

## On the occurrence of *Eudiaptomus gracilis* (G.O. Sars, 1863) (Copepoda: Diaptomidae) in Madeira (Portugal)

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### ABSTRACT

#### On the occurrence of *Eudiaptomus gracilis* (G.O. Sars, 1863) (Copepoda: Diaptomidae) in Madeira (Portugal)

Calanoid copepods belonging to the diaptomid genus *Eudiaptomus* Kiefer, 1932 were collected in an artificial water body on the island of Madeira, Portugal. The morphological and molecular identification of the collected specimens unequivocally proved that they belong to the allochthonous species *Eudiaptomus gracilis* (G. O. Sars, 1863), possibly unwarily introduced along with fish stockings or ornamental aquatic plants. In the sampled site, the species co-occurred with the non-native cyclopoid copepod *Acanthocyclops americanus* (Marsh, 1893) and some anomopod species. This is the first record of the species for Portugal and the whole Macaronesia, whereas the possible presence of *E. gracilis* in the Iberian Peninsula should be verified.

**Key words:** alien species, copepod, zooplankton, Madeira archipelago, Macaronesia

### RESUMEN

#### Sobre la ocurrencia de *Eudiaptomus gracilis* (G.O. Sars, 1863) (Copepoda: Diaptomidae) en Madeira (Portugal)

Copépodos calanoides pertenecientes al género de diaptómido *Eudiaptomus* Kiefer, 1932 fueron recolectados en una masa de agua artificial en la isla de Madeira, Portugal. Las identificaciones morfológicas y moleculares de los especímenes recolectados demostraron inequívocamente que pertenecían a la especie alóctona *Eudiaptomus gracilis* (G. O. Sars, 1863), posiblemente introducida inadvertidamente con introducciones de peces o plantas acuáticas ornamentales. En la localidad muestreada, la especie coexistía con el copépedo ciclopoide no nativo *Acanthocyclops americanus* (Marsh, 1893) y algunas especies de anomópodos. Esta es la primera cita de la especie para Portugal y para Macaronesia, aunque la posible presencia de *E. gracilis* en la Península Ibérica necesita verificación.

**Palabras clave:** especie exótica, copépedo, zooplancton, archipiélago de Madeira, Macaronesia

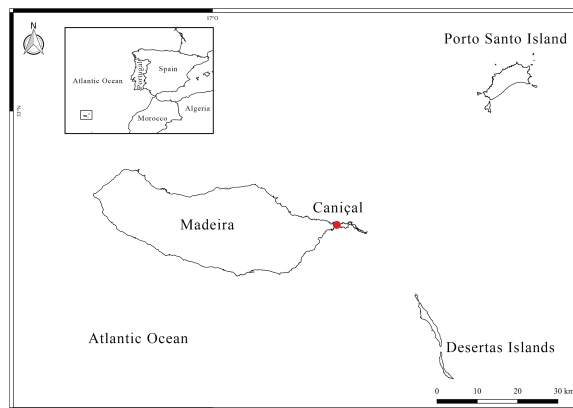
## INTRODUCTION

Biological invasions are unanimously considered one of the most serious threats to biological diversity worldwide (e.g. Havel *et al.*, 2015). In arid areas, as well as in temperate areas with a hot dry summer such as Madeira, artificial water reservoirs are often built to fulfil human needs (e.g. Zarfl *et al.*, 2015; Ollivier *et al.*, 2019; Naselli-Flores & Marrone, 2019). These artificial water bodies have the undesired side-effect of offering new colonization opportunities to non-indigenous species, and are known to facilitate the settlement of newcomers, especially euryecious, invasive taxa (Johnson *et al.*, 2008; Alfonso *et al.*, 2010; Havel *et al.*, 2015; Duggan & Payne, 2017). Such facilitation for biological invasions is particularly pernicious on oceanic islands, which often host a peculiar and disharmonic biota. Moreover, in those areas that lack exhaustive repositories of their native biodiversity, biological invasions might lead to the local extinction of autochthonous taxa even before they are ever censused or described. For these reasons, the attentive monitoring of the species occurring in potential “invasion hubs” is desirable. In this frame, the sampling of a man-made agricultural pond on Madeira island (Portugal) led to the finding of some non-native copepod taxa, whose identity and affinities are hereby discussed.

## MATERIALS AND METHODS

On 5<sup>th</sup> March 2018, a zooplankton sample was collected with a 200-micron mesh-sized hand net in a concrete reservoir located on Madeira Island, close to the town of Caniçal (WGS84 geographical coordinates: 32.746291 N, -16.723144 E; Fig. 1). The sample was fixed *in situ* in 90 % ethanol and crustaceans were sorted out in laboratory under a stereomicroscope. Branchiopods were identified according to Alonso (1996) and Elías-Gutiérrez *et al.* (2019); copepods were identified according to Dussart (1969), Kiefer (1978), Mirabdullayev & Defaye (2002), and Miracle *et al.* (2013).

A male and a female adult specimen of the collected calanoid copepod species were deposited in the Museo di Storia Naturale, Sezione di

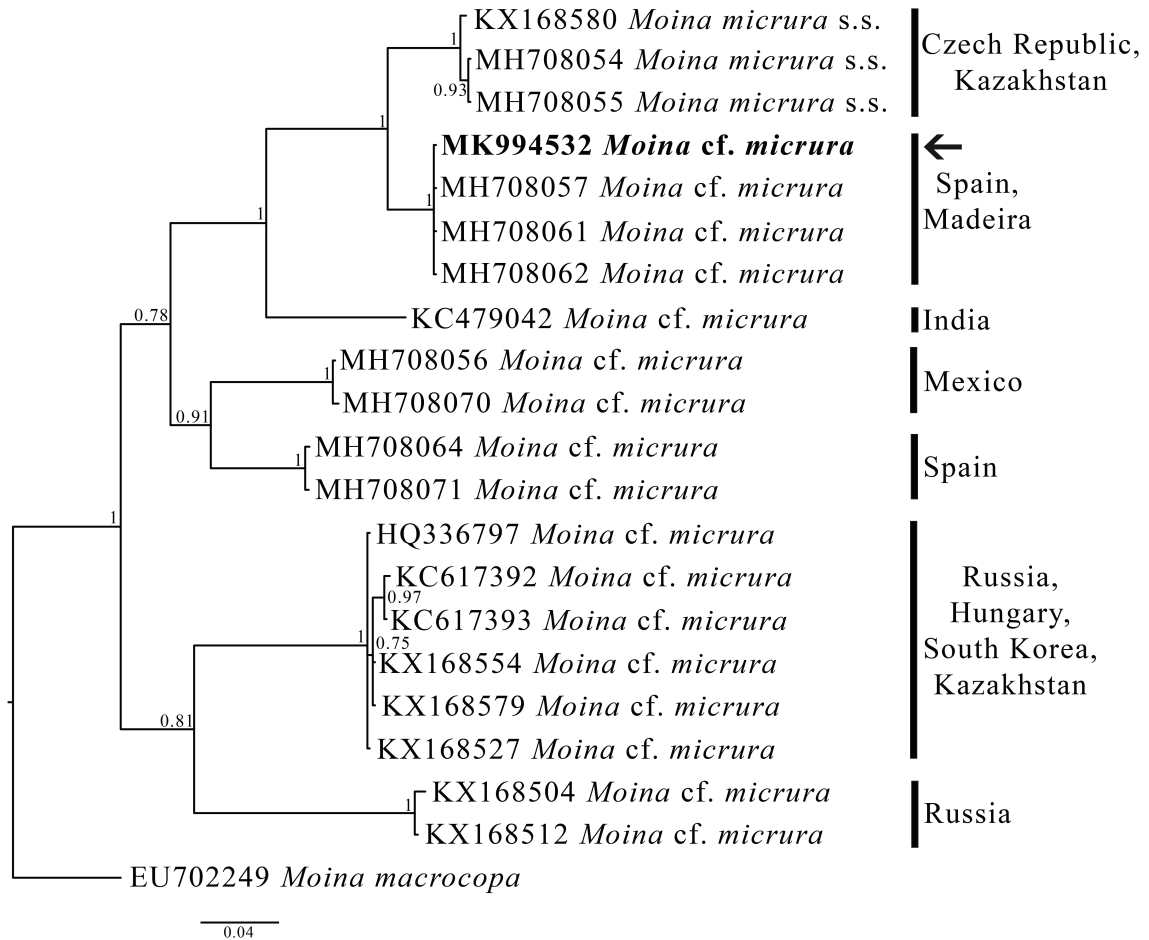


**Figure 1.** Location of the man-made water body sampled in the frame of this survey (red dot). *Localización de la masa de agua artificial muestreada en el marco de este estudio (punto rojo).*

Zoologia “La Specola”, Università di Firenze, Italy (MZUF) under the collection numbers MZUF 638 and MZUF 639, respectively. Other calanoid specimens and the co-occurring crustacean fauna are currently stored in FM’s collection at the Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche of the University of Palermo, Italy, and are available for loan on request.

In the light of the existence of a complex of closely related species currently lumped under the binomen *Moina micrura* (Elías-Gutiérrez *et al.*, 2019), a partial sequence of the cytochrome oxidase subunit I (COI) was obtained from a *Moina cf. micrura* specimen collected in Caniçal following the protocol described by Vecchioni *et al.* (2017). The obtained sequence was compared with selected *Moina micrura* s.l. sequences downloaded from GenBank, rooting the resulting tree on *Moina macrocopa* (Straus, 1820) (“*Moina* dataset”; see figure 2 for their origin and GenBank accession numbers).

Moreover, the same COI fragment was sequenced from two calanoid copepod specimens collected in Caniçal, one *Eudiaptomus gracilis* specimen from Sweden (“Lake Krankesjön”, Skåne County; WGS84 coordinates: 55.695444 N, 13.484944 E) and one *Copidodiptomus numidicus* (Gurney, 1909) specimen from Italy (“Stagno di Piano Perticone”, Sicily; WGS84 coordinates:

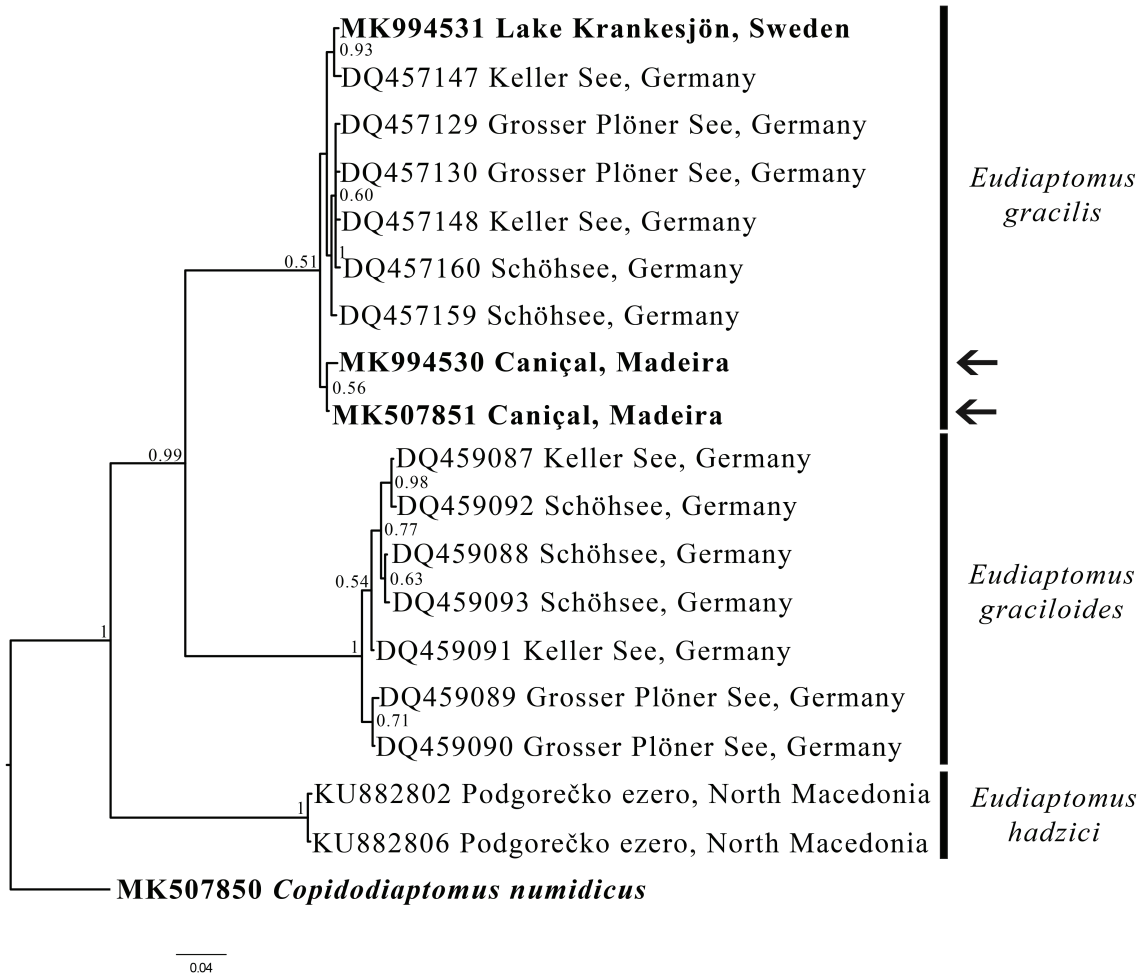


**Figure 2.** Bayesian consensus phylogram based on a 630-bp-long fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (mtDNA COI). Node support is reported as nodal posterior probability. GenBank accession number of the novel sequence is reported in bold. The arrow indicates the Madeiran *Moina* sequence. *Árbol filogenético de consenso a partir de la inferencia bayesiana basado en un fragmento de 630 pares de bases del gen mitocondrial codificando para la subunidad I de la citocromo oxidasa (mtDNA COI). El soporte de los nodos se presenta como la probabilidad posterior nodal. El número de acceso de GenBank de la secuencia nueva aparece en negrita. La flecha indica la secuencia de la Moina de Madeira.*

38.030891 N, 14.498671 E). In addition, 6 *Eudiaptomus gracilis* COI sequences, 7 COI sequences of *E. graciloides* (Lilljeborg, 1888) and 2 COI sequences of *Eudiaptomus hadzici* (Brehm, 1939) were downloaded from GenBank and included in the analyses of this “copepod dataset” (see figure 3 for their Accession Numbers).

The molecular identification of the studied specimens and the reconstruction of the phylogenetic relationships among the taxa were performed with Bayesian inference of phylogeny (BI) as implemented in MrBayes v. 3.2.6 (Ron-

quist *et al.*, 2012). The choice of the best evolutionary models was made using PartitionFinder v. 1.0.1 (Lanfear *et al.*, 2012) according to the Akaike information criterion (AIC). The BI analyses of both the “*Moina*” and “copepod” datasets were performed under a General Time Reversible model of sequence evolution for molecular data with gamma distributed rate variation among sites (GTR+G). Two independent mcmc analyses were run with 1000000 generations (temp.: 0.2; default priors), and 25 % of the saved trees were conservatively discarded as



**Figure 3.** Bayesian consensus phylogram based on a 437-bp-long fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (mtDNA COI). Node support is reported as nodal posterior probability. GenBank accession numbers of the novel sequences are reported in bold. The arrows indicate the Madeiran *Eudiaptomus gracilis* sequences. *Árbol filogenético de consenso a partir de la inferencia bayesiana basado en un fragmento de 437 pares de bases del gen mitocondrial codificando para la subunidad I de la citocromo oxidasa (mtDNA COI). El soporte de los nodos se presenta como la probabilidad posterior nodal. Los números de acceso de GenBank de las secuencias nuevas aparecen en negrita. Las flechas indican las secuencias de los Eudiaptomus gracilis de Madeira.*

“burn-in”. Nodes’ statistical support of BI trees was evaluated by their posterior probabilities.

## RESULTS AND DISCUSSION

The 630 bp-long COI sequence of the *Moina* from Caniçal belongs to a *Moina* haplotype already known for Spain, which shows a sister clade relationship with *Moina micrura* s.s. (Fig. 2). Accordingly, following Elías-Gutiérrez *et al.*

(2019), we conservatively refer here to this taxon as *Moina* cf. *micrura*.

For the copepod dataset, the BI tree obtained based on a 437-bp long fragment of mtDNA COI shows the presence of a well-supported clade, which includes all the German and Swedish *Eudiaptomus gracilis* sequences downloaded from GenBank along with those belonging to the *E. gracilis* specimens collected in Madeira (Fig. 3).

Accordingly, based on both the morphologi-

cal and molecular evidences, the studied zooplankton sample proved to include the anomopods *Bosmina longirostris* (Müller, 1776) and *Moina* cf. *micrura* Kurz, 1875, and the copepods *Acanthocyclops americanus* (Marsh, 1893) and *Eudiaptomus gracilis* (G.O. Sars, 1863). The collected anomopod species are new for Madeira (Báez, 1993; Sala *et al.*, 2015 and references therein) and they seem to be absent in the other Macaronesian archipelagos (Röben, 1976; Borges *et al.*, 2010; Cruz, 2013). However, they are known to occur both in the Iberian Peninsula and Morocco (e.g. Alonso, 1996; Mouelhi *et al.*, 2000; Elías-Gutiérrez *et al.*, 2019), and they are thus possibly native to Madeira, although this possibility should be further checked. In fact, the knowledge on anomopods in Madeira is very scarce and fragmentary, and to date only four more species are known (another moinid, *Moina brachiata* (Jurine, 1820), and the chydorids *Chydorus sphaericus* (Müller, 1776), *Alona quadrangularis* (Müller, 1776), and *Leydigia acanthocercoides* (Fischer, 1854; Fischer, 1851, 1854; Báez, 1993), all of them present also in other Macaronesian archipelagos. Regarding to copepods, cyclopoids are scarcely studied in Madeira, and only few species are known to date: *Eucyclops serrulatus* (Fischer, 1851), *Paracyclops chiltoni* (Thomson, 1882), *Tropocyclops prasinus* (Fischer, 1860), and *Metacyclops minutus* (Claus, 1863) (Fischer, 1860; Lindberg, 1962; Stauder, 1991). The presence of *Acanthocyclops americanus* in Madeira is interesting since this is the second confirmed population of this exotic species in Macaronesia, as it is already known from the Canary Islands sub *A. robustus* (Sars, 1863) (Röben, 1976) or *A. trajani* Mirabdullayev & Deyafe, 2002. *A. americanus* is a widely distributed species in the Iberian Peninsula and in other Mediterranean countries (Greece, Egypt, Tunisia, Algeria, Italy; Mirabdullayev & Deyafe, 2002; Hamaidi *et al.*, 2010; Miracle *et al.*, 2013; Schifani *et al.*, 2019), but to date it has not been recorded in Morocco (Mouelhi *et al.*, 2000). Finally, the diaptomid copepod *Eudiaptomus gracilis* is new for Madeira and the whole Macaronesia, and absent from continental Portugal and the whole Maghreb (Mouelhi *et al.*, 2000; Fernández de Puellas, 2015, and references there-

in; Marrone *et al.*, 2017). Only two more species of diaptomids are known from the Macaronesian archipelagos, i.e. *Arctodiaptomus wierzejskii* (Richard, 1888) from Azores, and *Neolovenula alluaudi* (Guerne & Richard, 1890) from Canary Islands (Bowman, 1989; Defaye & Dussart, 1991; Alfonso & Belmonte, 2013).

*Eudiaptomus gracilis* is one of the most widespread diaptomid species, with a native distribution range including central and northern Eurasia, and the circum-polar area, including Alaska (Reed, 1962; Dussart & Defaye, 2002; Błędzki & Rybak, 2016; Novichkova & Azovsky, 2017). In the west-Paelearctic biogeographical region, it is considered autochthonous north of the Alps and Pyrenees, whereas the populations reported for Italy and Turkey (Riccardi & Rossetti, 2007; Bozkurt & Akin, 2012) are considered non-native. The reports of the species from Spain (Pérez-Bote *et al.*, 2004; Tagliatalata *et al.*, 2014), Ethiopia (Imoobe & Akoma, 2008) and Hong Kong (Hodgkiss & Cham, 1976) are not adequately substantiated, and thus in need of confirmation. The occurrence of the species in Israel has to be excluded (see discussion in Marrone *et al.*, 2014).

The *Eudiaptomus gracilis* population occurring in Madeira is likely allochthonous, as suggested by its occurrence in a man-made water reservoir located on a remote island, thousands of kilometres away from the known native range of the species. Moreover, Madeira Island is characterised by a different climate from those characteristics of the areas inhabited by this temperate and circum-polar diaptomid species, and no permanent natural water bodies (i.e. the usual habitat of the species) occur on the island. The non-native status of *E. gracilis* on Madeira is also suggested by the topology of the molecular trees, which stresses the molecular homogeneity of the currently available *E. gracilis* sequences, thus further supporting the hypothesis of a recent, man-mediated introduction of this species on Madeira Island possibly linked with recreational fishing, aquaculture activities or the trade of ornamental aquatic plants, as already suggested for the allochthonous *E. gracilis* populations in Italy (Riccardi & Rossetti, 2007), and for other non-native diaptomids as *Neodiaptomus*

*schmackeri* (Poppe & Richard, 1892) in Albania (Alfonso *et al.*, 2014), *Sinodiaptomus valkanovi* (Kiefer, 1938) in Bulgaria and New Zealand (Makino *et al.*, 2010) and *Skistodiaptomus pallidus* (Herrick, 1879) in Germany and New Zealand (Branford *et al.*, 2017).

In Italy, *Eudiaptomus gracilis* showed pronounced colonization ability, often replacing the pre-existing native species in the invaded environments (Riccardi & Rossetti, 2007; Riccardi & Giussani, 2007; Visconti & Manca, 2010), and the possible impact of *E. gracilis* on native Madeiran crustacean biota should thus be carefully monitored.

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