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First record of the alien leech *Myzobdella lugubris* Leidy, 1851 (Hirudinea, Piscicolidae) in the Palearctic

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ABSTRACT

First record of the alien leech *Myzobdella lugubris* Leidy, 1851 (Hirudinea, Piscicolidae) in the Palearctic

In this study, we report the occurrence of the Nearctic leech *Myzobdella lugubris* in a brackish water body of the Riserva Naturale dello Stato “Le Cesine” (Apulia, Italy). Two specimens of the species were collected in October 2016 from the neck and the forelimbs of a European pond turtle, *Emys orbicularis hellenica*, and identified both based on morphology and molecular sequence data. In its native range, *M. lugubris* is known to be a host and vector of bacteria and viruses, among which some serious fish pathogens. In light of the possible noxious ecological effects exerted through pathogen spillover on autochthonous vertebrates, the distribution of the species and its possible role as a vector of pathogens in the invaded area should be urgently monitored.

Key words: *Emys orbicularis hellenica*, R.N.S. “Le Cesine”, Biological invasions, pathogen spillover

RESUMEN

Primer registro de la especie invasora *Myzobdella lugubris* Leidy, 1851 (Hirudinea, Piscicolidae) en el Paleártico

En este estudio reportamos la aparición de la sanguijuela Neártica *Myzobdella lugubris* en un cuerpo de agua salobre de la reserva natural Riserva Naturale dello Stato “Le Cesine” (Apulia, Italy). Dos especímenes de la especie fueron recolectados en el cuello y las extremidades anteriores de una tortuga de estanque europea *Emys orbicularis hellenica* en Octubre del 2016. La identificación de los dos especímenes fue basada en datos morfológicos y de secuenciación molecular. En su distribución natural, *M. lugubris* es conocida por ser huésped y vector de bacterias y virus, de los cuales algunos son patógenos de gravedad en peces. En vista de los posibles efectos nocivos, que ejercen sobre las poblaciones autóctonas de vertebrados a través de la propagación de patógenos, es urgente monitorear la distribución de la especie y su posible rol como vector de patógenos en las áreas invadidas.

Palabras clave: *Emys orbicularis hellenica*, R.N.S. “Le Cesine”, Invasión biológica, propagación de patógenos

INTRODUCTION

Myzobdella lugubris Leidy, 1851 (Hirudinea: Piscicolidae) is a common euryhaline leech occurring throughout North American fresh and brackish waters (Sawyer & Shelley, 1976; Klemm, 1985) (Fig. 1). The wide distribution of the species seems to be related to its salinity tolerance: *M. lugubris* has been collected in water bodies with salinity levels ranging from 0 to 26 ‰ (Sawyer *et al.*, 1975). Cocoons are able to hatch at salinity values comprised between 13 ‰ and 28 ‰ (Oren, 1981). During most of the year, *M. lugubris* is parasitic on a piscine host (usually

belonging to the families Ictaluridae, Mugilidae, Paralichthyidae or Fundulidae), but it leaves the fish host to lay its cocoons on decapods (Daniels *et al.*, 1975; Saglam *et al.*, 2018). In brackish areas, the cocoons are usually deposited on the blue crab (*Callinectes sapidus* Rathbun, 1896) (Daniels *et al.*, 1975).

Myzobdella lugubris is also known to be a host of bacteria (e.g. *Flavobacterium psychrophilum* (Bernardet & Grimont 1989)) (Schulz & Faisal, 2010) and viruses (Viral Hemorrhagic Septicemia virus) (Faisal & Schulz, 2009). The bacterium *F. psychrophilum* is a fish pathogen that causes “Bacterial Cold Water Disease” (Schulz *et al.*,



Figure 1. Geographic distribution of *Myzobdella lugubris* and origin of the studied samples. The symbols on the map indicate the samples included in the molecular analyses. The circle represents the Palearctic locality where *Myzobdella* specimens were collected. The grey dotted area shows the non-native distribution range of the species whereas the grey full area represents its native distribution range. *Distribución geográfica y origen de las muestras estudiadas de Myzobdella lugubris.* Los símbolos en el mapa indican las muestras que fueron incluidas en el análisis molecular. El círculo representa la localidad Peleártica en donde los especímenes de *Myzobdella* fueron colectados. El área punteada en gris indica el rango no nativo de distribución de las especies, mientras el área totalmente gris representa su rango de distribución nativo.

2010), although it is currently unknown whether *M. lugubris* can actually transmit the virus to fish hosts (Faisal & Schulz, 2009). In the area of Lake Erie (Michigan, USA), *M. lugubris* dominates the leech community and heavily parasitizes the channel catfish *Ictalurus punctatus* (Rafinesque, 1818) and the freshwater drum *Aplodinotus grunniens* Rafinesque, 1818 (Schulz & Faisal, 2010).

Although the species successfully invaded several regions of North and Central America (e.g. Font, 2003), to date, no records of the species out of the Nearctic region are available (Neubert & Nesemann, 1999; Minelli, 2005; Saglam *et al.*, 2018).

In the frame of the sampling activities aimed at investigating the phylogeography of the glossiphoniid leech *Placobdella costata* (Fr. Müller, 1846) in the western Palearctic, two leech specimens ascribable to the Nearctic piscicolid genus *Myzobdella* Leidy, 1851 were collected on a turtle host in southern Italy. These specimens were thus studied in order to identify them at the species level and to single out their possible origin and dispersal vector.

MATERIAL AND METHODS

Sampling was carried out in southern Italy – Riserva Naturale dello Stato Oasi WWF “Le Cesine” (Vernole, LE). The study site (40° 21' 12" N, 18° 20' 31" E) is a canal in a wide coastal wetland characterized by the presence of different habitats: canals, permanent and temporary water bodies, swamps and brackish areas.

The sampling, aimed at censusing the local population of the glossiphoniid leech *Placobdella costata*, was carried out in October 2016 with the permission of the managing authority. A single European pond turtle, *Emys orbicularis hellenica* (Valenciennes, 1832) was captured with a baited funnel trap left overnight in the sampling site. Two leech specimens were found on the neck and forelimb of this pond turtle and were collected and fixed *in situ* in 95 % ethanol. They were later identified as *Myzobdella lugubris* based on the morphological key of Moore (1946) and the description provided by Saglam *et al.* (2018); one of the collected specimens was deposited at the Museo di Storia Naturale, Sezione di Zoologia

“La Specola”, University of Firenze, Italy (Registration number: MZUF1929). One leech was also analyzed genetically by amplifying and sequencing a 540-bp-long fragment of the cytochrome oxidase subunit I mtDNA gene (COI), the DNA barcode, a marker which is widely used in Hirudinea systematics (e.g. Siddall *et al.*, 2005; Oceguera-Figueroa *et al.*, 2010; Marrone *et al.*, 2016; Saglam *et al.*, 2018). DNA extraction was carried out with BIORON Ron’s Tissue DNA mini kit following the manufacturer’s protocol. A partial sequence of the mitochondrial gene COI was amplified using the primers LCO1490 and HCO2198 described by Folmer *et al.* (1994).

PCR mix consisted of 2.5 µl of BIORON 10x ammonium buffer, 0.4 µl of dNTP at 10 mM, 0.5 µl of each 0.5 µl primer, 0.3 µl of BIORON DSF Taq enzyme, 1.5 µl of DNA template and 19.3 µl of double-distilled water, for a total reaction volume of 25 µl. The amplification consisted of an initial denaturation step of 94 °C for 5 min followed by 35 cycles of 94 °C for 45 s, 45 °C for 45 s and 72 °C for 60 s, followed by a final extension at 72 °C for 5 min.

The PCR product was checked on a 2 % agarose gel, purified using the Exo-SAP-IT kit (Affymetrix USB) and sequenced by Macrogen Inc. (Spain) with an ABI 3130xl (Applied Biosystems) sequencer with the same primers used for PCR amplification. The chromatogram was imported and edited with Chromas Lite 2.6.4 (Technelysium Pty Ltd, South Brisbane, Australia); MEGA7 (Kumar *et al.*, 2016) was used to translate the novel COI sequence to amino acids in order to check for the possible presence of frameshifts or stop codons, which would indicate the presence of sequencing errors or pseudogenes. The novel sequence was deposited in GenBank under the Accession Number MG820612.

All the *Myzobdella* sequences available on GenBank, and a single sequence of the piscicolid leech *Piscicola geometra* (Linnaeus, 1761) to be used as outgroup, were downloaded and included in the molecular analysis (see figure 2 for their Accession Numbers).

Novel and published GenBank sequences were aligned with ClustalX (Thompson *et al.*, 1997). Bayesian inference (BI) of the phylogenetic

ic analysis was then performed as implemented in MrBayes 3.2.6 (Ronquist *et al.*, 2012). The analysis was performed using a Generalized Time Reversible model of sequence evolution with a proportion of invariable sites (GTR + R), as selected by the Akaike information criterion in jModelTest 2.1.9 (Darriba *et al.*, 2012). Node supports were evaluated by their posterior proba-

bilities. The BI analysis was performed with two independent runs of 1000000 generations and four Markov chains using default heating values. Trees and parameter values were sampled every 100 generations resulting in 10 000 saved trees per analysis. An initial fraction of 2000 trees (20 %) was conservatively discarded as 'burn-in'. For all analyses, standard deviation of split frequencies

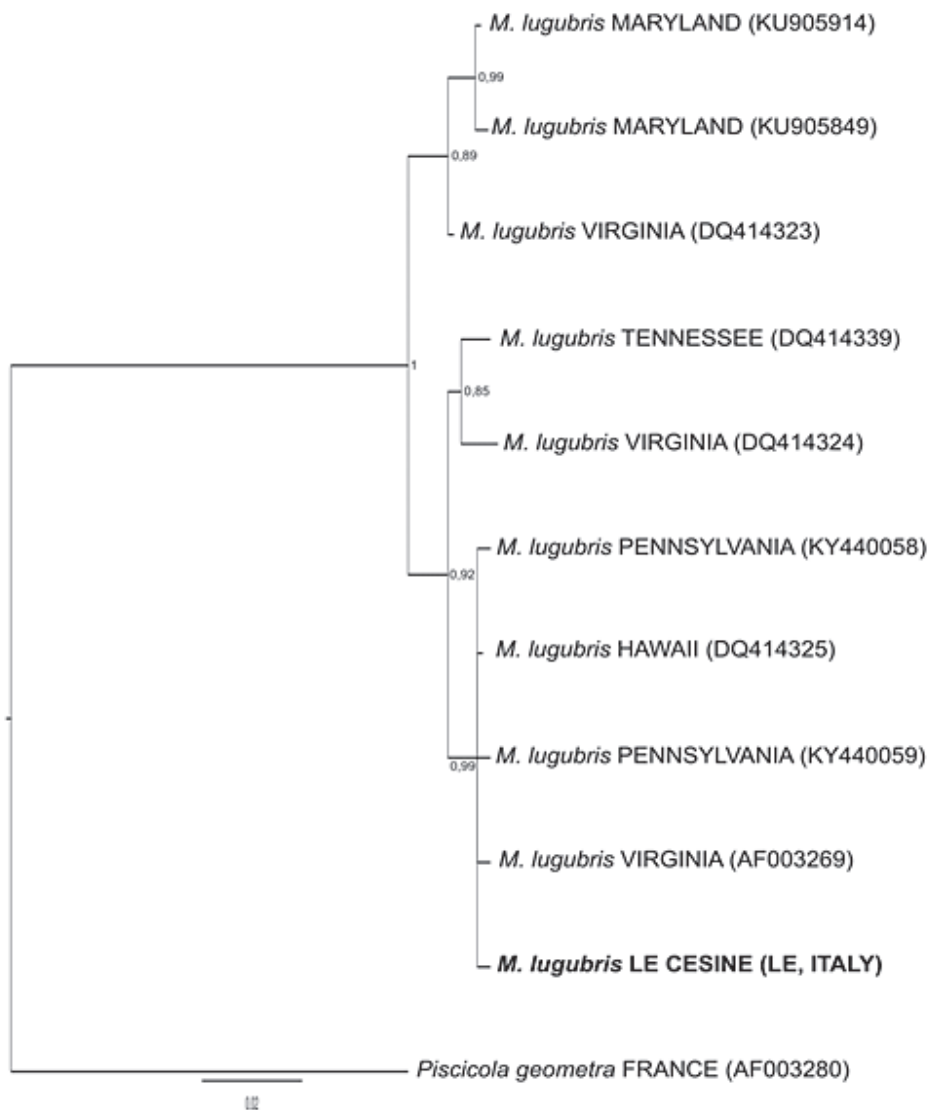


Figure 2. Bayesian phylogram based on a 540-bp-long fragment of the mtDNA gene for the cytochrome oxidase subunit I. Node support is reported as nodal posterior probabilities. The accession numbers of the sequences derived from GenBank are shown in brackets. *Filograma bayesiano basado en un fragmento de 540-bp-long del gen mtDNA del citocromo oxidasa subunidad I. El soporte al nodo es reportado como probabilidad nodal posterior. Los números de acceso de las secuencias derivados de GenBank están indicados entre paréntesis.*

reached values lower than 0.0078, and values of the potential scale reduction factor (PSRF) were between 1.000 and 1.006 for all parameters, thus indicating convergence of the runs.

RESULTS

Collected leeches were identified as *Myzobdella lugubris* on a morphological basis. The sequencing of the chosen marker and its comparison with the publicly available sequences confirmed its univocal identification, with the *Myzobdella* sequence from “Le Cesine” clustering within a *M. lugubris* clade including samples from Pennsylvania, the Hawaii and Virginia, i.e. both from native and non-native parts of its distribution range, in the BI tree (Fig. 2).

The topology of the phylogenetic tree based on BI thus confirmed the morphology-based identification of the leech collected in “Le Cesine” as *Myzobdella lugubris*.

DISCUSSION

Biological invasions are nowadays unanimously recognized as one of the main threats to the conservation of biological diversity worldwide, and inland waters are known to be highly vulnerable to either inadvertent or deliberate introductions of species and to their subsequent spread (Gherardi *et al.*, 2008). Several alien species of North American origin have successfully invaded southern Italian inland and coastal waters, often threatening the autochthonous biota (e.g. Gennaio *et al.*, 2006; Cianfanelli *et al.*, 2007; Gravili *et al.*, 2010; Marrone *et al.*, 2011; Mancinelli *et al.*, 2013; Bianco, 2014; Marrone & Naselli-Flores, 2015; Vecchioni *et al.*, 2017; Cilenti *et al.*, 2017, and references therein).

This work reports the first evidence of the presence of the leech *Myzobdella lugubris* in the whole Palearctic: to date, no *Myzobdella* species were known to occur in this biogeographical region (Peckarsky *et al.*, 1990; Hoffman, 1999; Fofonoff *et al.*, 2018) (see Fig. 1). The anthropogenic introduction of this leech out of its native range was possibly facilitated by its robust mechanisms for natural or anthropogenic passive dispersal (Saglam *et al.*, 2018). In the

Mediterranean area, the arrival of this Piscicolidae is possibly related to the commercial import of blue crabs (*Callinectes sapidus*) from the eastern U.S.A. and the Gulf of Mexico (Morado, 2007), facilitated by the strong adherence of *Myzobdella* cocoons to crustacean exoskeletons (Saglam *et al.*, 2018). It is possibly not by chance that the site where *M. lugubris* was observed is located just 13 km from the Aquatina lagoon, an Apulian site where *C. sapidus* specimens were found to be co-occurring with *Hematodinium* sp., a dinoflagellate parasite of crustaceans including *Callinectes sapidus*, typical of the Atlantic coasts (Mancinelli *et al.*, 2013).

Myzobdella lugubris is a potentially noxious taxon, associated with epidemic oral ulceration in the largemouth bass (*Micropterus salmoides* Lacépède, 1802) (Noga *et al.*, 1990) and with the bacterium *Flavobacterium psychrophilum*, which is the causative agent of the “Bacterial Cold-Water Disease” and of the “Rainbow Trout Fry Syndrome”. Both these diseases cause high mortality in salmonids and increase their susceptibility to other diseases (Nematollahi *et al.*, 2003). *M. lugubris* is also known to be a vector of the Viral Hemorrhagic Septicemia virus (VHSV), which causes high mortality among fish communities.

The present report is the first evidence of *Myzobdella lugubris* occurring on a reptile; it remains unclear, however, if the two specimens were actually feeding on the pond turtle, or were rather there for cocoon deposition or other non-trophic reasons, as demonstrated in the glossiphoniid genus *Helobdella* (Marrone *et al.*, 2016). However, analogously to what was stressed for the system “*Haemogregarina stepanowi* - *Placobdella costata* - *Emys trinacris*” by Arizza *et al.* (2016), the possible presence of pathogen spillover related to *M. lugubris* on the vertebrates occurring in “Le Cesine” and neighboring areas must be monitored.

The finding of two adult *Myzobdella lugubris* specimens on the single pond turtle captured suggests that a significant population of the species might be present in the study area, but further investigations are needed to test this hypothesis, including sampling of fishes, decapods and other potential hosts along the Apulian coasts.

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