

## Rapid Communication

## Molecular confirmation of the North American leech *Placobdella ornata* (Verrill, 1872) (Hirudinida: Glossiphoniidae) in Europe

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### Abstract

Specimens of the North American leech, *Placobdella ornata* (Verrill, 1872) were confirmed from the Donkmeer, a freshwater lake in the province of East Flanders, Belgium, by morphological and molecular analysis. Leech specimens from Belgium were morphologically consistent with the syntype series and description of *P. ornata* by Verrill (1872). Molecular comparison of the Belgian specimens to specimens of *P. ornata* from the type locality (New Haven, Connecticut, USA) using the cytochrome c oxidase subunit I (COI) gene revealed a similarity of 99.5%. *Placobdella ornata* from Belgium is the first documentation of a second species of the genus *Placobdella* in Europe.

**Key words:** Hirudinea, Rhynchobdellida, Donkmeer, alien species, molecular analysis

### Introduction

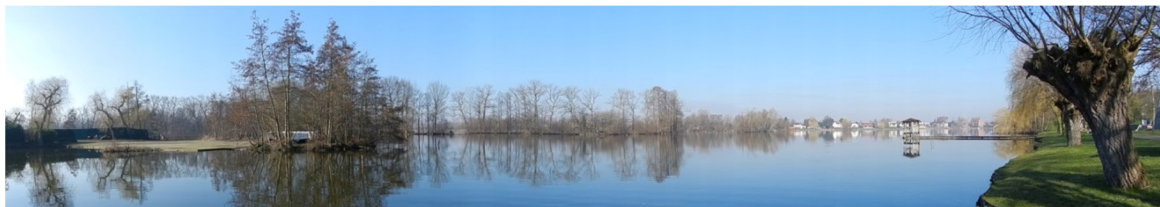
The Donkmeer (Figure 1), located in the municipality of Berlare in the East Flanders province in Belgium, is a shallow (max depth: 3.20m), 86 hectare, anthropogenically modified oxbow lake (paleopotamon) of the estuarine river Zeeschelde. The ‘silted up’ part of the old meander was used for peat extraction from the late 17<sup>th</sup> century to the early 19<sup>th</sup> century. After flooding of the peat pits, the extant lake was formed. The Donkmeer is a heavily utilized and disturbed lake with regular introductions of European eel *Anguilla anguilla* (Linnaeus, 1758), common roach *Rutilus rutilus* (Linnaeus, 1758), common bream *Abramis brama* (Linnaeus, 1758), ide *Leuciscus idus* (Linnaeus, 1758), northern pike *Esox lucius* (Linnaeus, 1758) and tench *Tinca tinca* (Linnaeus, 1758) for recreational fishing (Van Thuyne 2009).

Sampling activity to investigate environmental targets established by the European Water Framework Directive, revealed the presence of the North American leech *Placobdella ornata* (Verrill, 1872) from the Donkmeer. *Placobdella ornata* was originally described by Verrill (1872) from the West River, New Haven, Connecticut, United States. Most species of the genus *Placobdella* are found in North America. The only previously reported European representative of the genus is *Placobdella costata* (Fr. Müller, 1846) a native species in Europe, Western Asia, Arabian Peninsula and North Africa.

### Materials and methods

#### Collection of leeches

As part of a monitoring network to determine the biological quality of Vlaamse Milieumaatschappij/



**Figure 1.** The Donkmeer in landscape view. Photograph by Jan Soors.

Flemish Environment (VMM) and a sample scheme to investigate the requirement set by the European Water Framework Directive, macroinvertebrates were collected at 3 different stations (WGS84, Station 541000: latitude 51.042520, longitude 3.980225; Station 542010: latitude 51.042897, longitude 3.973723. Station 541500: latitude 51.037082, longitude 3.979624) in the Donkmeer East Flanders Province, Belgium from 2008–2014. Free-living *P. ornata* were collected underneath branches, wood, plastic and rocks on 7 July 2004, 14 May 2008, and 26 September 2011. On 4 March 2014, twenty specimens were collected and fixed in 100% ethanol. The leech specimens were deposited in the collection of the Royal Belgian Institute of Natural Sciences, Brussels (RBINS), Peabody Museum of Natural History (YPM), Yale University, New Haven, Connecticut and the Smithsonian Institution, National Museum of Natural History (USNM), Washington, District of Columbia.

#### DNA Analyses

Molecular analyses were conducted on one specimen of the newly collected material following the protocol of Richardson et al. (2010). Purified PCR products were sequenced using the HCO2198 primer and the LCO1490 primer for the Cytochrome c oxidase subunit I products by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University. The DNA sequences were aligned using Clustal W version 2 (Larkin et al. 2007), checked manually using SeaView 4 (Gouy et al. 2010), analyzed using PAUP\* 4.0b10 (Swofford 2002), deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>), and compared to other leech DNA sequences contained within Genbank. Uncorrected p distance was calculated using PAUP\*.

#### Results

*Placobdella ornata* was most abundant on plastic sheeting that was used to protect the shore against

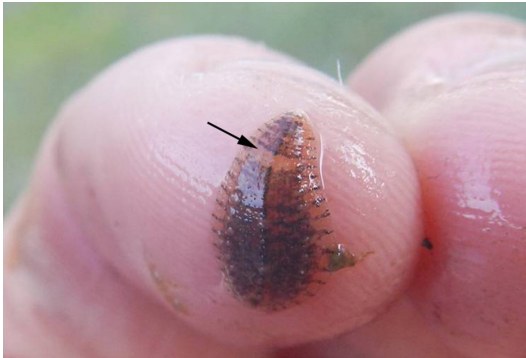
erosion. Live specimens of *P. ornata* ranged in length from 5 mm to 15 mm. Leech specimens from the Donkmeer were consistent with the syntype series and description of *P. ornata* by Verrill (1872) (Moser et al. 2012). Leech specimens from the Donkmeer had two coalesced eye spots; an unpigmented nuchal band, an unpigmented genital bar, and an anal patch; three rows of dorsal papillae; and two rows of five pre-anal papillae (Figures 2–4).

Molecular comparison of 603 nucleotides of CO-1 revealed a 99.5% similarity from a specimen of *P. ornata* from the Donkmeer Belgium (GenBank KP176597) to five specimens of *P. ornata* collected from the type locality (West River, New Haven, Connecticut, USA) (GenBank JQ812128 – JQ812132) and a 99.2% to 99.5% similarity to four specimens of *P. ornata* collected from the type locality (Shivericks Pond, Falmouth, Barnstable County, Massachusetts, USA) of *Placobdella phalera* (junior synonym of *P. ornata*) (JQ812133–JQ812136). *Placobdella ornata* from the Donkmeer is identical to *P. ornata* from its type locality (West River, New Haven, Connecticut, USA) and *P. ornata* from Shivericks Pond, Falmouth, Barnstable County, Massachusetts, USA with the exception of 3 to 5 nucleotides. A difference of 14.4% (87 nucleotides) was found between a specimen of *P. ornata* from the Donkmeer, Belgium (GenBank KP176597) and the European congener *Placobdella costata* (Fr. Müller, 1846) (AY962461).

Other exotic or alien species collected in the course of this study include: the Ponto-Caspian mysid shrimp *Limnomysis benedii* (Czerniavsky, 1882), the brown planarian *Girardia tigrina* (Girard, 1850), the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817), the aquatic gastropod *Physella acuta* (Draparnaud, 1805) and the New Zealand mudsnail *Potamopyrgus antipodarum* J. E. Gray, 1843. The pumpkinseed sunfish *Lepomis gibbosus* (Linnaeus, 1758), the red-eared slider *Trachemys scripta elegans* Wied-Neuwied, 1839 and Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards,

**Table 1.** Morphological comparison of *Placobdella ornata* (Verrill, 1872) and *Placobdella costata* (Fr. Müller, 1846).

	<i>Placobdella ornata</i> (Figure 2–4)	<i>Placobdella costata</i> (Figures 5)
Size	16.3–26.3 mm	20–70 mm
Eyes	two coalescent eyes	two coalescent eyes
Dorsal papillation	three rows (medial and two paralateral)	five rows (medial, two paramedial and two paralateral)
Dorsum colour	reddish brown with unpigmented nuchal (transverse) band, genital bar, and anal patch	dark greenish, brownish
Pre-anal papillation	two rows of five pre-anal papillae	two rows of three pre-anal papillae



**Figure 2.** Dorsal surface of living specimen of *Placobdella ornata* from the Donkmeer, Belgium. Note the characteristic white genital patch in the anterior third (see arrow). Photograph by Jan Soors.



**Figure 3.** Dorsal surface of preserved specimen of *Placobdella ornata* from the Donkmeer, Belgium. Scale bar equals 2 mm (arrow: nuchal band, asterix: genital patch). Photograph by Eric Lazo-Wasem.



**Figure 4.** Posterior end of *Placobdella ornata* from the Donkmeer, Belgium showing the papillar pattern of two rows of five pre-anal papillae (see arrows). Photograph by Jan Soors.



**Figure 5.** Dorsal surface of preserved specimen of *Placobdella costata* from Driebergen, The Netherlands. Scale bar equals 5 mm. Photograph by Ton van Haaren.

1853 were found in the Donkmeer in low numbers during a fish stock assessment completed by the Research Institute of Nature and Forest (INBO) (Van Thuyne 2009).

Other leeches that were collected at the Donkmeer during this investigation were *Glossiphonia complanata* (Linnaeus, 1758), *Hemiclepsis marginata* (O. F. Müller, 1774), *Erpobdella octoculata* (Linnaeus, 1758), *Haemopsis sanguisuga* (Linnaeus, 1758) and Piscicolidae indet., which are all common and widespread European species.

## Discussion

This report of *P. ornata* is the first documentation of a second species of the genus *Placobdella* from Europe. A congener, *Placobdella costata* is widely distributed throughout Europe (Sawyer 1986; Bielecki et al. 2012). In addition to molecular differences, *P. ornata* also differs from *P. costata* morphologically. *Placobdella ornata* is distinguished from *P. costata* by its possession of five pair of pre-anal papillae, three rows of dorsal papillae, and unpigmented nuchal band,

genital bar and anal patch, compared to three pair of pre-anal papillae, five rows of dorsal papillae, and an interrupted dorsal medial line in *P. costata* (see Table 1). No host of *P. ornata* has been documented. Although there is a large amount of literature reporting *P. ornata* as a turtle parasite in North America, the identifications of specimens are based on a series of taxonomic errors (Moser et al. 2012). *Placobdella ornata*, re-described by Moser et al. (2012) based upon syntype series and contemporary specimens collected from the type locality, appears to be restricted to the northeastern region of the United States.

Specimens of *P. ornata* may have been brought into the Donkmeer with the introduction of its host. Moser et al. (2005) reported the eastern North American leech species *Placobdella parasitica* (Say, 1824) from California for the first time and surmised that the leech was introduced with its host. Introductions of alien blood-feeding leeches with their hosts have the risk of introducing blood parasites (e.g. trypanosomes, haemogregarines, etc.) which are transmitted by these leeches.

To date, *P. ornata* has a confirmed distribution in the US states Connecticut and Massachusetts (Moser et al. 2012), and now the Donkmeer in Belgium. Although Graf (1899) suggested that *P. ornata* is parasitic on the common musk turtle *Sternotherus odoratus* Latreille, 1801, no host has been confirmed.

Knowledge on freshwater leeches is rather limited in Belgium compared to the well-studied neighboring countries Germany and The Netherlands. In both countries, the presence of at least four non-indigenous leech species has been confirmed: *Helobdella europea* Kutschera, 1987, *Caspiobdella fadejewi* Epshtein, 1961, *Barbronia weberi* (Blanchard, 1897) and *Dina punctata* Johansson, 1927 (van Haaren et al. 2004). *Placobdella costata* is still to be found in Belgium but it is also present in other countries without freshwater turtles (Bielecki 2012; Elliott and Tullet 1982). Amphibians, mammals of which *Castor fiber* (Linnaeus, 1758) is the most suspected and birds are potential hosts of *P. costata* (Sawyer 1986; Grosser 1996).

In Belgium, there is a very recent record of the Asian leech *Barbronia weberi* (pers. comm. Nobby Thys) and in 2005, one specimen of the southeast European leech *Hirudo verbana* (Carena, 1820) was found in Edegem (province of Antwerp) (Vercauteren and Isate 2005). This medicinal leech was possibly released or escaped after having been used in a medicinal therapy; no further observations on this species are known.

Further investigation is needed to determine if *P. ornata* occurs in other localities in Europe.

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