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## *Monacha claustralis* (ROSSMÄSSLER 1834), a hygromiid snail new to Germany

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**Abstract:** Colonies originally identified as *Monacha cartusiana* have often turned out to be *M. claustralis*. This was the case with a sample collected in 2016 from near Jena, and thus the first record of *M. claustralis* from Germany. We compare these animals' genital anatomy against several distinguishing characters advocated in the literature. A partial-COI sequence indicates a close genetic relationship with Polish colonies near Gdansk and Kielce. Although originally from western Turkey and adjacent parts of the Balkan Peninsula, *M. claustralis* is liable to have established itself at other sites in Germany, maybe sometimes hidden in mixed colonies with *M. cartusiana*, from which it is indistinguishable using external characters.

**Keywords:** COI, first occurrence, genital anatomy, introduced species, *Monacha cartusiana*

**Zusammenfassung:** Ursprünglich als *Monacha cartusiana* determinierte Vorkommen haben sich oft nachträglich als *M. claustralis* erwiesen. Dies ist nun auch der Fall für eine Serie, die 2016 nahe Jena gesammelt wurde und den Erstfund von *M. claustralis* für Deutschland darstellt. Wir vergleichen die Genitalanatomie der Tiere mit Unterscheidungsmerkmalen, die in der Literatur angegeben werden. Eine COI-Teilsequenz indiziert eine enge Beziehung zu polnischen Kolonien nahe Gdansk und Kielce. *Monacha claustralis* stammt ursprünglich aus der westlichen Türkei und angrenzenden Gebieten der Balkan-Halbinsel. Es ist aber anzunehmen, dass die Art sich auch an anderen Lokalitäten in Deutschland angesiedelt hat, evtl. manchmal in gemischten Kolonien zusammen mit *M. cartusiana*, von der sie anhand äußerer Merkmale nicht unterschieden werden kann.

### Introduction

*Monacha* is a genus of hygromiid snails containing some 100 or so species, with its centre of diversity in Turkey (NEIBER & HAUSDORF 2017). *Monacha cantiana* (MONTAGU 1803) and *M. cartusiana* (O. F. MÜLLER 1774) have long been present in western Europe (KERNEY 1999, PIEŃKOWSKA & al. 2018a), but a recent series of papers has established that in Poland and elsewhere colonies assumed to be *M. cartusiana* often turn out to be the externally indistinguishable species *M. claustralis* (ROSSMÄSSLER 1834) or a mixture of the two species (PIEŃKOWSKA & al. 2015, 2016, 2018b). *Monacha claustralis* is widespread in western Turkey and in Bulgaria (HAUSDORF 2000, IRIKOV 2008), so these areas together with adjacent parts of Greece are probably its original range, but it has now been recognised also from (in chronological order) Albania, the Crimea, Macedonia, the Czech Republic, Poland, Georgia, Montenegro, and Bosnia-Herzegovina (HAUSDORF 2000, PIEŃKOWSKA & al. 2015, 2018b). Within Poland its spread appears to be continuing (PIEŃKOWSKA & al. 2016). The typical habitat of *M. claustralis* is rough grassland, and even within its native range it often occurs synanthropically (HAUSDORF 2000, IRIKOV 2008).

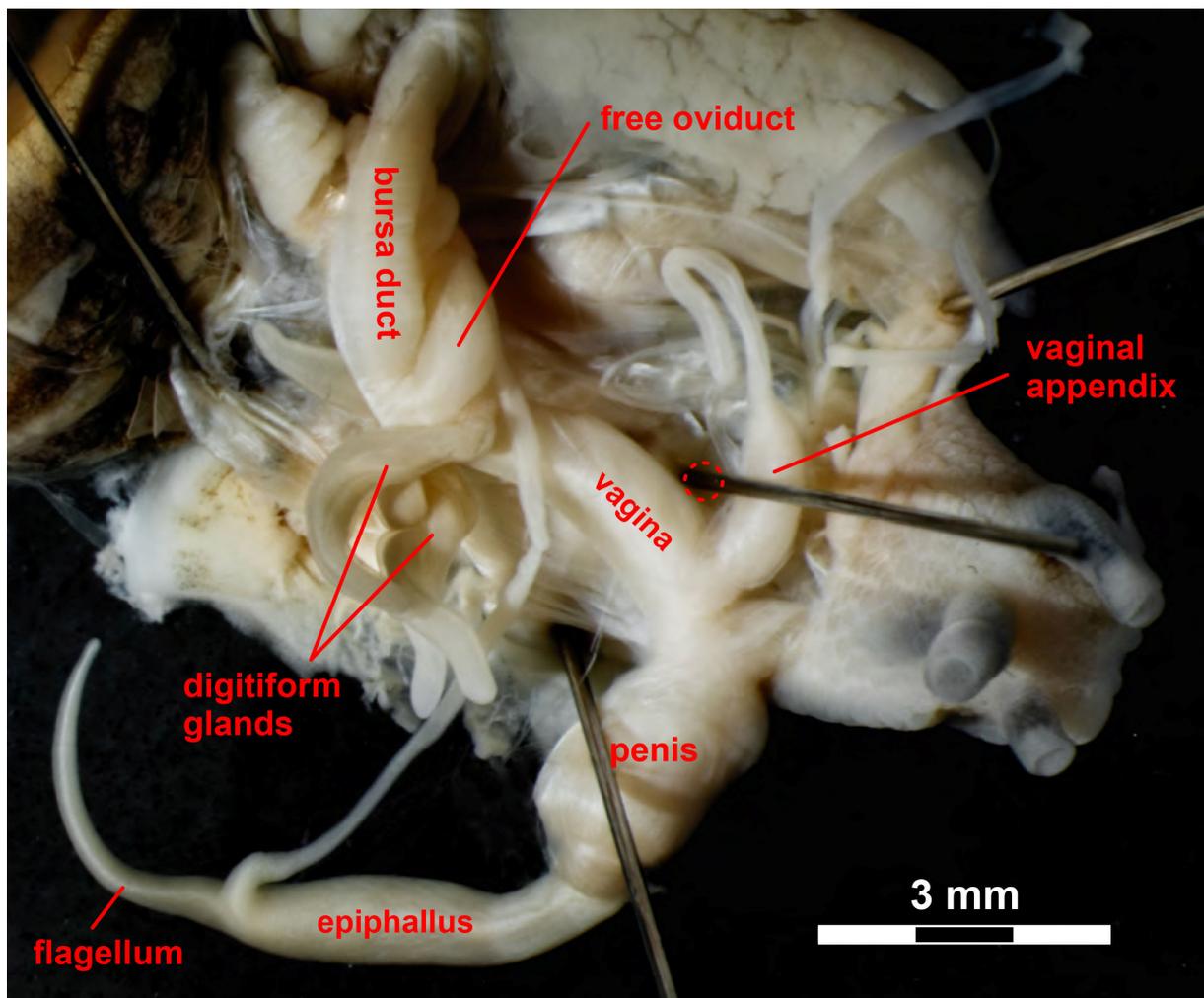
PIEŃKOWSKA & al. (2018b) examined three populations from Germany (from Cologne, Bonn and near Hannover) and confirmed that they really were *M. cartusiana*. Here we provide the first report of *M. claustralis* in Germany. It was collected during the autumn 2016 meeting of the Deutsche Malakozologische Gesellschaft (KNORRE & BÖSSNECK 2017). The original purpose of our collection was to provide shells of *M. cartusiana* with which students could practise using identification keys. Only later did we become aware of the recent discoveries of *M. claustralis* in adjacent countries. Fortunately we had deposited some alcohol-preserved specimens in our museum collection, allowing us to make the new identification based on both anatomy and DNA sequence.

## Materials and Methods

*Monacha* was plentiful in rough grassland along a valley bottom and field margin. This was behind an area of housing in the small village of Nennsdorf, Thüringen, 5 km southwest of the centre of Jena (50.8913° N 11.5399° E, or possibly 70 m to the west of this). HEIKE REISE and JOHN M. C. HUTCHINSON collected the sample on 26<sup>th</sup> September 2016. Eighteen specimens are preserved in alcohol in the collection of the Senckenberg Museum für Naturkunde Görlitz (SMNG, catalogue number p19105).

Two specimens were dissected to reveal the genital anatomy. We avoided cutting the genitalia out of the animal, and photographed the critical features in situ. Thus Fig. 1 shows the genitalia from the opposite (dorsal) side to the drawings and photographs in PIENKOWSKA & al. (2015, 2018).

Using foot-sole tissue from the specimen in Fig. 1, we sequenced the standard barcoding section of the mitochondrial COI gene. DNA was extracted using a Roti-Prep Genomic DNA MINI kit (Carl Roth, Karlsruhe). For DNA amplification, we used Taq-polymerase, buffer from Peqlab (VWR, Darmstadt), and the standard barcoding primers LCO1490 and HCO2198 (FOLMER & al. 1994). PCR was carried out with total volumes of 10 µl,  $T_a = 40^\circ\text{C}$ , 38 cycles. The DNA fragments were gel purified with Roti-Prep Gel Extraction kit (Carl Roth, Karlsruhe) and then sequenced in both directions at the Senckenberg BIK-F Laborzentrum (Frankfurt a. Main). We checked the sequencing electropherograms manually.



**Fig. 1:** Genital anatomy of *Monacha claustralis* from Nennsdorf, near Jena (identity of individual confirmed by partial-COI sequence). The dashed circle indicates where the vaginal sac would be visible in *M. cartusiana*, although attaching on the far (ventral) side of the vagina. Image contrast has been enhanced for clarity.

## Results

HAUSDORF (2000) provided two characters to distinguish *Monacha claustralis* from *M. cartusiana*. First, only *M. cartusiana* has a lateral bulge on the vagina, elsewhere called the vaginal sac (PIEŃKOWSKA & al. 2015). Second, in *M. claustralis* the epiphallus is shorter relative to other parts of the genitalia. HAUSDORF (2000) stated that its epiphallus is 1.0-2.3 times as long as the penis (cf. 2.3-3.5 in *M. cartusiana*) and 0.6-1.8 as long as the vagina (cf. 1.5-2.6 in *M. cartusiana*, so the distributions overlap). IRIKOV (2008) reported similar ratios in three populations of *M. claustralis* from Bulgaria: 1.1-2.1 and 0.6-1.1 respectively. However, PIEŃKOWSKA & al. (2015) found that the epiphallus was only 1.6 (sd = 0.4) times as long as the penis in their populations of *M. cartusiana*, so this ratio is also overlapping. To us, looking at published illustrations, the most obvious species difference in proportions is the longer vagina in *M. claustralis*. PIEŃKOWSKA & al. (2015) indeed found the absolute length of its vagina to be consistently longer than in *M. cartusiana*. However, our population showed considerable variation in adult size (judging adulthood from the shell's lip and internal rib), so it would seem preferable to base identifications on relative, rather than absolute, dimensions.

Both our specimens lacked the vaginal sac found in *M. cartusiana*. The epiphallus was 1.2 and 0.9 as long as the penis, and 1.0 and 0.5 as long as the vagina (or 1.1 and 0.7 if, like PIEŃKOWSKA & al. 2015, measuring the vagina to the branching of the digitiform glands rather than of the bursa duct). These ratios consistently indicate *M. claustralis*. The overall appearance of a long vagina also lead us to identify the species confidently as *M. claustralis* (Fig. 1).

PIEŃKOWSKA & al. (2015) gave three further distinguishing characters, which are not highlighted by HAUSDORF (2000). They seem less satisfactory. One such character is that the flagellum is longer in *M. claustralis* than in *M. cartusiana*. PIEŃKOWSKA & al.'s (2015) measurements of absolute lengths confirmed that the means were statistically different, but the distributions overlapped. In our specimens the flagellum was clearly longer than the epiphallus, which agrees with PIEŃKOWSKA & al.'s (2015) illustrations of *M. claustralis* in contrast to those of *M. cartusiana*. However, HAUSDORF (2000) found in his Turkish samples of *M. claustralis* that the flagellum was usually shorter than the epiphallus (the range of epiphallus : flagellum ratios is given as 0.9-1.8 on p. 80 but 1.3-1.8 in Tab. 8). IRIKOV (2008) found the same in Bulgarian samples (ratio 1.1-2.0).

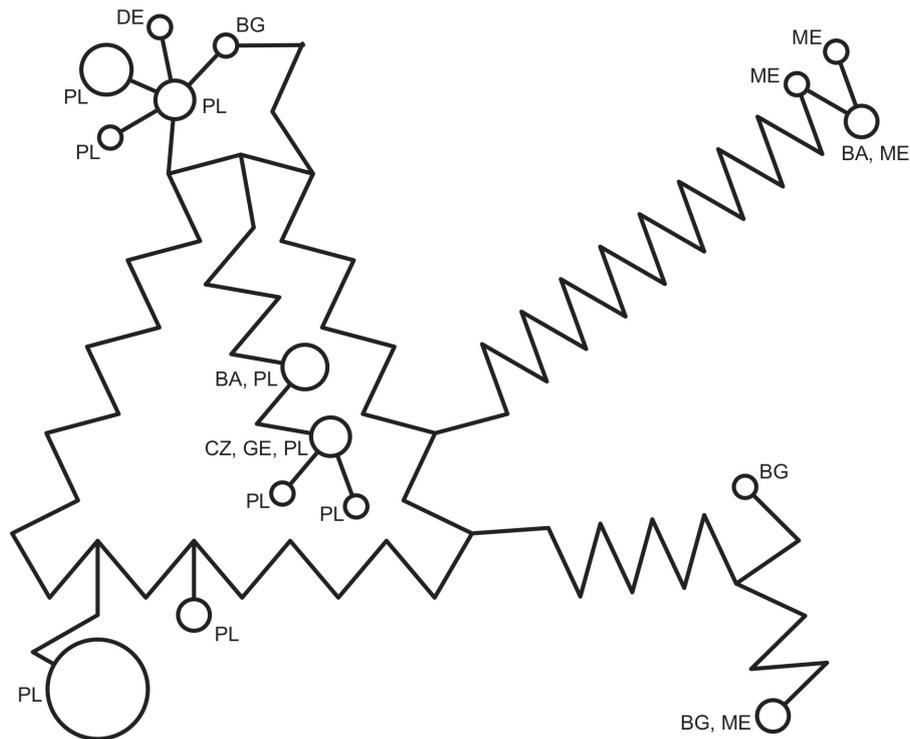
PIEŃKOWSKA & al. (2015) stated that in *M. cartusiana* the vaginal appendix is sharply differentiated into a short bulbous basal part and slender apical part, whereas the transition is more smoothly tapering in *M. claustralis*, with the basal part longer than in *M. cartusiana*. In contradiction with this, we interpreted our Fig. 1 as showing a sharp constriction from the wider to slenderer part. The *M. claustralis* illustrated in fig. 5 of PIEŃKOWSKA & al. (2018) shows a similarly sharp division, little different to the *M. cartusiana* illustrated in fig. 11 of PIEŃKOWSKA & al. (2015). HAUSDORF's (2000) and IRIKOV's (2008) sketches of *M. claustralis* show even sharper constrictions, but it may not have been a feature on which they were focusing. In the second specimen that we dissected, the slender part was totally absent (not just invaginated).

Lastly, PIEŃKOWSKA & al. (2015) stated that inside the genital atrium *M. cartusiana* had thin low pleats whereas *M. claustralis* had a wide spongy pleat separating penis from vaginal appendage. In the place of what looks like this wide pleat in their illustration (fig. 17), the single specimen that we examined has a smooth furrow. However, there are other strong pleats in the atrium and these fit PIEŃKOWSKA & al.'s (2015) drawing of *M. claustralis* better than their drawing of *M. cartusiana*.

We obtained a partial-COI sequence of 648 base pairs, out of the 655 base pairs between primers (Genbank accession number MK284228). Genbank provided 310 other partial-COI sequences of *Monacha*, of which 140 were labelled as *M. claustralis* and 87 as *M. cartusiana*. Our sequence and the *M. claustralis* sequences formed a group of 16 distinct haplotypes within which the most different haplotypes differed by 33 base pairs (5 %, not untypical of intraspecific variation in terrestrial pulmonates: PARMAKELIS & al. 2013). The most similar sequence from another *Monacha* species differed by 68 base pairs (10 %). Thus the molecular evidence confirms the identification as *M. claustralis*.

The program TCS (CLEMENT & al. 2000) constructed a haplotype network of *M. claustralis* partial-COI haplotypes (Fig. 2). Our sequence is unique, but included in a haplogroup composed of five hap-

lotypes. Four of these, including ours, each differ by a different single base pair from the fifth haplotype (with our sequence we rechecked the electropherograms to confirm this difference). Such a star-like genealogy is a typical consequence of recent demographic expansion (SLATKIN & HUDSON 1991). Other members of this haplogroup have been reported from eight localities. One of these is in Bulgaria, the other seven in Poland clustered in two regions, one near Kielce (north of Cracow), the other on the Baltic coast northwest of Danzig. Members of this haplogroup have not been reported from other regions from which *M. claustralis* is known, including Poznan and Prague, two closer regions from which *M. claustralis* has been sequenced (PIEŃKOWSKA & al. 2018; Prague is the nearest occurrence, 220 km from Nennsdorf).



**Fig. 2:** Haplotype network of *Monacha claustralis* based on 141 partial-COI sequences, constructed by the program TCS (CLEMENT & al. 2000). Open circles indicate haplotypes observed, with the area proportional to the number of localities where the haplotype has been found. Each straight segment on the line connecting two haplotypes indicates an inferred point mutation, but shorter direct transformations between non-adjacent haplotypes may exist. Countries where each haplotype has been found are indicated with two-letter codes: BA = Bosnia and Herzegovina, BG = Bulgaria, CZ = Czech Republic, DE = Germany, GE = Georgia, ME = Montenegro, PL = Poland. The single German haplotype is at the top left.

## Discussion

Besides being the first report from Germany, our record is the furthest west. NEIBER & HAUSDORF (2017) proposed that global warming was responsible for the range expansion of this species. However, in this case the presumed direction of spread, from Poland or the Czech Republic to Germany, is from a more continental climate towards a milder Atlantic-influenced one. There seems no obvious climatic impediment to a spread further west, likely limited more by the vagaries of human-assisted dispersal. In this respect it is perhaps unexpected that it is in the east of the country that the species was first noted. One might think that the likeliest route into Germany would be via the Turkish population, who are settled predominantly in the former West Germany, bringing plants and produce back from visits to family in Turkey. Instead, the limited genetic evidence suggests a route from Bulgaria via Poland to Germany.

It should now become routine in Germany to check the anatomy of all colonies that would hitherto have been identified as *M. cartusiana* on the basis of external appearance. The most obvious candidate to check is another report from the same field meeting of “*M. cartusiana*” from Blankenhain, 12 km away (KNORRE & BÖSSNECK 2017). Note that mixed populations of *M. cartusiana* and *M. claustralis* are known from Poland, the Czech Republic and Bosnia (PIEŃKOWSKA & al. 2018), so ideally several specimens from a site should be dissected. For this purpose, it would be useful to investigate external characters that are indicative of species identity even if not foolproof guides. An eye should also be kept out for further *Monacha* species: for instance, ANDERSON & al. (2018) reported a British colony of *M. ocellata* (ROTH 1839) from a site near a container port.

We prefer not to invent an artificial vernacular name for *M. claustralis* to be added to the list of German mollusc species (JUNGBLUTH & KNORRE 2008) since the use of alternative names, especially unfamiliar ones, can impede communication. Unfortunately with the Latin name there appears to be continuing disagreement about the appropriate taxonomic authority. HAUSDORF (2000) derived the name from MENKE’s (1828) mention of *claustralis* as a synonym of a variety of *Helix carthusianella*, the name having been made available by MOUSSON (1859) using it for a variety of *H. cartusiana*. WELTER-SCHULTES (2012a, b; see also <http://www.user.gwdg.de/~fwelter/iczn.htm>, accessed 12.xii.2018) explains why he rejects this and instead derives the same name from its use by ROSS-MÄSSLER (1834), again for a variety of *H. carthusianella*. Whereas PIEŃKOWSKA & al. (2015, 2016, 2018a, b) follow WELTER-SCHULTES, we note that NEIBER & HAUSDORF (2017) retain MENKE as the authority.

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