Lauterbornia 65: 69-75, D-86424 Dinkelscherben, 2008-11-15

On the taxonomic status of *Barbronia wuttkei* (Kutschera, 2004) n. comb. – a leech species (Hirudinea: Salifidae) from a German aquarium

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With 2 figures and 1 table

Keywords: Erpobdella, Barbronia, Hirudinea, taxonomy, annulation, gonopore, morphology, anatomy, redescription, molecular genetics
Schlagwörter: Erpobdella, Barbronia, Hirudinea, Taxonomie, Annulation, Geschlechtsöffnung, Morphologie, Anatomie. Wiederbeschreibung, Molekulargenetik

***Barbronia wuttkei* (Kutschera, 2004) was described as *Erpobdella wuttkei*, allegedly showing typical characteristics of the genus *Erpobdella* (fam. Erpobdellidae). Investigations on two specimens of this leech, obtained from the author, revealed that the description is inaccurate and partly wrong. Results of morphological, anatomical and molecular phylogenetic analyses clearly place it in the genus *Barbronia* (fam. Salifidae). Since available morphological, anatomical and molecular data do not permit a clear and unambiguous synonimization with any of the hitherto described *Barbronia* species, we propose to retain it as a nominal species of this genus.**

1 Introduction

Erpobdella wuttkei was described from a freshwater aquarium in Germany (Kutschera 2004). The natural distribution is unknown. Kutschera suspects Asia or South America as possible regions of origin. It is very likely that this species will be found as a neozoon in European freshwaters. Some other species like *Barbronia weberi* (Blanchard, 1897) or *Helobdella europaea* Kutschera, 1987, that are known from aquaria were recorded free-living from several places in Europe (Nesemann & Neubert 1999, Haaren et al. 2004). This implies that the new species be readily identifiable by fieldworkers. But the problem with the description is that it is inaccurate and partly wrong, and therefore cannot be used for unambiguous determination. Following the description precisely, inevitably leads to wrong results. On one hand specimens of this species cannot be clearly identified using the original description, and on the other hand specimens of other species could be mistaken for *E. wuttkei*. For example, the description is more suitable for young specimens of *E. testacea* with a reduced number of eyes than for *E. wuttkei*. Therefore it is important to discuss its genuine characteristics in order to avoid incorrect records. A second problem is that

the generic and familial assignment as proposed by the author is wrong. In this paper we address both problems and propose a re-classification.

2 Material and Methods

External morphology

Two specimens obtained from Prof. Kutschera (20 Sept 2005, size: 17 x 2.5 mm; 15 x 1.5 mm) and the holotypus from Senckenberg-Museum, Frankfurt/ Main, Germany (voucher SM 13711), were examined. Their morphology was studied with the help of a stereomicroscope (Novex, enlargement 6.5-45 x). The number and position of eyes, the annulation and the position of gonopores were examined according to the description (Kutschera 2004). The assessment of colouration was based on figures from the description (Kutschera 2004 Fig. 1 A to C). Photos depicting the annulation and position of gonopores (Fig. 1) were taken with a microscope camera (Euromex, VC 3031C). The size was measured with a ruler. We consider the precision of such measurement as sufficient, because the size of the body varies considerably depending on its contraction.

Anatomy

The anatomy was only studied on the larger specimen obtained from Kutschera. Unfortunately, it was not well preserved internally. The inside of the body was bloated and showed signs of decay. In order to determine the number of testisacs and whether or not pharyngeal stylets and post caeca were present we used the same technical equipment as for external morphology. The pharynx and stylets of *Barbronia weberi*, *Salifa* (*Nematobdella*) *biharensis* Nesemann, Sharma & Sinha, 2004, and *Odontobdella polaneci* Nesemann, 1995 were investigated before examining the stylets in *Erpobdella wuttkei*.

Molecular phylogenetics

The only available DNA sequence of *Erpobdella wuttkei* (Pfeiffer et al. 2005) was scrutinized in a wider phylogenetic context with other Erpobdelliform cytochrome oxidase subunit one (COI) gene sequences from GenBank. These included leeches of the families Erpobdellidae, Salifidae and Americobdellidae as putative sister to the monophylum composed by the first two families. (e.g. Borda & Siddal 2004). Bayesian inference under MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) was performed to search for optimal trees under a pre-specified model of nucleotide substitution. The model selected with the help of Modelgenerator (Keane 2006) was a GTR model with gamma distributed rate heterogeneity (approximated by six discrete categories). The parameters of the selected model were optimized during searches. Two independent Mark-

ov chain Monte Carlo (MCMC) searches were run under default prior settings for 10^6 generations, each search consisting of one cold and three hot chains. The sampling frequency was 100 generations. Convergence of the log-likelihood values of the cold chains was examined graphically and corroborated by inspection of the posterior probability variances. A 50 % majority rule consensus tree was obtained from a combined last 7,000 trees from each run.

3 Results

All external characteristics were clearly visible on the large specimen, and verified by comparison with the holotypus. Important differences between the original description of *E. wuttkei* (Kutschera 2004) and the characters found in the present investigation concern the annulation pattern and the presence of accessory gonopores (Fig. 1, Tab. 1). An anatomical difference compared to the original description was the presence of pharyngeal stylets (Tab. 1).

Tab. 1: Comparison of characteristics of *Erpobdella wuttkei* described by Kutschera 2004 and characteristics found in this study

Original description (Kutschera 2004)	This study
Size at rest 15-19 mm in length to 2.5 mm in width	Size 15-17 mm in length, 1.5-2.5 mm in width
6 eyes	6 eyes
Homonomous annulation	Complex heteronomous annulation (Fig. 1)
Gonopores separated by 4 annuli	Gonopores separated by 4.5-5.5 annuli, dependent on the subdivision of the broadened annulus before the female gonopore
Accessory gonopores not mentioned	Accessory gonopores present (Fig. 1), male accessory pore 5.5 annuli anterior to male gonopore and female accessory pore 5 annuli posterior to female gonopore
Papillae absent	Papillae absent
Whitish spots absent	Whitish spots absent
Reticulum of dark pigment below the dorsal surface, which form two brown-black parallel lines running longitudinally from the anterior region of the clitellum to the posterior sucker	Incomplete pigmentation; laterally darker brownish with irregular restriction, median portion brighter
Pharyngeal stylets absent	Pharyngeal stylets present, but very small
Testisacs not mentioned	7 testisacs on each side visible
Postcaeca not mentioned	Postcaeca absent

The phylogenetic position of *E. wuttkei*, as revealed by Bayesian analysis of mitochondrial COI gene sequences in a wider taxonomic context, is clearly within the genus *Barbronia* (Fig. 2). No erpobdellid leech species shows any tendency to group with *E. wuttkei*. Given the well-established sister-relationship and mutual monophyly of the Erpobdellidae and Salifidae (e.g. Borda & Siddall 2004) it seems safe to say that *E. wuttkei* actually belongs to the Salifidae. The

fact that the presented topology renders the Erpobdelidae paraphyletic can be explained by homoplasy of the hypervariable 3rd positions in the COI gene coding sequence. When restricted to 1st and 2nd positions of the codons, a monophyletic Erpobdellidae was recovered (tree not shown) sister to *Barbronia* (Salifidae).

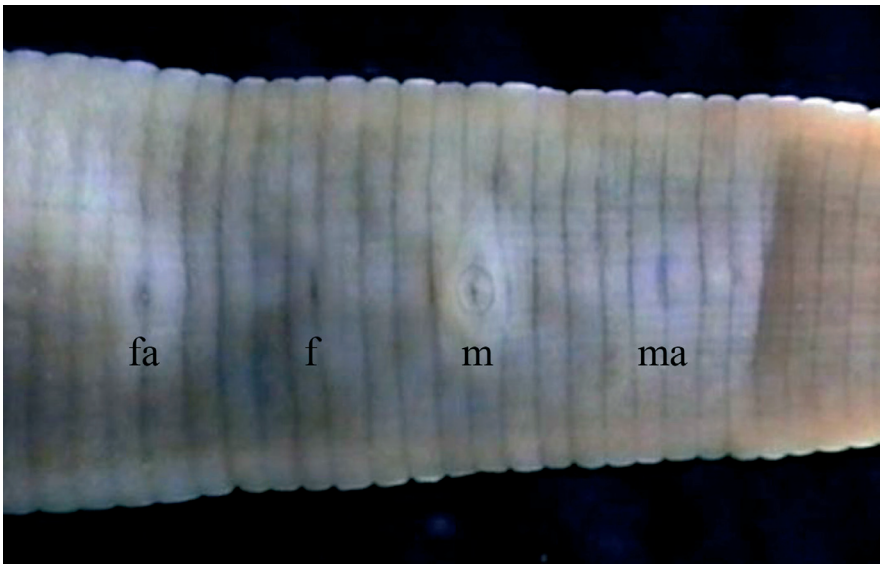

Fig. 1: *Erpobdella wuttkei*. Clitellum with situation of gonopores and annulation. ma = male accessory pore, m = male pore, f = female pore, fa = female accessory pore

4 Discussion

The genus Erpobdella Blainville, 1918 (Erpobdellidae Blanchard, 1894) is morphologically and anatomically characterised by five-annulate somites with annuli approximately equal in size, numerous testisacs, lacking pharyngeal stylets (Sawyer 1986), and usually four pairs of eyes (Soós 1966). Accessory gonopores are absent. The characteristics of the genus Barbronia Johansson, 1918 (Salifidae Johansson, 1910) are accessory copulatory pits on venter at somites X/XI and XIII/XIV. Some specimens have only one accessory gonopore or even none at all (Moore 1924). The midbody somites are heteronomously subdivided into six or seven annuli: b1 + b2 + a2 + b5 + b6 (c11 + c12), or b1 + b2 + a2 (b3 + b4) + b5 + b6 (c11 + c12) (Soós 1966, Nesemann & Neubert 1999). Usually they have three pairs of eyes (Soós 1966). Anatomical characteristics of Salifidae are only few testisacs and pharyngeal stylets (Sawyer, 1986).

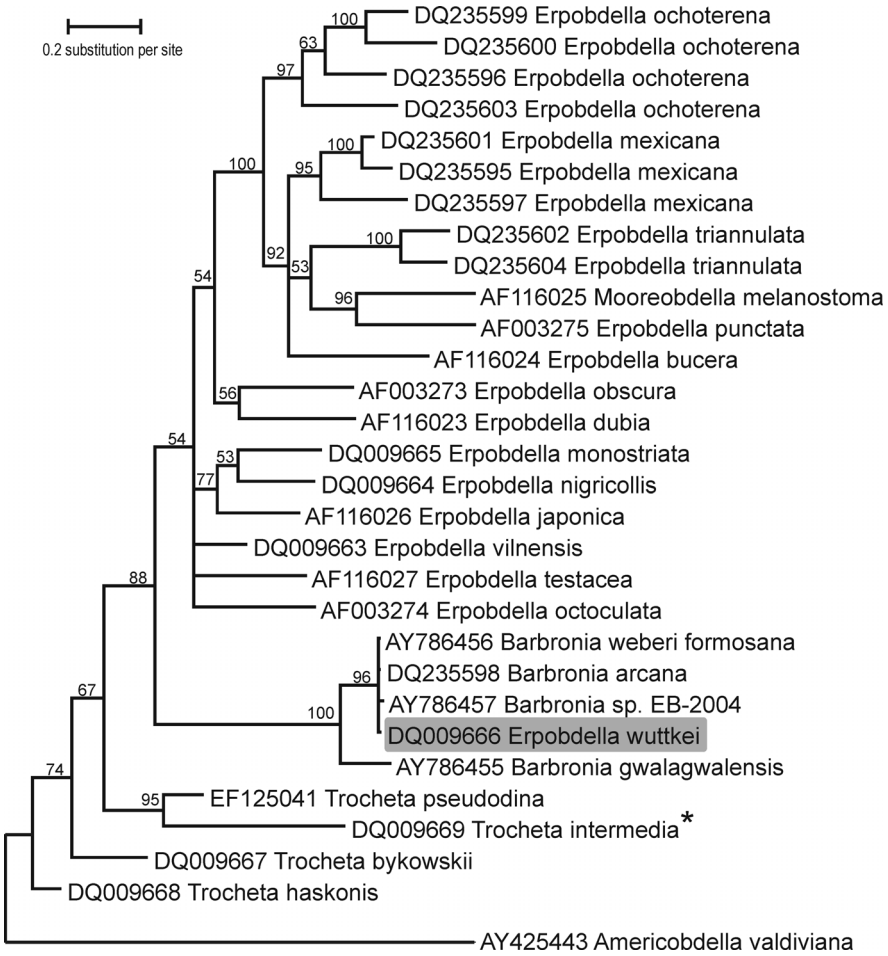

Fig. 2: Bayesian tree of publicly available erpobdelliform COI gene sequences. The taxon labels are composed of the accession number and the taxon label as deposited in GanBank. Numbers on branches are Bayesian posterior probabilities in percent. The position of the taxon described as *Erpobdella wuttkei* (highlighted) clearly justifies its transfer to the genus *Barbronia*. Notes: (1) The paraphyly of the Erpobdellidae is only weakly supported and can be explained as the consequence of homoplasy at 3rd codon positions. (2) *Trocheta intermedia*, indicated by asterisk, is a nomen nudum; a description was not published, only the name with the COI gene sequences submitted to GenBank by Kutschera (24. July 2006)

The leech species described as *E. wuttkei* is characterised by the following complex of morphological and anatomical character states: six eyes, accessory gonopores, gonopores separated by 4.5 annuli (or 5.5 because the annulus anterior to the female gonopore is slightly subdivided), heteronomous annulation, small pharyngeal stylets, seven testisacs on each side in the examined specimen, and absence of post caeca. All characteristics, especially the anatomy, must be verified on more specimens. In addition to its convincing molecular phylogenetic placement this demonstrates that the leech is a member of the genus *Barbronia*. There are two possible ways to rectify the taxonomic error.

First, the leeches from the aquarium might belong to an invasive species already described, like for example *Barbronia weberi*. This common Asian species (Moore 1930, Nesemann et al. 2007) is also known from many parts of the world (e. g. Europe, South America) and aquaria. Sawyer (1986) listed four species of the genus: *B. rouxi* Johansson, 1918, *B. arcana* (Richardson, 1970), B. *weberi* Blanchard, 1897 with forma *formosana* Oka, 1929 and *B. assiuti* Hussein & El-Shimy, 1982. He correctly assigned *B. delicata* Moore, 1939 to the genus *Salifa* Blanchard, 1897. Recently, *B. gwalagwalensis* Westergren & Siddall, 2004 with an exact anatomical investigation, *B. nepalensis* Nesemann & S. Sharma, 2007 and *B. shillongensis* Nesemann, 2007 added to the list. It has to be emphasized that the differences between the COI gene sequences submittend to GenBank under the names *Barbronia weberi formosana* (AY786456), *Barbronia arcana* (DQ235598), *Barbronia* sp. EB-2004 (AY786457) and *Erpobdella wuttkei* (DQ009666) are minute, and do not permit their distinction at even the lowest taxonomic level. However, a single gene sequence from a single specimen should not be taken as evidence to synonymize morphologically well established and distinguishable species (c.f. Sites & Marshall 2004). Only a thorough revision with specimens from type localities, sequenced at two or more independent loci, and studied anatomically could provide the grounds to do so. At the current state of salifid systematics this is not possible.

This leaves us with the second – possibly provisional – solution. Since none of the descriptions of other *Barbronia* species completely matches the new description presented here, we propose to retain the nominal species, and re-classify it to *Barbronia wuttkei*. Distinct or not, this taxon is certainly not the smallest erpobdellid leech, and any conclusions based on this premise (Kutschera 2004, Pfeiffer et al. 2005) have to be reconsidered.

Acknowledgements

We are thankful to Prof. Kutschera, Kassel/Germany, for furnishing material, Mr. Jueg, Ludwigslust/Germany, for discussing the results, Dr. Zettler, Rostock/Germany, for providing the holotypus, and Mr. Nesemann, M.S. by Research (Environmental Sciences), Kathmandu/Nepal, for sending samples of different Salifidae species.

References

Borda, E. & M. E. Siddall (2004): Review of the evolution of life histroy strategies and phylogeny of the Hirudinida (Annelida: Oligocheta).- Lauterbornia 52: 5-25, Dinkelscherben

Haaren, van T., H. Hop, M. Soes & D. Tempelman (2004): The freshwater leeches (Hirudinea) of The Netherlands.- Lauterbornia 52: 113-131, Dinkelscherben

Keane, T. M., Creevey, C. J., Pentony, M. M., Naughton, T. J. & McInerney, O. J. (2006): Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified.- BMC Evolutionary Biology, 6, 29, London

Kutschera, U. (2004): Description of a new leech species, Erpobdella wuttkei nov. sp. (Hirudinea: Erpobdellidae).- Lauterbornia 52: 147-151, Dinkelscherben

Lukin, E. (1976): Pijavki.- Fauna SSSR. Vol. 1, Akademie der Wissenschaften der SSSR, Zoologisches Institut. Neue Serie 109, Leningrad

Moore, J. P. (1924): Notes on some Asiatic leeches (Hirudinea) principally from China, Kashmir, and British India.- Proceedings of the Academy of Natural Sciences Philadelphia 76: 343-388, Philadelphia

Moore, J. P. (1930): Leeches (Hirudinea) from China with descriptions of new species.- Proceedings of the Academy of Natural Sciences Philadelphia 82: 169-192, Philadelphia

Moore, J. P. (1938): Additions to our knowledge of African Leeches (Hirudinea).- Proceedings of the Academy of Natural Sciences Philadelphia 90: 297-360, Philadelphia

Nesemann, H. (1995): On the Morphology and Anatomy of the Asian Leeches (Hirudinea: Erpobdellidae, Salifidae).- Acta Zoologica Academiae Scientiarum Hungaricae 41(3): 165-182, Budapest

Nesemann H. & E. Neubert (1999): Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea.- In: Schwoerbel, J. & P. Zwick (eds): Süßwasserfauna von Mitteleuropa. Begründet von A. Brauer 6/2, 178 pp., (Spektrum) Heidelberg

Nesemann, H., S. Sharma, G. Sharma, S. N. Khanal, B. Pradhan, D. N. Shah & R. D. Tachamo (2007): Aquatic Invertebrates of the Ganga River System (Mollusca, Annelida, Crustacea [in part]), Vol. 1, 1-263, Chandi

Sawyer, R. T. (1986): Leech biology and behaviour, Vol. 1-3, 1-1065, (Oxford University Press) Oxford

Sites, J. W. & J. C. Marshall (2004): Operational criteria for delimiting species.- Annual Review of Ecology, Evolution, and Systematics 35: 199–227, Palo Alto, Calif.

Soós, A. (1966): Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. III. Family: Erpobdellidae. Acta Zoologica Academiae Scientarum Hungaricae 12(3/4), 371-407, Budapest

Pfeiffer, I., B. Brenig, & U. Kutschera (2005): Molecular phylogeny of selected predaceous leeches with reference to the evolution of body size and terrestrialism.- Theory in Biosciences 124: 55–64, Berlin

Ronquist, F. & J. P. Huelsenbeck (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574, Oxford

Westergren, S. & M. E. Siddall (2004): Two new species of Salifid Leeches (Arhynchobdellida: Erpobdelliformes: Salifidae) from South Africa and Madagascar.- American Museum Novitates 3456: 1-6, New York

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Received: 2008-10-29