Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 90 (2019): e902572

Taxonomy and systematics

First report of *Chaetogaster limnaei* (Annelida: Naididae) in Chile based on samples retrieved from an invasive freshwater snail

Primer registro de Chaetogaster limnaei *(Annelida: Naididae) en Chile con base en muestras obtenidas de un caracol de agua dulce invasor*

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Received: 15 diciembre 2017; accepted: 24 septiembre 2018

Abstract

Some naidid oligochaetes establish commensal relationships with species of molluscs worldwide. In the present study, we report the finding of *Chaetogaster limnaei* in Illapel River, northern Chile. This worm was found inhabiting the mantle cavity of the freshwater gastropod *Physa acuta*, an invasive species in this country. The taxonomic status of *C. limnaei* was confirmed by molecular analysis based on mitochondrial 16S ribosomal RNA gene.

Keywords: Aquatic molluscs; Invasive species; Naidids; Oligochaetes; *Physa acuta*

Resumen

Los oligoquetos Naididae pueden establecer relaciones comensales con especies de moluscos alrededor del mundo. En el presente estudio se informa el hallazgo del oligoqueto *Chaetogaster limnaei* en el río Illapel, norte de Chile. Los animales se encontraron habitando la cavidad del manto del gasterópodo de agua dulce *Physa acuta*, una especie invasora en este país. El estado taxonómico de *C. limnaei* se confirmó mediante el análisis molecular basado en secuencias de ADN del gen mitocondrial del ARN ribosomal 16S.

Palabras clave: Molusco acuático; Especie invasora; Naididae; Oligoquetos; *Physa acuta*

ISSN versión electrónica: 2007-8706; Universidad Nacional Autónoma de México, Instituto de Biología. https://doi.org/10.22201/ib.20078706e.2019.90.2572

Introduction

Naididae Ehrenberg, 1828, is a family of small aquatic oligochaetes that play an important role in many marine and freshwater ecosystems worldwide (Brinkhurst & Jamieson, 1971). The group includes detritivorous, grazers and carnivorous species (Pinder & Ohtaka, 2014). One member of the family is the genus *Chaetogaster* von Baer, 1827 which, in addition to free-living worms, includes *Chaetogaster limnaei* von Baer, 1927, an ectosymbiont species for which a close commensal association with several mollusc taxa, including gastropods and bivalves, has been broadly reported (Barbour, 1977; Buse, 1974; Conn et al., 1995; Gelder, 1980; Gruffydd, 1965a, b; Ibrahim, 2007; Streit, 1974).

Chaetogaster limnaei has no dorsal chaetae and the ventral chaetae, up to 14-20 per bundle, are absent in segments 3 to 5 (Gruffydd, 1965a; Brinkhurst, 1986). The species is also characterized by having a vestigial prostomium and chaetae with strongly curved teeth (Gruffydd, 1965a; Gelder, 1980; Brinkhurst, 1971, 1986). These features, as well as its habitat, separate *C. limnaei* from the other species of the genus. Two subspecies have been proposed for *C. limnaei*, based on habitat and feeding differentiation, *C. limnaei limnaei* Von Baer, 1927 and *C. limnaei vaghini* Gruffydd, 1965. *Chaetogaster limnaei limnaei* inhabits the mantle cavity of snails, where it reduces parasite infection by feeding on trematode larvae in addition to other organisms, so it has been considered a commensal (Gruffydd, 1965a, b; Khalil, 1961; Michelson, 1964; Rodgers et al., 2005; Zimmermann et al., 2011). However, Gamble and Fried (1976) reported *C. limnaei limnaei* consuming host snail epithelial tissue, indicating a parasitic relationship. *Chaetogaster limnaei vaghini*, on the other hand, lives in the renal system of the host, where it feeds on kidney cells, so it has been considered as endoparasitic (Gruffydd, 1965a, b). This subspecies separation is not supported by recent molecular phylogenetic analysis based on COI sequences, which provides evidence that these forms belong to the same taxonomic entity (Smythe et al., 2015). In South America, *C. limnaei* has been found associated with snails *Aplexa rivalis* (Maton & Rackett, 1807) (Physidae), *Biomphalaria straminea* (Dunker, 1848) (Planorbidae) and *Pseudosuccinea columella* (Say, 1817) (Lymnaeidae) in Brazil (Callisto et al., 2005; Martins & Alves, 2008, 2010). Di Persia (1980) found *C. limnaei* in Argentina. In Chile, Gluzman (1990) and Valdovinos (2008) included *Chaetogaster* sp. in their checklists without specifying habitat or host, while Fuentealba-Jara (2011) recorded this worm associated with the freshwater snails of the genus *Uncancylus* Pilsbry, 1913. Only *Chaetogaster diastrophus* (Gruithuisen, 1828) has been recorded in freshwater ecosystems at the Chilean Altiplano (SINAB, 2017).

In the present study, we report the first finding of *C. limnaei* in Chile based on samples retrieved from the gastropod *Physa acuta* Draparnaud, 1805, an invasive snail recently reported in the country (Bousset et al., 2014; Collado, 2017).

Materials and methods

Eleven adult physid snails were sampled from the Illapel River (Fig. 1), Coquimbo Region, Northern Chile (31°37'55.30" S, 71°09'20.30" W) on March 2016 and preserved in ethanol. The snails were assigned to *Physa acuta* following Collado (2017). The worms were isolated by dissection of the mantle cavity of snails and observed using stereo and light microscopes (Motic). Photographs of the chaetae were obtained using a scanning electron microscope (SEM) Hitachi SU3500. The specimens were identified as *C. limnaei* according to taxonomic keys (Brinkhurst, 1971, 1986; Kathman & Brinkhurst, 1998) and additional literature regarding the morphology of the species (Cichy et al., 2016; Gelder, 1989; Gruffydd, 1965a, b; Khalil, 1961). Genomic DNA was extracted using the CTAB method (cetyltrimethyl ammonium bromide) (Winnepennickx et al., 1993) of one specimen assigned to this species. The mitochondrial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers 16Sar-L and 16Sbr-H (Palumbi, 1996). PCR profile was described by Collado and Méndez (2012). Nucleotide sequences were obtained by automatic sequencing in forward and reverse directions at Macrogen Company (South Korea). DNA sequences were edited in the software BioEdit (Hall, 1999) and aligned with Clustal X (Thompson et al., 1997). Phylogenetic analyses were

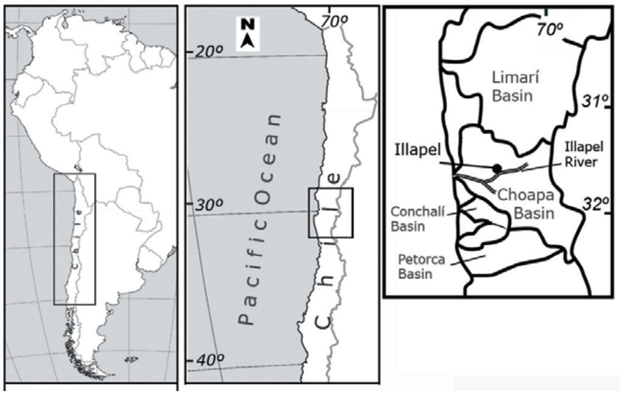

Figure 1. Illapel River in the Choapa River Basin, locality of occurrence of *Chaetogaster limnaei* in northern Chile.

performed using maximum parsimony (MP) and Bayesian inference (BI) methods. The MP analysis was based on heuristic search, the TBR algorithm and the random addition of sequences using the program PAUP* 4.0 (Swofford, 2003). The statistical confidence of the nodes was evaluated performing 100 bootstrap pseudoreplicates (Felsenstein, 1985). The BI analysis was performed in the software MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003), previously selecting the best model of sequence evolution with jModelTest (Posada, 2008). The analysis was run for 3 million generations sampling every 1,000 generations; posterior probabilities were obtained using a burn-in period of 10%. The analyses were performed together with 16S rRNA sequences from other congeners and naidid taxa (Bely & Sikes, 2010; Envall et al., 2006, 2012; Sjölin et al., 2005).

Genomic DNA of *C. limnaei* is housed in the Laboratorio de Malacología y Sistemática Molecular, Universidad del Bío-Bío, Chillán, Chile. The 16S sequence obtained in the present study was deposited in GenBank (accession number: KY436156).

Results

Specimens of *C. limnaei* (Fig. 2A-C) were found living in the mantle cavity of the invasive freshwater snail *P. acuta* sampled in Illapel River. The worms were characterized by the absence of dorsal chaetae, the absence of ventral chaetae in anterior segments and the presence of an inconspicuous prostomium. Other worm species were not present in the host snails. The incidence of *C. limnaei* on *P. acuta* was estimated to be 27.3%.

Amplification of the 16S gene in the specimen of *C. limnaei* from Illapel River produced a 503 base pair fragment. BLASTn analysis indicated 100% identity with a sequence of *C. limnaei* from Sacramento, USA (accession number: GQ355405) (Bely & Sikes, 2010). The MP analysis recovered a single tree (not shown) similar to the topology recovered by the BI analysis (Fig. 3). In both trees our sequence of *C. limnaei* formed a monophyletic group (1.00 posterior probability under BI; 100% bootstrap under MP) together with *C. limnaei* from Sacramento, USA, within a well-supported clade (1.00 posterior probability under BI; 87% bootstrap under MP) composed by other species of the genus *Chaetogaster* for which relationships are poorly supported.

Discussion

The present study allowed the identification of *C. limnaei* in the invasive *P. acuta* in northern Chile. The molecular approach was useful to confirm the accuracy of the species identification based on morphological characters. Although the 16S rRNA sequence obtained in the present study is identical to that of *C. limnaei* from Sacramento, USA (Bely & Sikes, 2010), further molecular investigation is required to establish the route of introduction of *C. limnaei* to Chile. Besides, it is important to note that the

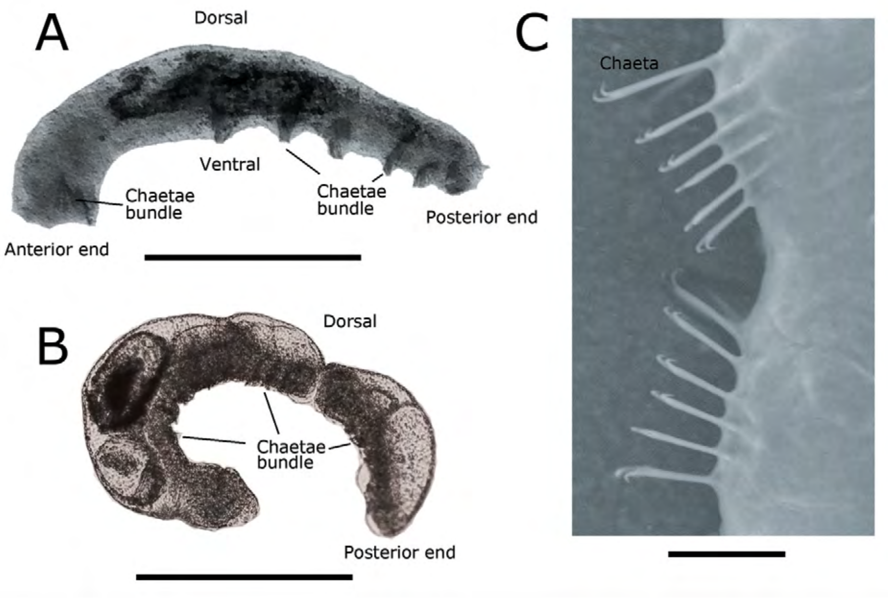

Figure 2. *Chaetogaster limnaei*, Illapel River, Chile. A, B, Lateral view of living specimens using stereo and light microscope, respectively; C, 2 bundle of chaetae imaged using SEM. Scale bar: A, B = 0.5 mm; C = 30 µm.

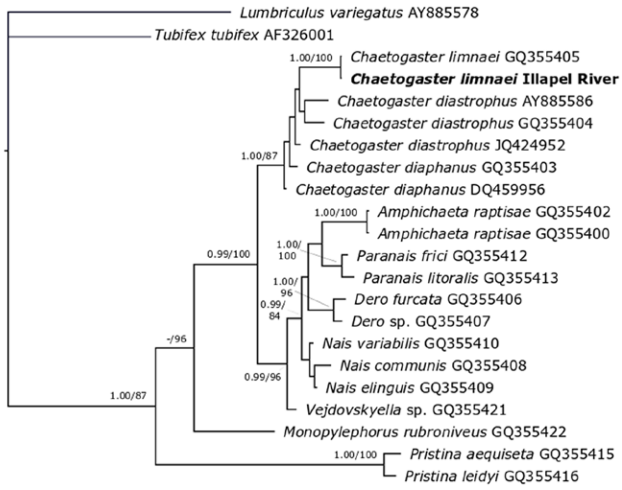

Figure 3. Phylogenetic tree obtained by the BI analysis. Number at nodes showed the posterior probabilities (BI) and bootstrap values (MP). The specimen of *Chaetogaster limnaei* sequenced in the present study is shown in bold.

route of introduction of *P. acuta* to this country is also unknown, although more than one introduction event of the species has been suggested (Collado, 2017).

As a commensal, *C. limnaei* feeds in a variety of prey including diatoms, filamentous algae, ciliates, flagellates, foraminiferans, rotifers and trematode cercariae, although its omnivory is limited by particle size (Buse, 1974; Conn et al., 1996; Gelder, 1989; Gruffydd, 1965a; Khalil, 1961; Michelson, 1964; Stoll et al., 2013; Streit, 1974). In the present study, we speculate the existence of a commensal relationship between *C. limnaei* and *P. acuta* in Illapel River since the examination of the snails showed no damage to the paleal tissues, a hypothesis that needs to be further investigated. Stoll et al. (2013), on the other hand, hypothesized an epizoic antibiosis relationship between *C. limnaei* and *P. acuta* at high infestation rates as suggested by the lower growth rates of the infected snail hosts. A strong positive correlation between the size of *P. acuta* and the infection intensity of *C. limnaei* has also been observed (Mitchell & Leung, 2016).

Chaetogaster limnaei has been considered a cosmopolitan species (Brinkhurst & Jamieson, 1971; Callisto et al., 2005). It has been found associated to more than 40 species of freshwater snails from at least 10 families, mainly members of Lymnaeidae, Physidae, and Planorbidae (Smythe et al., 2015). In the present study we found *C. limnaei* within a population of the invasive *P. acuta* in Chile. Potential snail hosts in this country include native species of the genus *Biomphalaria* Preston, 1910, *Uncancylus*, *Chilina* Gray, 1828, *Lymnaea* Lamarck, 1799, *Heleobia* Stimpson, 1865, *Physa* Draparnaud, 1801 and *Potamolithus* Pilsbry, 1896. A more extensive sampling is necessary to test the occurrence of *C. limnaei* in species of these genera, as well as other freshwater ecosystems in Chile.

Acknowledgements

We thank the Subsecretaría de Pesca y Acuicultura, Ministerio de Economía, Fomento y Turismo, República de Chile for authorization to collect samples. We also thank CONICYT-FONDEQUIP Program (No EQM-140088) for the acquisition of Hitachi Scanning Electron Microscope (SEM). This work was partially funded by the projects DIUBB 153309 2/R and CONICYT-FONDECYT No. 11130697.

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