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*Bithynia leachii* (SHEPPARD 1823) and *B. troschelii* (PAASCH 1842),  
two distinct species?

By

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With 5 figures and 1 table.

**Introduction.**

PAASCH, who described *Bithynia troschelii* (*Paludina troschelii*) in 1842, thought that *B. leachii* and *B. troschelii* were of one and the same species. So it was impossible for him to point out the specific distinguishing shell characters. As *Bithynia leachii* had already been described by SHEPPARD in 1823, *Bithynia troschelii* widely fell into oblivion after this first description had been noticed, henceforth it was used only as a synonym (CLESSIN 1876) or was not even mentioned at all (CLESSIN 1887). In 1886, WESTERLUND was the first to add *B. troschelii* to the list as a distinct species and to put emphasis on the different shell characters of this particular species. GEYER (in 1923) regarded *B. troschelii* but as a bigger form of *B. leachii*, indicating, however, that the aperture was slightly blunt-edged above, whereas the aperture of *B. leachii* is of a rounded egg-like shape. EHRMANN (1933) pointed out the differences in the characters of the apertures, too. However, since JAECKEL (1961) only differences in the heights of the shells were considered, and *B. troschelii* was taken to be a geographical subspecies (GLÖER & MEIER-BROOK 2003, GLÖER 2002, 2002b). For the first time again, FALNIOWSKI (1989) admitted *B. leachii* and *B. troschelii* to be looked at as distinct species, and, also for the first time, he scrutinized the penis morphology as well as the anatomy and further characters (FALNIOWSKI 1989, 1990).

On account of the general uncertainty which species was actually meant by the name *B. troschelii*, GLÖER (2002) declared a lectotype of *B. troschelii* and argued that it was a subspecies of *B. leachii* that prevailed in eastern regions.

In the following we shall investigate whether this case is in fact a matter of two distinct species, or whether the two taxa are conspecific.

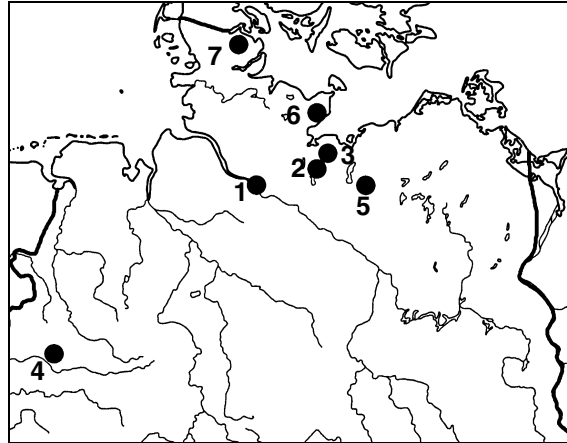
**Material and Methods.**

From seven different sampling sites we gathered living material of *B. leachii* (map 1, 4-7) and *B. troschelii* (map 1, 2-3) in the summer of 2003 by applying a Drahtsiebkescher from the shore. At sampling site #1 we were able to collect both taxa which are living syntopically in this brook. For comparison, *B. tentaculata* was also collected at each site.

The living snails had to be sent from Hamburg (Germany) to Kraków (Poland) for electrophoretic investigation, and they had to be alive still when they arrived: a task that was not at all without difficulty. No parcel service was willing to accept living animals for transport. We were forced, thus, to falsely declare the snails to be documents, and hence they were put on the way to Poland "by express". As enquiries turned out later, this "snail-mail" was kept for one day in Hamburg for safety inspection. On the following day it swiftly went to Cologne, from there via Brussels to Berlin and finally directly to

Kraków. That is to say the snails were on their way for 2-3 days, and not all of them survived the hard transport conditions in the summer heat.

The snails' allozymes were investigated by means of electrophoresis, and examinations of anatomy were carried out. Further the penis morphology and the shell characters were registered and intercompared.



Map 1. The sampling sites of the material studied.

- 1: Reitbrooker Sammelgraben (a brook), Hamburg (sympatric population of *B. leachii* and *B. troschelii*),
- 2: Lankower See (a lake), Mecklenburg Vorpommern (*B. troschelii*),
- 3: Lake at Klein Siemz, Mecklenburg Vorpommern (*B. troschelii*),
- 4: NSG Bever (natural reserve), Nordrhein-Westfalen (*B. leachii*),
- 5: Brook at Crivitz (Mecklenburg-Vorpommern) (*B. leachii*),
- 6: Klostergraben (a brook) at Cismar, Schleswig-Holstein (*B. leachii*),
- 7: Südensee (a lake), Schleswig-Holstein (*B. leachii*).

In addition another 500 samples from Hungary could be included, which were studied by GLÖER & FEHÉR (2004) in order to produce distribution maps.

## Results.

### The Shell.

The typical shell of *B. troschelii* (Fig. 1.1) has a height of 8 mm to 10 mm, and the aperture as well as the operculum show an obtuse angle, and at the point of contact with the last whorl they are concave.

The typical shell of *B. leachii* (Fig. 1.3) on the other hand has a height from 4 mm to 6 mm, and the aperture as well as the operculum are ovally rounded.

Besides these typical shells apparent hybrid characters are to be found in syntopic populations (Fig. 1.2, Fig. 2.3-4).

In total 328 shells from samplings of the years 2001 and 2002 out of the Reitbrooker Sammelgraben were examined and divided into typical and atypical forms according to the illustrations above. The variability among the shells of *B. leachii* was very small so that it was not registered here.

We reached at the following results.

Table 1. Frequencies of typical and atypical shells of *B. leachii* and *B. troschelii* in a syntopic population.

Reitbrooker Sammelgraben Hamburg (Germany)	<i>B. leachii</i> typical	<i>B. troschelii</i> atypical	<i>B. troschelii</i> typical	Sum
2001	1 (0.7 %)	40 (27.2 %)	106 (72.1 %)	147
2002	34 (18.8 %)	56 (30.9 %)	91 (50.3 %)	181
<b>Sum</b>	<b>35 (10.6 %)</b>	<b>96 (29.3 %)</b>	<b>197 (60.1 %)</b>	<b>328</b>

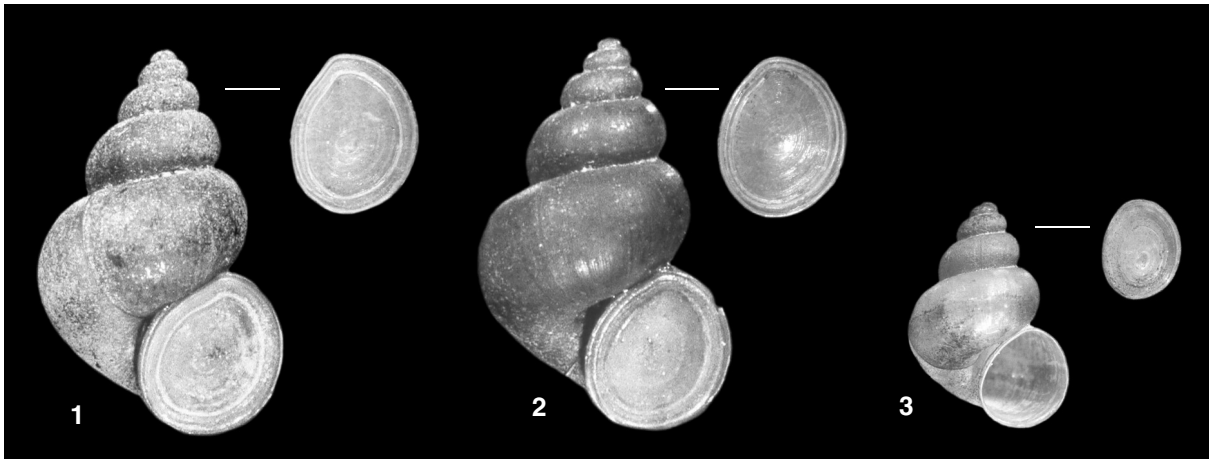


Fig. 1. The shell of *B. leachii* and *B. troschelii*. Reitbrooker Sammelgraben, Hamburg. Magnification = 5 : 1.  
1.-2. =*Bithynia troschelii*, 3=*Bithynia leachii*.

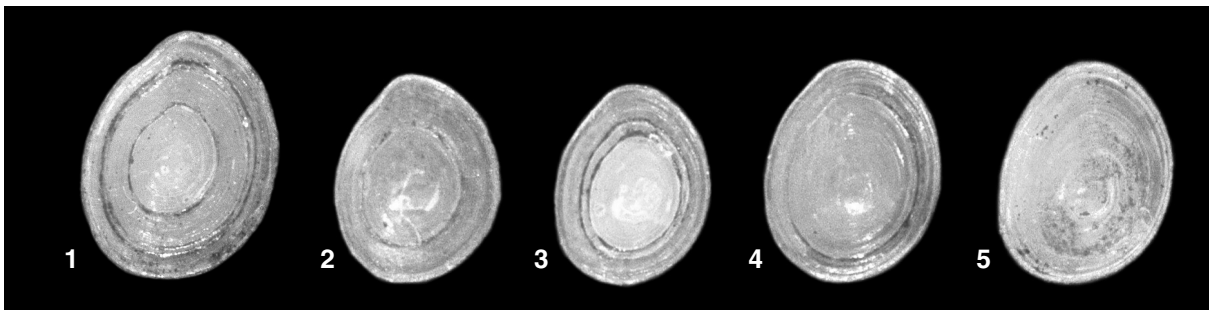


Fig. 2. The operculum. 1-4.=*Bithynia troschelii*, Reitbrooker Sammelgraben, Hamburg; Magnification = 10:1. 5=*Bithynia leachii*, Reitbrooker Sammelgraben, Hamburg; Magnification = 15:1.

GLÖER (in 2002) found, that the typical height reached by *B. leachii* in isolated populations amounts to 4-6 mm, with a peak in the size-frequency curve at 5 mm. In the case of *B. troschelii*, the frequency of heights in allopatric populations lies between 8 mm and 10 mm, with a peak at 9 mm. In sympatric populations, however, the heights of the shells of both taxa were found to range from 5 mm to 9 mm. Under non-optimal conditions populations with smaller shells prevail in both taxa, of course.

In order to rule out that *B. troschelii* is but a representation of abnormal growth caused by parasitic castration, 10 animals from each examined population have been dissected. No larva of trematodes could be found, and the copulatory organs were of normal development.

If the results from the examination of the form of the aperture and the operculum as well as the shell height are interpreted from the point of view of hybrid characters, species distinctness seems doubtful. Another possibility would be to assume that *B. troschelii* is of a higher variability than *B. leachii*, which becomes apparent especially in Hungary. There is a morph of *B. troschelii* the shells of which are of a conical to globular shape (GLÖER & FEHÉR 2004). In this case one could interpret the results in the way that the deduced variation breadth for *B. troschelii* is induced by ecological factors, so coming to the conclusion that the assumption of distinct species is compelling.

### The Protoconch.

From the macroscopic point of view, the protoconches between *B. tentaculata*, *B. leachii* and *B. troschelii* are identical. Considering the microstructure, the protoconch of *B. leachii* is granulated, whereas it is even in the case of *B. troschelii* (FALNIOWSKI 1990).

### The Mantle.

Studying the mantles of the two taxa we found significant differences in the respective pigmentations. While *B. leachii* has a black background with big white spots and a typical white bold line near the mantle edge, the mantle pigmentation of *B. troschelii* is of a black background with smaller round white spots and, in addition, one or two large white gaps.

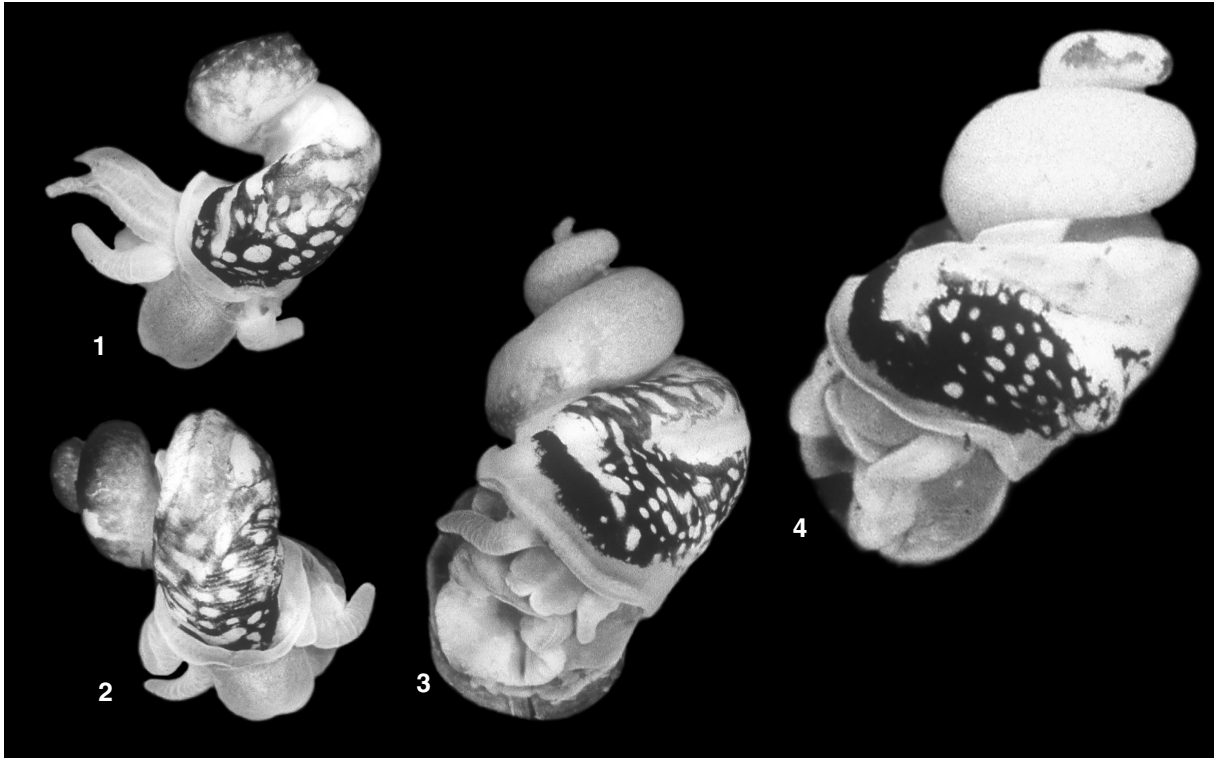


Fig. 2. Mantlepigmentation of *B. leachii* and *B. troschelii*; Magnification = 8:1.

1-2. *Bithynia leachii* from Südensee, Schleswig-Holstein, 3-4. *Bithynia troschelii* from Lankower See, Mecklenburg-Vorpommern.

### Anatomy.

Although FALNIOWSKI (1990) found pronounced differences between the respective length ratios of the flagellum to the penis between *B. leachii* and *B. troschelii*, all other anatomical characters were identical.

### The Penis.

In the morphology of the penis, marked differences are conspicuous. While the penis appendix (Drüsenrute, accessory lobe (PONDER 2003)) branches off from the distal third of the penis (Fig. 4.4-5) in the case of *B. leachii* (Fig. 4.1-3), it is the proximal third of the penis in *B. troschelii*.

*B. troschelii* has in the contrast too *B. leachii* a sucker at the tip of the penis appendix (Fig. 4.5) as *B. tentaculata* (Fig. 4.7).

From the point of view that both taxa are conspecific this could be considered as a case of allometric growth that resulted in a change of the proportions. However, no intermediate forms could be found so far (Fig. 4.8).



Fig. 3. Snails relaxed with Pentobarbital and fixed in 75% Ethanol. Shell and mantle edge removed. Magnification = 25 : 1. (p = penis, p1 = penis appendix, s = snout, su = sucker, t = tentacle). 1-3=*B. leachii*, Südensee, Schleswig-Holstein, 4-5=*B. troschellii*, Lankower See, Mecklenburg-Vorpommern, 6.=*B. troschellii*, Reitbrooker Sammelgraben, Hamburg, 7=*B. tenaculata*, Lankower See, Mecklenburg-Vorpommern, 8=*B. troschellii* juv., Reitbrooker Sammelgraben, Hamburg.

## The Enzymes.

Electrophoretic investigations of allozymes on cellulose acetate gels were carried out according to the notes of RICHARDSON & al. (1986). There is no general rule how profound the differences in the allozymes must be to confirm a species distinctness, thus the specimens of *B. tentaculata* from a couple of sites were also assayed on the same gels with *B. leachii* and *B. troschelii*. We were applying the strategy of searching for fixed differences (loci fixed on different alleles in different taxa). For each taxon from each site no less than five specimens were assayed, considering 23 enzyme systems (enzyme nomenclature and numbers according to MURPHY & al. 1996), represented by 34 loci (the list is available from the co-author on request).

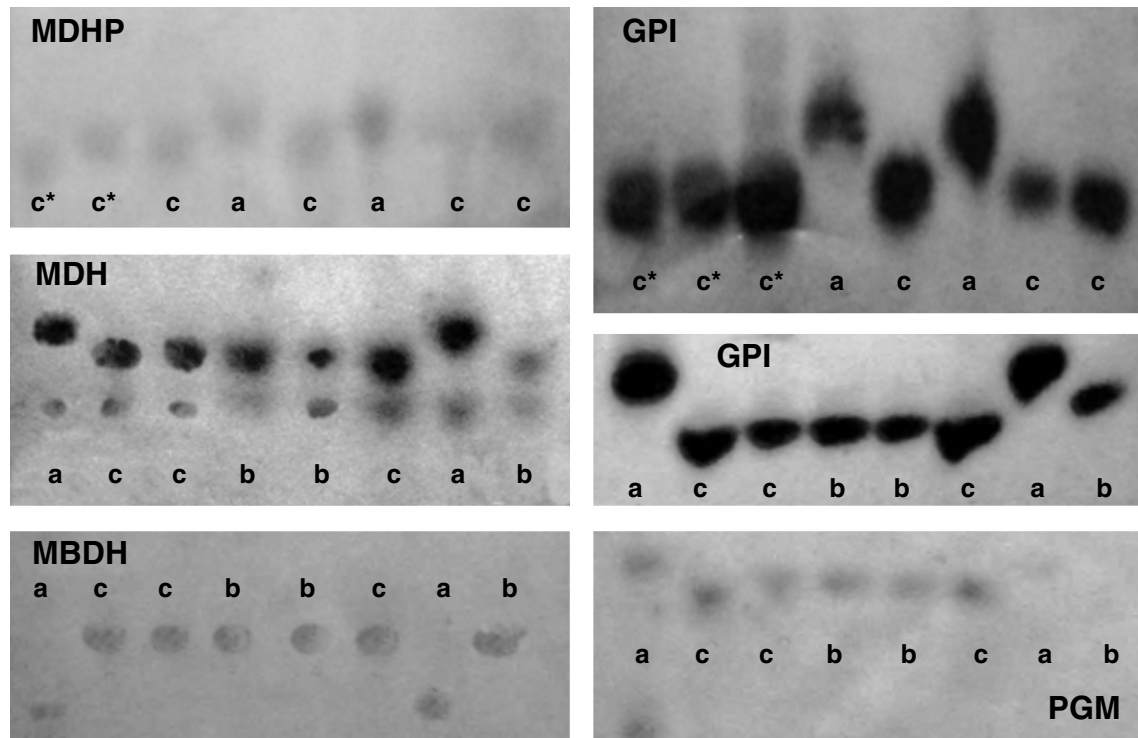


Fig. 1. Enzymograms of a: *Bithynia tentaculata*, b: *B. leachii*, c: *B. troschelii*, c\*: *Bithynia troschelii* (Reitbrooker Sammelgraben).

We found fixed differences between *B. leachii* and *B. tentaculata* in six (20.5% of all studied) enzyme systems, coded by seven loci: Glucose-6-Phosphate Isomerase (EC 5.3.1.9; Gpi), Hydroxybutyrate Dehydrogenase (EC 1.1.1.30; Hbdh), Isocitrate Dehydrogenase (EC 1.1.1.42; Idh-2), Malate Dehydrogenase (EC 1.1.1.37; Mdh), Malate Dehydrogenase (NADP+) (EC 1.1.1.40; Mdhp), and Phosphoglucosmutase (EC 5.4.2.2; Pgm-1, Pgm-2) (FALNIOWSKI & al., submitted).

At the same time, we did not find a constant difference between *B. leachii* and *B. troschelii* no matter from which particular sites the specimens originated. In addition, the specimens from site #1 (Reitbrooker Sammelgraben), which had previously assumed to be hybrids, were allozymatically identical with both *B. leachii* and *B. troschelii*.

All the results listed above confirm the molecular identity of *B. troschelii* and *B. leachii*. This means that the taxa are either one species or two species that are allozymically almost indistinct. The latter was observed in gastropods several times. As is always the case in such studies, it cannot be ruled out that the assayed loci were not representative.

## The Distribution.

*B. leachii* lives primarily in the western European lowlands, but it is commonly found in the lowlands of central Europe as well. A population at the Chiemsee (Bavaria) seems to be an isolated one. The exact eastern boundary of its habitat is unknown to date. *B. troschelii* also populates the lowlands, but it spreads out farther into the east (EHRMANN (1933), JAECKEL (1962), FALNIOWSKI (1989), GLÖER (2002a). The western boundary at present is Hamburg. The Hungarian and Romanian (Carpathian plains) populations of both taxa seem to be isolated from the others. Throughout the entire habitat, *B. troschelii* is less abundant than *B. leachii*.

In the regions of continental climate both taxa the distinctive markings of which should display intermediate differences had the gene flow not been interrupted, exist sympatrically. On the other hand, syntopically populations are very rare, in Hungary only 38 (7.6 %) populations from 500 examined sites were found in which the two species occurred together. There, *B. troschelii* appears with a broader variability. There are, perhaps, ecological factors that prevent further syntopy so that the two taxa can coexist within this large area even as subspecies.

## Ecology.

Both taxa live in small brooks up to big lakes, but are found in shallow areas of slowly running waters, too. As far as the degree of pollution of the water is concerned, *B. troschelii* seems to be less particular than *B. leachii*, as we found in a dammed-up part of a river in Hamburg abundances of 1300 individuals m<sup>-2</sup> coexisting with 2680 individuals of *B. tentaculata* m<sup>-2</sup> (0.25 m<sup>2</sup> counted and extrapolated to 1 m<sup>2</sup>).

Both taxa prefer the lowlands, with *B. troschelii* preferring continental climate, in contrast with *B. leachii* that lives in maritime climates of west European sites as well (GLÖER 2002a). The warmer spring and warmer summer allows *B. troschelii* to become larger (COSTIL 1994) owing to an increased metabolism (BRENDENBERGER & Jürgens 1993). *B. troschelii* on the other hand, needs low temperatures in winter so that it does not suffer too great a degrowth of tissue. *B. graeca* e. g. lost 40% of dry mass of its tissue in winter (ELEUTHERIADES & LAZARIDOU-DIMITRIADOU 1995).

## Discussion of the Results.

Distinct species require distinctive markings, which ones do not have to be always visible, geographical races require a different distribution, and ecological races need different ecological requirements to be able to syntopically exist as subspecies.

The only stable distinctive mark that really discriminates between both species is provided by the morphology of the penis. Now and then are the differences in the respective shells only slight or even fluid. PONDER (2003), too, emphasizes the poorly distinct differences between the particular species of Bithyniidae in Australia.

However, as long as we interpret all the deviations from the typical distinctive marks of both taxa as resulting from natural variability instead of being manifestations of hybrid characters, we may speak of two distinct species. At the same time, the different morphologies of the penis provide evidence for a reproductive isolation. The sometimes great similarity of the two species shows that they splitted not too long ago, possibly in a saltational way.

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