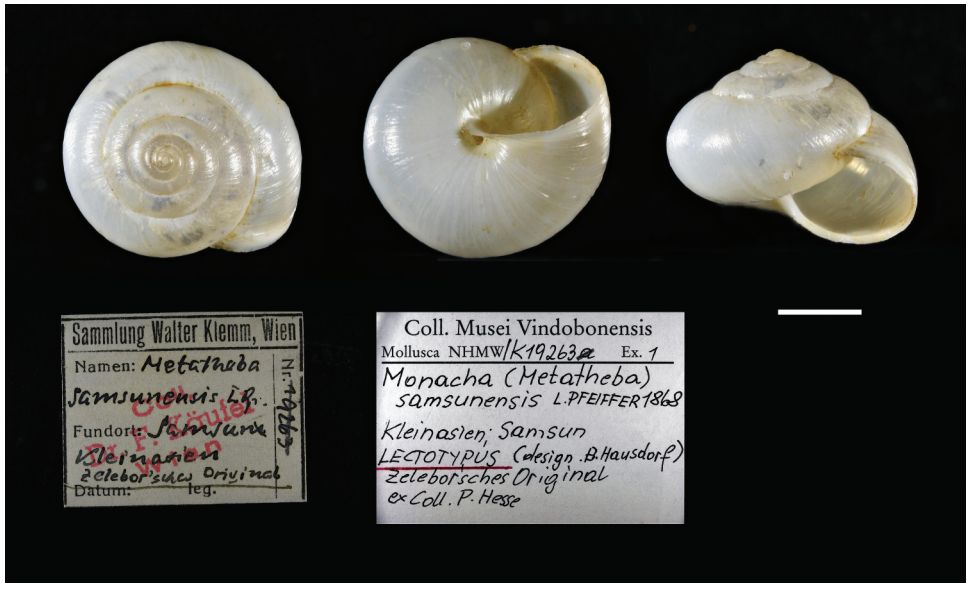


Figure 27. Shell of the *Monacha samsunensis* lectotype designated by Hausdorf (2000a), kept in the Naturhistorischen Museum Wien (© NHMW, the inventory number: NHMW-MO-79000/K/19263) (photo by Sara Schnedl obtained by courtesy of Anita Eschner, NHMW). Scale bar 5 mm.

Table V. Ranges of K2P genetic distances between COI sequences of *M. atacis* and *M. samsunensis* populations (mean value in parenthesis).

Comparison	COI (%)
Within *M. atacis* (southern France)	0.0–1.4 (0.6)
Within *M. samsunensis* (Anatolia Atakum/Samsun)	0.0–6.5 (3.5)
Within *M. samsunensis* (Anatolia Kastamonu)	0.3–0.8 (0.7)
Within *M. samsunensis* (Anatolia Kürtün, Gümüshane)	n/a
Between *M. atacis* (southern France) and *M. samsunensis* (Anatolia Atakum/Samsun)	1.2–6.9 (2.8)
Between *M. atacis* (southern France) and *M. samsunensis* (Anatolia Kastamonu)	3.3–4.3 (3.8)
Between *M. atacis* (southern France) and *M. samsunensis* (Anatolia Kürtün, Gümüshane)	4.1–4.8 (4.4)
Between *M. samsunensis* (Anatolia Atakum/Samsun) and *M. samsunensis* (Anatolia Kastamonu)	3.1–7.0 (4.4)
Between *M. samsunensis* (Anatolia Atakum/Samsun) and *M. samsunensis* (Anatolia Kürtün, Gümüshane)	4.1–7.6 (5.0)
Between *M. samsunensis* (Anatolia Kastamonu) and *M. samsunensis* (Anatolia Kürtün, Gümüshane)	4.5–4.8 (4.6)

The introduction of *M. samsunensis* to Western Europe is not an isolated case among the *Monacha* hygromiids. A population of *M. ocellata* was recently found in England, where it was accidentally introduced from the Istanbul area, Turkey (Anderson et al. 2018).

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Disclosure statement

The authors declare that they do not have any conflict of interests.

Supplementary material

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