Rapid Communication

A species on the rise in Europe: *Sinodiaptomus sarsi* (Rylov, 1923) (Copepoda, Calanoida), a new record for the Romanian crustacean fauna

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Abstract

This paper represents the first record in Romania of the calanoid copepod *Sinodiaptomus sarsi* (Rylov, 1923), native to Eastern Asia. It was identified in nine ponds in north-east Romania, in a fish farming region recently declared a Natura 2000 protected area (Lakes Fălticeni). More than one thousand individuals were found in qualitative samples in November 2018 and June 2019. The water pools were constructed more than forty years ago by the damming of the natural course of the Șomuz River. Previously unpublished research on zooplankton from the area did not record any calanoid copepods, so *S. sarsi* likely arrived there at a later date, probably from Western Ukraine, where it was reported in 2014. Possible vectors for introduction include fish stocking, migratory birds or human release. Future studies are required to clarify the impact of this species on native lentic fauna and its routes of introduction, since this species has potential to become invasive in Europe.

Key words: Diaptomidae, first record, invasive potential, Lakes Fălticeni, microcrustaceans, non-native species

Introduction

Calanoid copepods are a successful group in freshwater, brackish and marine habitats (Reid 2001; Kiørboe 2010; Bledzki and Rybak 2016), either natural or man-made (Merrix-Jones et al. 2013). 175 calanoid species were found in the Palaearctic zoogeographic region, with 144 belonging to the family Diaptomidae (Boxshall and Defaye 2008). Together with other microcrustaceans (cladocerans, cyclopoid and harpacticoid copepods), they represent primary and secondary consumers, being considered an important link in pelagic food webs (Dussart and Defaye 2001).

Many cases of intercontinental invasions in freshwater environments by calanoid copepods have been documented (Reid and Pinto-Coelho 1994; Bollens et al. 2002; Ferrari and Rossetti 2006; Brandorff 2011; Papa et al. 2012; Alfonso et al. 2014). The introduction of non-native species often occurs by passive transport due to: i) human-mediated circumstances, such as transfer of fish stocks for aquaculture, shipping of tropical plants for horticulture or agriculture, and ballast water (Reid and Pinto-Coelho 1994; Reid 2001; Havel and Shurin 2004; Boxshall and Defaye 2008; Alfonso et al. 2014); ii) wind, rain, and flowing surface waters (Cáceres and Soluk 2002; Havel and Shurin 2004), and; iii) live animals, especially migratory birds (Havel and Shurin 2004; Frisch et al. 2007; Khomenko and Shadrin 2009; Hessen et al. 2019). Calanoid copepods are perfect candidates for passive transportation, either as active individuals or as resting stages (Reid and Pinto-Coelho 1994), since many species produce diapausing eggs that can remain dormant for long periods of time (Bledzki and Rybak 2016); diapause is strongly linked to invasion of inland water (Hairston and Bohonak 1998).

Native to Eastern Asia, the diaptomid *Sinodiaptomus sarsi* (Rylov, 1923) was described in fish ponds and reservoirs from eastern China and south-eastern Russia (Rylov 1923, 1930; Afonina 2018; Li et al. 2018). Over time, this species has also been found in the east (Korea: Chang and Kim 1986; Chang 2014; Japan: Ueda and Ishida 1997), south (Vietnam: Ho et al. 2008; Tran et al. 2016; the Phillipines: Lagbas et al. 2017; Indonesia: Sari et al. 2014), north (Mongolia: Borutsky 1959; other parts of Russia: Afonina 2013, 2018), and west (Iran: Löffler 1961; Azerbaidjan: Kasymov et al. 1972; Borutsky et al. 1991; Turkey: Gündüz 1998; Kazahstan: Krupa et al. 2016; Ukraine: Mykitchak 2016; Mykitchak and Koval 2018).

Sinodiaptomus sarsi was identified in nine artificial ponds from a fish farm near Fălticeni, north-east Romania, during 2018 and 2019. The species is new to the Romanian calanoid fauna, which previously included 24 species and 10 genera (Demeter and Marrone 2009; Samchyshyna 2011). The aims of the present paper are to: (i) describe the *S. sarsi* populations found for the first time in Romania and (ii) discuss the invasion potential of the species.

Materials and methods

Sinodiaptomus sarsi was found in several small ponds, located in a fish farm region called "Lakes Fălticeni", where native and Asian carp species are cultivated: common carp *Cyprinus carpio* Linnaeus, 1758, grass carp *Ctenopharyngodon idella* (Valenciennes, 1844), silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), and bighead carp *Hypophthalmichthys nobilis* (Richardson, 1845). The lakes are located in Suceava County, north-eastern Romania, on the Șomuzul Mare River, a tributary of the Siret River (part of the lower Danube catchment). These are all artificial reservoirs, created by damming the river natural course between 1962 and 1978, including Moara reservoir (site 2), built in 1976 (Olariu et al. 2015). The 11 sampled aquaculture ponds are also part of two Natura 2000 protected areas: ROSPA 0064 Lakes Fălticeni, designated in 2007 for the protection of 19 bird species, and ROSCI0310 Lakes Fălticeni, created in 2011 for the protection of 3 mammal species and 5 species of amphibians and reptilians

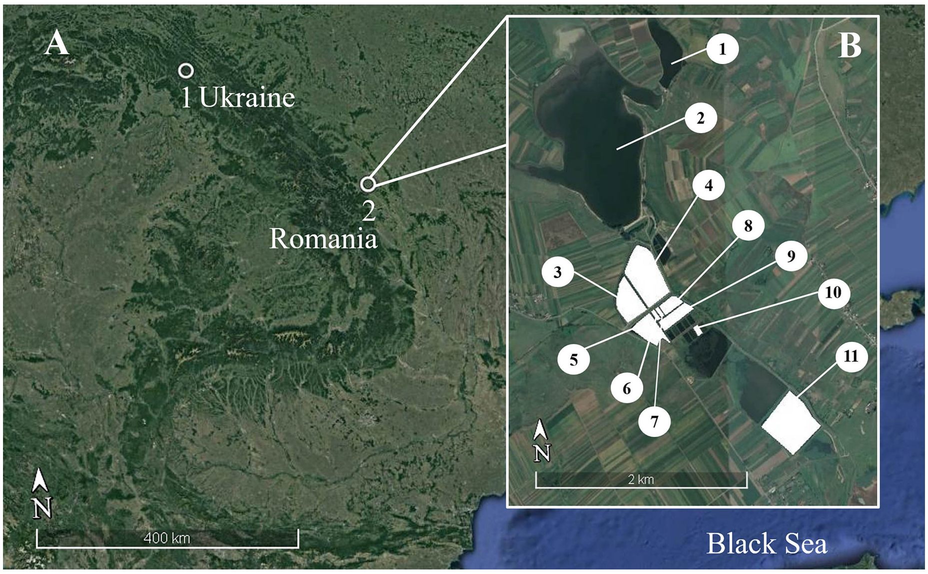

Figure 1. Location of *Sinodiaptomus sarsi* in Europe, West from the Black Sea; A: general view (1 – Ukraine, according to Mykitchak 2016; 2 – Romania, present study); B: detailed view over Lakes Fălticeni, Romania (1–11: sampling codes; white areas: ponds with *S. sarsi*) (Image source: Google Earth Pro 2019).

(Brînzan 2013). No public access for recreational activities is permitted in or near the fish farms.

Sampling took place on 1 and 2 November 2018 and on 19 and 26 June 2019. All sites (1–11, Supplementary material Table S1) were sampled twice, in November 2018 and June 2019, using a benthic dredge with a 550 µm net. Only ponds 7 and 8 were sampled with a 55 µm plankton net on 26 June 2019. Most samples were taken from nursery/rearing ponds, except for site 2, Moara reservoir, a production pond for all four cultivated carp species. All samples were considered qualitative. The main physical and chemical parameters were measured in the field using a Hanna HI98194 multiparameter meter. Location and characteristics of sampled ponds, together with the approximate location of the *S. sarsi* population in Ukraine, are presented in Table S1 and Figure 1.

Samples were preserved in 96% ethanol and processed in the laboratory using standard procedures. Planktonic microcrustaceans were analyzed using the following keys: Damian-Georgescu (1963); Negrea (1983); Einsle (1993); Bledzki and Rybak (2016). The taxonomic identification of *S. sarsi* followed Bledzki and Rybak (2016) and subsequently confirmed using Ueda and Ohtsuka (1998). An Olympus BX51 microscope and the *cell*^{D} Olympus imaging software for life science microscopy were utilized for light microscopy photographs. Scanning electron microscope (SEM) images were obtained from individuals processed using the turbomolecular pumped coater Quorum Q150T ES, and examined at SEM Hitachi SU8230 (LIME-INCDTIM, Cluj-Napoca, Romania). Undissected individuals of *S. sarsi* (20 females and 20 males) were deposited in the collections of the Zoological Museum of the Babeş-Bolyai University, Cluj-Napoca, Romania, under the code: COPE01101.

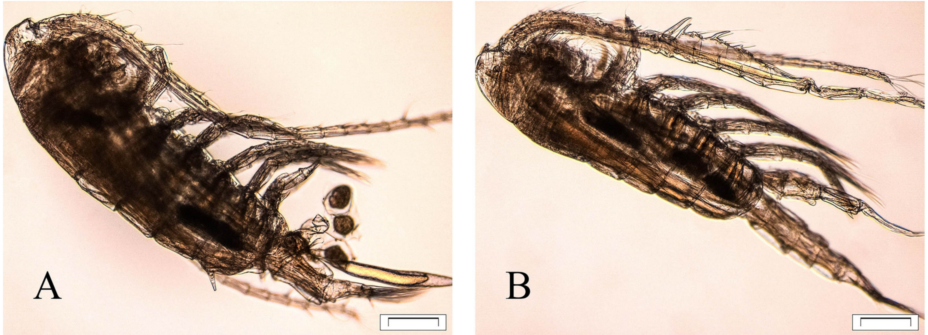

Figure 2. *Sinodiaptomus sarsi*, general habitus: female (A) and male (B) (the line represents 200 µm). Photos by Karina Battes and Mirela Cîmpean.

Results

Sinodiaptomus sarsi*: morphological aspects*

More than 1000 individuals of *S. sarsi* were identified in the fish ponds from Lakes Fălticeni. This is the first citation of *S. sarsi* in Romania. The species is easily distinguished from *Sinodiaptomus valkanovi* Kiefer, 1938 based on the following morphological characteristics: shorter antennules in both sexes, a long projection on the right antennule segment 15 of the male, and a short (non thumb-like) posterodistal basal projection of the male left leg 5. (Ueda and Ohtsuka 1998; Makino et al. 2010). The most important characteristics used for the identification of *S. sarsi* were: (1) the swimming legs in both males and females, namely: the first pair P1with a two-segmented endopodite and a three-segmented exopodite, having outer spines on the exopodite first and last segments; the swimming leg pairs P2–P4 with three-segmented endopodites and exopodites; (2) the male right antennule A1, with 4 segments behind the geniculation, with segments 13–18 dilated and with a comb-like process on its antepenultimate (third from the end) segment (Figures 2B, 4); (3) the male left swimming leg P5 with the second segment of exopodite ending in a lamellate thumb-like process (Figures 3B, 6A, B), and the male right swimming leg P5 with the endopodite reaching the posterior edge of the first exopodite segment (Figure 3B); (4) the female last thorax segment with a dorsal bulge, looking narrow in the dorsal view and long in the side view (Figures 2A, 5A). All females examined for the present study had spinules on both margins of the end claw of the fifth leg exopodite (Figures 3A, 5B), unlike the Korean type (Chang and Kim 1986; Ueda and Ohtsuka 1998).

Sinodiaptomus sarsi*: ecological aspects*

Sinodiaptomus sarsi was identified in 9 out of the 11 ponds sampled in 2018 and 2019, in shallow water pools of less than 20 ha, usually rich in riparian vegetation, used as nursery or rearing ponds. These were all located between Moara reservoir in the north and Pocoleni reservoir in the south (Table S1). Nine cladoceran and four cyclopoid copepod species were

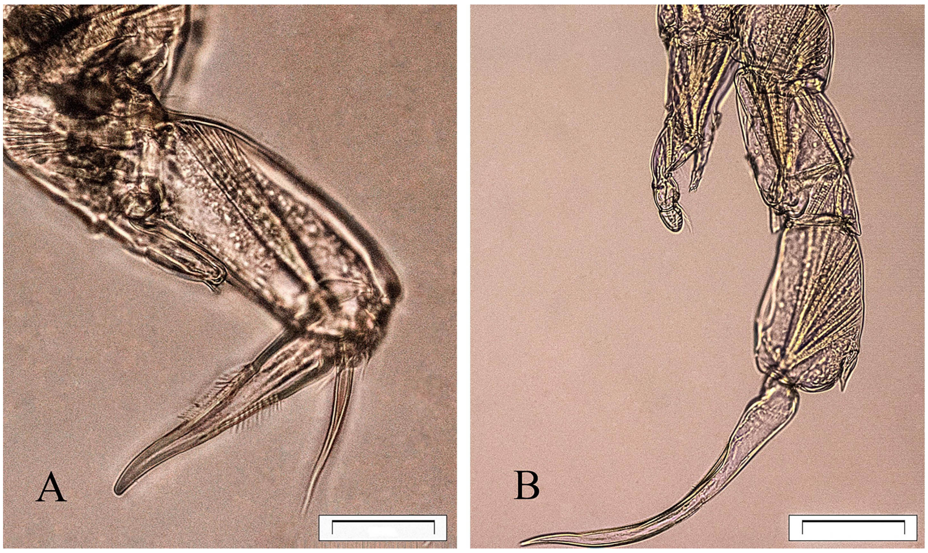

Figure 3. The fifth swimming legs P5 of *Sinodiaptomus sarsi*: female (A) and male (B) (the line represents 50 µm in A and 100 µm in B). Photos by Karina Battes and Mirela Cîmpean.

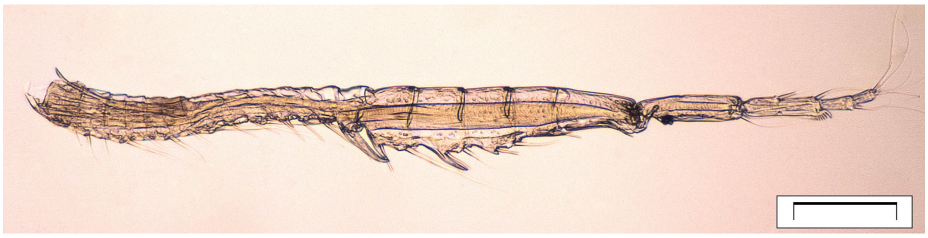

Figure 4. *Sinodiaptomus sarsi*, the male right antennule A1 (the line represents 200 µm). Photos by Karina Battes and Mirela Cîmpean.

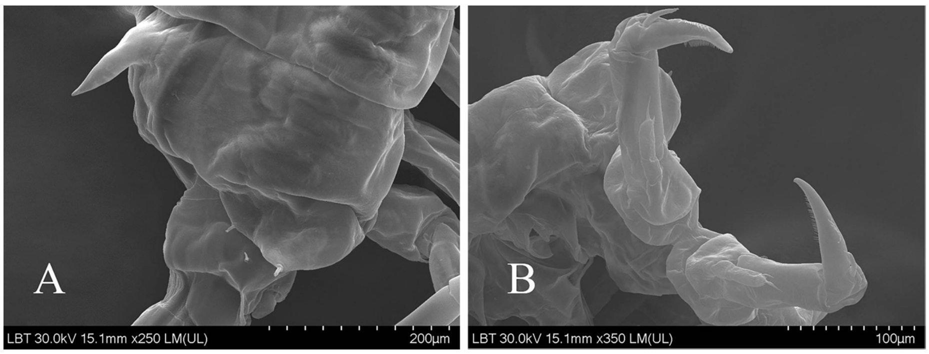

Figure 5. *Sinodiaptomus sarsi*, female: dorsal bulge (A) and the fifth pair of swimming legs P5 (B). Photos by Lucian Barbu-Tudoran.

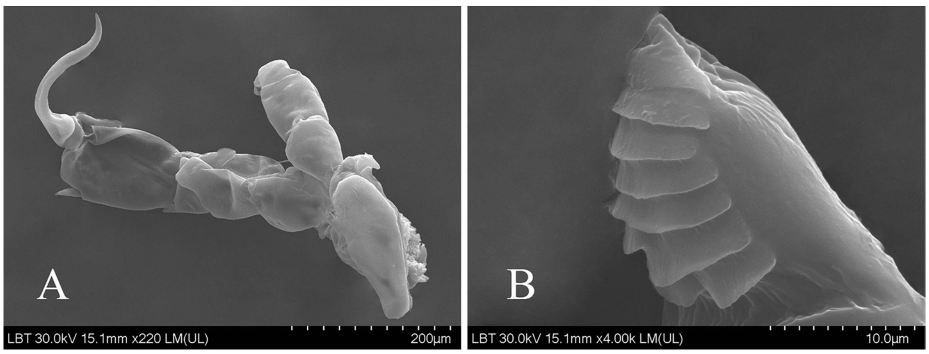

Figure 6. *Sinodiaptomus sarsi*, male: the fifth pair of swimming legs P5 (A) and the lamellate process at the end of exopodite 2 in the left leg P5 (B). Photos by Lucian Barbu-Tudoran.

identified in 2018 and 2019 together with *S. sarsi* in the fish ponds (Table S1). Frequently occuring taxa in the samples were: the cladoceran *Daphnia galeata* Sars, 1863 (63.64%) and the cyclopoid copepods *Acanthocyclops robustus* Sars, 1863 and *Cyclops vicinus* Ulianine, 1875 (54.55% each). The latter species was recorded in higher numbers in November 2018.

No previous zooplankton studies were found for the area, except for unpublished research conducted in 1988 and 1989 by the Laboratory for Aquaculture and Aquatic Ecology, Piatra-Neamţ, now part of Alexandru Ioan Cuza University, Iaşi (I. Cărăuş *pers. comm.*; R. Rujinschi *unpublished data*). That study investigated the trophic potential for aquaculture of several water pools from Suceava county. These unpublished results reported the following species in Fălticeni fish farms (Moara and Pocoleni reservoirs): the cladocerans *Bosmina longirostris* (O.F. Muller, 1776), *D. galeata, Moina brachiata* (Jurine, 1820); the cyclopoid copepods *Acanthocyclops vernalis* (Fischer, 1853), *C. vicinus*, and the rotifers: *Asplancha priodonta* Gosse, 1850, *Brachionus angularis* Gosse, 1851, *Brachionus calyciflorus* Pallas, 1776, *Brachionus diversicornis* (Daday, 1883), *Euchlanis triquetra* Ehrenberg, 1838, *Polyarthra vulgaris* Carlin, 1943, and *Trichocerca birostris* (Minkiewicz, 1900). No calanoid copepods were recorded.

The planktonic sample taken from pond 7 on 26 June 2019 revealed a dominance of *S. sarsi*, including both adults (males, ovigeorus and non-ovigerous females) and juveniles (copepodites and nauplii). Cyclopoid copepods were sporadic or rare. Large cladocerans like *D. galeata* or *Simocephalus vetulus* (O.F. Muller, 1776) were common in the sample, together with small-bodied species like *Chydorus sphaericus* (O.F. Muller, 1776). The percentage of copepods in pond 7 was 84% (juveniles included), while cladocerans reached 16%. No rotifers were found. In pond 8, the cladoceran *Moina micrura* Kurz, 1875 was the dominant taxon on 26 June 2019, next to juvenile copepods, while *S. sarsi* and *A. robustus* were both also common. In the ponds investigated in 1988/1989, rotifer percentages ranged between 30 and 60%, while cladocerans varied between 9 and 30% and cyclopoid copepods between 15 and 40%.

Alkaline pH values were recorded in ponds 7 and 8 in June 2019 (8.3–9.2). Conductivity values ranged between 0.611 and 0.912 mS cm^{-1}, while dissolved oxygen values were low (3.05–4.85 mg L^{-1}) due to high water temperatures (31.2–32.3 °C).

Discussion

Current distribution of Sinodiaptomus sarsi

Sinodiaptomus sarsi is native to eastern Asia: eastern China and south-eastern Russia (Rylov 1923, 1930; Borutsky et al. 1991; Afonina 2018; Li et al. 2018). It occurs all year round, especially in warm seasons (Chang and Kim 1986; Chang 2014) in small ponds and fishponds abundant in aquatic vegetation (Chang and Kim 1986), small eutrophic water bodies (Ueda and Ishida 1997; Chang 2014; Mingming et al. 2014) and even large dam reservoirs (Ueda and Ishida 1997).

Unlike its congeneric species, *S. valkanovi*, endemic to Japan and cited in three isolated sites in Bulgaria, California and New Zealand (Ueda and Ohtsuka 1998; Duggan et al. 2006; Makino et al. 2010), *S. sarsi* has expanded its distribution from its native range to Mongolia (Borutsky 1959), Iran (Löffler 1961), Azerbaidjan (Kasymov et al. 1972), Korea (Chang and Kim 1986; Chang 2014), the north Caucasus, Russia (Borutsky et al. 1991), Japan (Ueda and Ishida 1997), East Anatolia, Turkey (Gündüz 1998), Vietnam (Ho et al. 2008; Tran et al. 2016), the Transbaikal region, Russia (Afonina 2013), Indonesia (Sari et al. 2014), the Bering Islands, Russia (Novichkova and Chertoprud 2016), Kazahstan (Krupa et al. 2016) and the Phillipines (Lagbas et al. 2017). A review of this distribution was published by Afonina (2018).

The first report of *S. sarsi* in Europe, west from the Black Sea, was from Ukraine on 8 August 2014, from three fish ponds in the Rivna Mountains, the Uzh River basin (the Ukrainian Carpathians): two males and one female (Mykitchak 2016; Mykitchak and Koval 2018). Two previous records of the species in Bulgaria (Kiefer 1938) and in California (Light 1939; Wilson 1959) have since been reassessed: there, *S. sarsi* was later determined to be *S. valkanovi* (Ueda and Ohtsuka 1998). Both cases were isolated accidental introductions from Asia with exotic aquatic plants like water lilies, and both populations perished at a later date (Kiefer 1978; Reid and Pinto-Coelho 1994; Marrone et al. 2017).

Invasive potential of Sinodiaptomus sarsi *in Europe*

Sinodiaptomus sarsi is considered non-native to the western Palearctic, according to Marrone et al. (2017). The species gradually expanded its range westwards, from eastern China to the Caucasus, Anatolia, Ukraine and now Romania. Broad environmental tolerances have been used to explain the spread in this species' distribution (Gündüz 1998; Krupa et al. 2016; Afonina 2018). However, the invasive character of this species has never discussed in previous literature. The present record of *S. sarsi* from Lakes Fălticeni must be linked to its future invasion potential in Europe. This potential is indicated by: i) its presence in most ponds investigated during the study (nine out of eleven sampled pools); ii) its presence in both sampling seasons in high numbers; and iii) its status as a "new arrival" in the area (no record of calanoid copepods was made in the previous unpublished study from 1988/1989).

In this invasive context, the effects of the species on native populations must be investigated, since they can become catastrophic for local biodiversity (Leppäkoski et al. 2002; Papa et al. 2012; Havel et al. 2015; Branford and Duggan 2017). Other frequent microcrustaceans identified with *S. sarsi* were *D. galeata* and *C. vicinus*. Similar cladoceran and cyclopoid copepods were found in 1988 and 1989 in Lakes Fălticeni, together with a high percentage of rotifers. The absence of rotifers in plankton samples taken in June 2019 requires further examination. Diaptomid copepods are generally described as herbivorous filter-feeders (Dussart and Defaye 2001), but they can also undertake predatory feeding, preying on other crustaceans, protozoans and rotifers (Brandl 2005). Subsequent studies are required to clarify this aspect.

The role of *S. sarsi* as food for fish cannot be overlooked. Calanoid copepods have a higher nutrition value for juveniles fish compared with cyclopoids, because of their larger size and their earlier development in lentic plankton (Damian-Georgescu 1966). Lu et al. (2002) reported inverse variations of densities for *S. sarsi* and the planktivorous silver carp *Hypophthalmichthys molitrix*. These findings have great significance in Lakes Făltinceni, since *H. molitrix* in one of the four cultured species in these fish farms. Other studies suggest that wild zooplankton has more desirable nutritional characteristics for fish larval diets than laboratory cultured *Artemia* nauplii (Manickam et al. 2017). Thus, an intentional introduction of *S. sarsi* as food item for fish juveniles must be investigated further. Fish stocking may represent the main vector of *S. sarsi* introduction in Romania. Similar cases of calanoid invasions related to aquaculture activities are cited in Europe: *Boeckella triarticulata* (Thomson, 1883) in Italy (Ferrari and Rossetti 2006), or *Neodiaptomus schmackeri* (Poppe & Richard, 1892) in Albania (Alfonso et al. 2014), but also in North America: *Arctodiaptomus dorsalis* (Marsh, 1907) (Reid 2007) and New Zealand: *Skistodiaptomus pallidus* (Herrick, 1879) (Duggan et al. 2006; Duggan and Pullan 2017). Branford and Duggan (2017) found direct correlations between the translocation of grass carp *Ctenopharyngodon idella* and the introduction of "hitchhiking" zooplankton, including non-native calanoid copepod species. Since grass carp was introduced in Europe, including Romania, for aquatic macrophyte control, together with other Chinese carp species since 1959 (Copp et al. 2005), aquacultural activities remain the most probable explanation for the presence of *S. sarsi* in Lakes Fălticeni. Nevertheless, *S. sarsi* has not previously been recorded in aquaculture facilities, according to a recent global review of zooplankton in freshwater aquaculture facilities (Pearson and Duggan 2018).

The species appearance in Ukraine could potentially also be explained by human introduction in association with salmon juveniles from reservoirs of the region (Mykitchak and Koval 2018). Another possibility includes migratory birds, since 77% from the 39 bird species protected in Natura 2000 area ROSPA0064 Lakes Fălticeni are migratory (Natura 2000 Standard data form 2019).

Several characteristics of *S. sarsi*, like its high tolerance to ecogeographically diverse conditions (Gündüz 1998), or its ability to develop in different trophic conditions, even during cyanobacterial blooms (Afonina 2018), make it a perfect candidate for invasion. Non-indigenous zooplankton have been found to more easily colonize constructed waters (like canals, dam reservoirs or ornamental ponds) than natural pools (Taylor and Duggan 2012; Havel et al. 2005, 2015), due to low biotic resistance found in man-made habitats. Aquaculture activities in particular carry a high risk of invasion from zooplankton "hitchhikers" through intentional or accidental introductions (Duggan and Pullan 2017). Since zooplankton species are simply regarded as fish food by aquaculture workers, systematic research in this area is scarce (Pearson and Duggan 2018), making it difficult to determine the extent and spread of *S. sarsi* invasion in Europe by this industry.

The interconnection of river basins in Europe raises the issue of spread through migration corridors (Bij de Vaate et al. 2002). From this point of view, *S. sarsi* must be kept under surveillance, especially within the framework of current climatic changes, since temperature is a critical parameter influencing aquatic invasions (Havel et al. 2002, 2015; Bates et al. 2013). Subsequent quantitative and molecular studies must follow this first record in Romania, to elucidate the introduction routes and future spread of this species.

Conclusions

1. The East Asian calanoid copepod species *S. sarsi* has been recorded for the first time in Romania. Possible vectors of introduction of this non-native species are fish stocking, migratory birds or intentional release.
2. Further research on *S. sarsi* should target community ecology, in order to describe the dynamics and effects on native plankton. Also, molecular investigations are required to clarify the disjunct character of the distribution of the species world-wide and its routes of introduction in Europe.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Location, characteristics and the presence of planktonic microcrustaceans (cladocerans and copepods) in the eleven sites (1–11) sampled in 2018 and 2019.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2020/Supplements/BIR_2020_Battes_etal_Table_S1.xlsx