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First record of *Oligochoerus limnophilus* (Acoela, Acoelomorpha) from British waters

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Abstract

First record of Oligochoerus limnophilus *(Acoela, Acoelomorpha) from British waters.*— We report the occurrence of the acoel *Oligochoerus limnophilus* (Acoelomorpha) from the British Islands, based on specimens captured in the river Thames (locally known as the river Isis) in Oxford, England, thereby considerably widening the distributional range of the species that had formerly been reported only from continental Europe. We further present live images and CLSM–projections of systematically informative structures that corroborate a close relationship with the genus *Convoluta* Ørsted, 1843.

Key words: Acoela, *Oligochoerus*, Limnic, Thames.

Resumen

Primera cita de Oligochoerus limnophilus *(Acoela, Acoelomorpha) en aguas británicas*.— Informamos de la existencia de poblaciones del acelo *Oligochoerus limnophilus* (Acoelomorpha) en las islas Birtánicas, basándonos en los especímenes capturados en el río Támesis (también conocido localmente como río Isis) a su paso por Oxford, Inglaterra, ampliando así considerablemente el área de distribución de la especie, restringida hasta ahora al continente europeo. La información gráfica que aportamos, imágenes de especímenes vivos y proyecciones CLSM de estructuras seleccionadas por su valor sistemático, corrobora su estrecha relación con los miembros del género *Convoluta* Ørsted, 1843.

Palabras clave: Acoela, *Oligochoerus,* Límnico, Támesis.

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Introduction

Acoels are small and predominantly marine flatworms. Of the approximately 400 described species only two are known from freshwater. *Limonoposthia polonica* (Kolasa & Faubel, 1974) occurs in lakes in Poland (Kolasa & Faubel, 1974) while *Oligochoerus limnophilus* Ax & Dörjes, 1966 has been found in a variety of environments such as lakes, irrigation ditches, and rivers in continental Europe: in Germany (Ax & Dörjes, 1966; Müller & Faubel, 1993), France (Ax & Dörjes, 1966), the Netherlands (Dörjes & Young, 1975), and Romania (Nastasescu & Popescu–Marinescu, 2004) (fig. 1A). Interestingly, the six other species of the genus *Oligochoerus* Beklemischev, 1963 occur exclusively in the Caspian Sea, suggesting that the brackish waters of the Pontic–Caspian have been a steppingstone for the species to invade freshwater (Ax & Dörjes, 1966).

Material and methods

Specimens were found on the lower surface of stones on an artificial platform above the water level of the river Thames (fig. 1B) in Oxford (–1,2557780º W 51,745882º N; 59 m: collection date: 26 IX 11). They were transferred with a fine brush from the stones into 15 ml falcons filled with water from the collection site and subsequently transported to the laboratory. Live animals were observed in squeeze preparations using a Leica MZ16F compound microscope and photographed using a Wild stereomicroscope with a Deltapix InfinityX camera. Several specimens were prepared to reveal F–actin using the following protocol: anaesthetization by slowly adding drops of 100% ethanol, fixation with 4% paraformaldehyde in 0.1 M PBS for 1 h, three washes with 0.1 M PBS containing 0.1% Triton–X (PBS–T) for 5 min each, incubation in Alexa Fluor 488 Phalloidin (Molecular Probes, Eugene, OR) in PBS–T (concentration 1:100) for 1 h, three washes with PBS for 5 min each, mounting with 80% glycerol, and sealing with nail polish. Specimens were scanned using either a Leica SPE or SPII confocal microscope. Images were processed using the software Photoshop 7.0.

Results and discussion

The specimens (figs. 2A, 2B) can be safely designated to the species *Oligochoerus limnophilus* Ax & Dörjes, 1966 by the following characters: presence of a pharynx on the ventral side in front of the statocyst and close to the anterior end (fig. 2C), male copulatory organ consisting of a glandular penis that is invaginated into a weak muscular bulb (fig. 2D), presence of multiple bursal nozzles, each with its own seminal bursa, presence of prostatoid organs with needles on the lateral rim in variable but species–typical numbers (figs. 2C, 2E), presence of a pair of eyes, and occurrence in fresh water.

In accordance with Ax & Dörjes (1966) we are confident about the placement of the species in the genus *Oligochoerus*. The genus *Oligochoerus* resides within the family Convolutidae (Achatz et al., 2010) but its phylogenetic relationships remain unresolved due to the lack of sequence data. In the original description of the species (Ax & Dörjes, 1966) a sistergroup relationship with the genus *Polychoerus* Mark, 1882 was proposed based on the differentiation of the ovary into a germative and a yolk–producing part. However, the 'vitellaria' have been shown to be misinterpreted lobes of maturing oocytes (Achatz et al., 2007). We propose a close relationship of the genera *Oligochoerus* and *Convoluta* Ørsted, 1843 based on the shape of the body, the morphology of the male copulatory organ, and especially the occurrence of prostatoid organs, which furthermore have actin–rich needles at the terminal tip in common with *Convoluta convoluta* (Abildgaard, 1806) (fig. 2E; see figure 2 in Achatz et al., 2010).

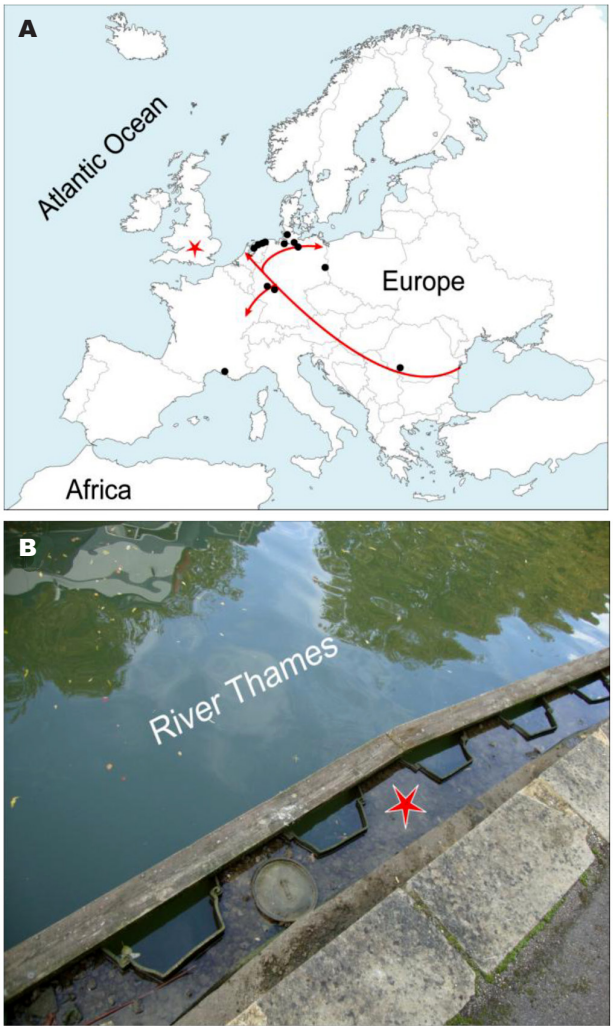

Fig. 1. A. Geographic range of *Oligochoerus limnophilus.* Black dots, previously known localities for the species (data extracted from literature and the Turbellarian Taxonomic Database [Tyler et al., 2006–2012]). Red star, this study. Red arrows indicate the South Central migration corridor of Pontic–Caspian aquatic invertebrates that connects the Danube and the Rhine water basins through the Main–Danube Canal (Ketelaars, 2004). B. Picture of the habitat at Oxford. Red star: artificial substrate where individuals of *O. limnophilus* were collected.

Fig. 1. A. Distribución geográfica de Oligochoerus limnophilus. *Los puntos negros indican las localidades donde era conocida previamente la presencia de la especie (datos extraídos de la literatura y de la Turbellarian Taxonomic Database [Tyler et al., 2006–2012]). La estrella roja corresponde a este estudio. Las flechas rojas indican el corredor central sur de migración de invertebrados acuáticos póntico–caspios que conecta las cuencas del Danubio y el Rin a través del canal principal del Danubio (Ketelaars, 2004). B. Fotografía del hábitat en Oxford. Estrella roja: sustrato artificial donde se recolectaron los especímenes de* O. limnophilus.

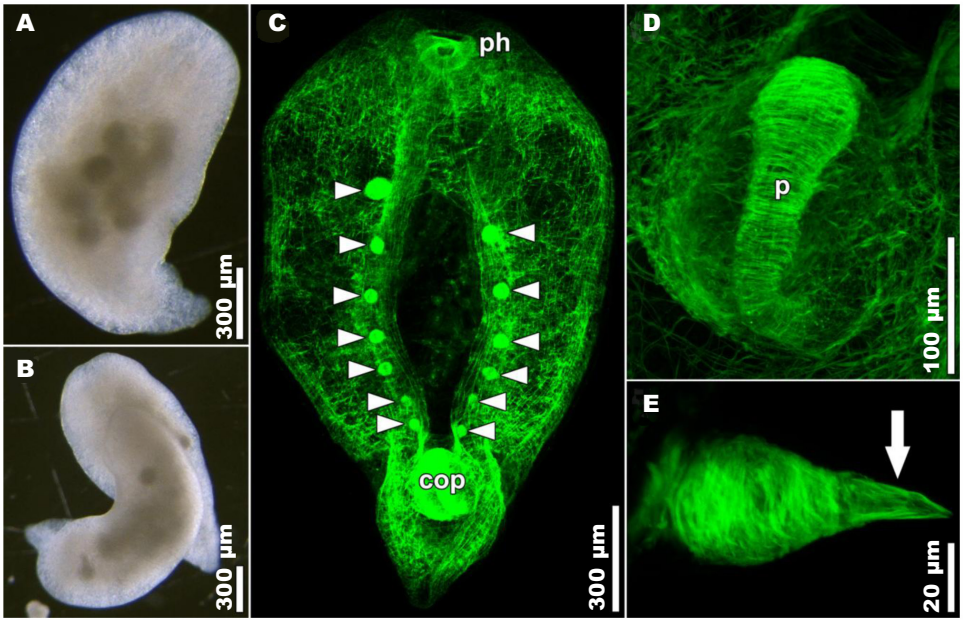

Fig. 2. A–B. Live images of adult specimens of *O. limnophilus*, dark spheres in the center of the body are eggs; C–E. CLSM projections of preparations stained with fluorophore–tagged phalloidin; C. Ventral side of the whole body, arrowheads point to prostatoid organs; D. Male copulatory organ, note that there is no distinct muscular sheet of the seminal vesicle but instead the parenchymal musculature is absent around the penis and slightly less dense in the adjacent area; E. Prostatoid organ, arrowhead points to F–actin–rich needles at distal tip of the organ. (Abbreviations: cop. Male copulatory organ; p. Penis; ph. Pharynx.)
Fig. 2. A–B. Imágenes de especímenes adultos vivos de O. limnophilus*, las esferas oscuras situadas en el centro del cuerpo son huevos; C–E. Proyecciones CLSM de preparaciones teñidas con faloidina conjugada con fluoróforo; C. Vista ventral del cuerpo, las puntas de flecha señalan los órganos prostatoides; D. Órgano copulador masculino, obsérvese que la vesícula seminal no presenta una capa muscular diferenciada y que en cambio la musculatura parenquimática es inexistente en torno al pene y ligeramente menos densa en el área adyacente; E. Órgano prostatoide, la flecha señala agujas ricas en actina F en el extremo distal del órgano. (Abreviaturas: cop. Órgano copulador masculino; p. Pene; ph. Faringe.)*

As has been described for other sampling sites, specimens were abundant in well–defined spots (on the underside of stones on the artificial platform) but were apparently absent in the proximity (Ax & Dörjes, 1966). Due to this selective occurrence within the habitat and to the small size and translucent appearance of the animals, we assume that specimens have often been overlooked and that the known range of the species is an underestimate. For example, the isolated record from Romania suggests a continuous distribution in the Danube basin from southern Germany to Romania. Interestingly, the other six species of the genus *Oligochoerus* occur exclusively in the Caspian Sea (Beklemischev, 1963) and

therefore the evolutionary origin of the genus most likely lies in this area. With regards to the new record from England it would be of great interest to know how and when *O. limnophilus* expanded through Europe and the British islands. More precisely, we need to clarify whether the current distribution of the species in Europe is due to a post–glacial migration, as proposed by Ax & Dörjes (1966), or whether it is a more recent event driven by human activity as in the case of other Ponto–Caspian species, *e.g.* the flatworm *Dendrocoelum romanodanubiale* (Codreanu, 1949) (Nastasescu & Popescu–Marinescu, 2004). Keteelars (2004) considered that *O. limnophilus* expanded its range through the South Central fauna corridor that runs from the Danube to the Rhine and branches into the Netherlands, Northern Germany and Rhone river, precisely the areas where the populations of *O. limnophilus* are more abundant (fig. 1A). If this is true the colonization of the species in England could well be a recent event mediated by human activity.

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