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Cladocera in the valley of the river Sûre (Luxemburg and Belgium), with a review of *Chydorus brevilabris* in Western Europe

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Abstract
Cladocerans were investigated in the valley of the river Sûre (Belgium and Luxembourg) in August 2017. A total of 28 species was found, of which nine were new to the fauna of Luxembourg and three to the fauna of Belgium. The known range of *Eubosmina berolinensis* was widened to the southwest by 550 km. The American ball waterflea, *C. brevilabris*, is new to Belgian fauna. *C. brevilabris* is an exotic species in Europe. In this paper, its present status in Western Europe is discussed and additional data on its ecology is presented.

Keywords: floral calendar, honey bee, melliferous plands, mellissopalynology

Introduction
The cladoceran fauna of Luxembourg is very poorly known (Meisch & Massard, 2015) [1]. In contrast, the cladoceran fauna of Belgium is better known (Louette *et al*., 2007) [2]. The Sûre is a small river on the border of Belgium and Luxembourg. It originates in the hills of the Ardennen in Belgium, forming the border of Belgium and Luxembourg between Martelange and Tintange. The dam at Esch-sur-Sûre resulted in a large and deep freshwater reservoir of 380 hectare, with a maximum depth of 32 meters. Large, deep, freshwater lakes are not naturally occurring in this part of Europe. The lake is fed from the watershed of the Sûre. Several small ponds are present along the border, providing a valuable opportunity to locate more species in the two countries. In August 2017, the author investigated the lake and ponds in the watershed for the cladoceran fauna.

Material and methods
Samples were taken with a zooplankton net (EFE & GBnets), with mesh of 150 µm width. To gather the samples, the net was pulled through the water and through the vegetation, if present, and scraped over hard substrate such as logs and stones. Following the hauling of the net, the residual material and animals were rinsed out of the cup and collected in a bottle (Fig. 1). The samples were preserved in 95% ethanol (denaturated bio-ethanol GN 2207.20). Acidity (Milwaukee pH 600), depth (Secchi-disc), and electric conductivity (HM digital EC-3) were measured. Vegetation cover was estimated.

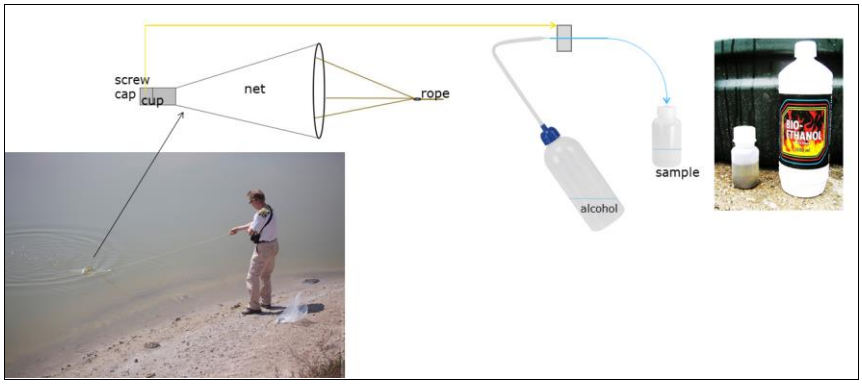

Fig 1: Schematic representation of the sampling method (photo left: Joris de Raedt)

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Twelve samples were taken at 10 locations (Fig. 2). The locations were as follows: (1) Esch sur Sûre (49°54′42″N; 5°55′60″E); (2) Lac de la Haut-Sûre (49°54′15″N; 5°52′57″E); (3) Rambrouch, pond (49°49′28″N; 5°45′14″E) and temporary water (49°49′30″N; 5°45′15″E) in Luxembourg; (4) Tintange (49°52′34″N; 5°44′21″E); (5) Martelange (49°49′55″N; 5°44′10″E); (6) Forêt D'Anlier (49°49′50″N; 5°44′00″E); (7) River Sûre (49°49′50″N; 5°44′08″E); (8) Bodange (49°51′07″N; 5°41′03″E); (9) Fauvilliers north (49°51′15″N; 5°39′55″E); and (10) Fauvilliers south, two connected ponds (49°50′55″N ; 5°39′37″E and 49°50′54″N ; 5°39′33″E) in Belgium (Fig. 2).

Fig 2: Sample locations in the valley of the river Sûre

These samples were used for further analysis. A sub-sample was counted in a cuvet (Hydrobios 5ml). One hundred animals from each sample were identified to the species level. Counting was done using an inverted microscope (Olympus IX70), at 100x magnification. Animals were selected for detailed observation under a binocular microscope (Olympus SZX12). They were mounted in a drop of glycerin on a slide and covered with a cover glass. Detailed observations were made using an Olympus BX51 microscope at 400 or 600x magnification. Photographs were taken using Olympus CellSense.

The samples remain in the collection of the author.

Additional data was used to clarify the ecology of *Chydorus brevilabris*. The data was gathered from samples taken by the author in the Netherlands and the United States.

Results

Blue-green algae were present in Lac de la Haut-Sûre (2) and Martelange (5). The pond in Forêt D'Anlier (6) was covered with Common water-plantain (*Alisma plantago-aquatica*) (25%) and Lesser duckweed (*Lemna minor*) (5%). The pond in Bogange had a vegetation cover of 12% Western waterweed (*Elodea nuttallii*). The pond at Fauvilliers north (10) was covered with 2% Cat's-tail (*Typha*). The small pond at Fauvilliers south (11) was covered with 40% Water horsetail (*Equisetum fluviatile*). The larger pond at Fauvilliers south (12) was covered with Cat's-tail (10%), Western waterweed (*Elodea nuttallii*) (5%), and Lesser duckweed (2%). Rudd (*Scardinius erythrophtalmus*) inhabits both isolated ponds at Fauvillier south, and was probably introduced here.

In total, 28 species were found, including 15 in Luxembourg and 19 in Belgium. Sample 8 contained just two cladocerans. The results of the measurements and the species composition are given in Table 1.

Table 1: Results and species composition Water type: R – River, L – Lake, P – Pond, T – Temporary pond.

Location	**1**	**2**	**3**		**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Sample	1	2	3	4	5	6	7	8	9	10	11	12
Land	L	L	L	L	B	B	B	B	B	B	B	B
Water type	R	L	P	T	P	P	P	R	P	P	P	P
EC (µS/cm)	215	175	438	486	160	694	162	187	153	380	198	133
pH	8.0	9.45	8.15	6.5	7.7	7.4	6.8	8.1	8.1	9.8	8.4	7.8
Vegetation cover	0%	0%	2%	0%	0%	0%	30%	0%	12%	2%	40%	17%
Depth (m)	0.5	30	6.0	0.7	2.0	0.6	0.3	1.0	1.0	0.8	0.5	1.0
Secchi-disc (m)	0.5	1.0	1.0	0.6	1.0	0.2	0.3	0.6	0.8	0.4	0.5	0.7
Acroperus harpae											9	2
Alona affinis			18		1				3		4	1
Alona quadrangularis					1							
Alonella nana					1							
Bosmina cornuta						100		2		2		
Bosmina longirostris			3									

Bosmina longirostris similis	97											
Ceriodaphnia megops							99					
Ceriodaphnia pulchella		2	62						29	88	1	5
Chydorus brevilabris									1		20	10
Chydorus sphaericus									1			9
Daphnia ambigua					4				28	4		
Daphnia cucullata	1	6			76							
Daphnia hyalina	2	1										
Daphnia longispina							1					
Daphnia pulex				100								
Diaphanosoma brachyurum		32										
Diaphanosoma orghidani					14							
Disparalona leei					2							
Disparalona rostrata									1		1	1
Eubosmina berolinensis		38										
Ilyocryptus agilis											1	
Pleuroxus denticulatus		1										
Pleuroxus truncatus									1		52	63
Polyphemus pediculis		18										
Pseudochydorus globosus		1										
Scapholeberis mucronata		1	14		1				36	6	11	7
Sida crystallina			1									
Simocephalus vetulus			2								1	2
Total	100	100	100	100	100	100	100	2	100	100	100	100

The American Ball Waterflea (*Chydorus brevilabris*) was found in three samples. This is an exotic species in Europe, and almost nothing is known of its ecology. To lift the veil of its ecology in Western Europe, additional data on electric conductivity, acidity, depth, secchi-depth, temperature, and vegetation cover were added, based on observations in the Netherlands (Table 2).

Table 2: Properties of waters inhabited by *C. brevilabris* in Belgium and the Netherlands n = number of measurements

Measurements	minimum	maximum	mean	sd	n
EC 25 (µS/cm)	60,0	1850.0	485.6	473.0	13
pH	4.7	8.8	7.8	1.1	15
Depth (cm)	25.0	200.0	85.6	55.7	17
Secchi-depth (cm)	25.0	160.0	65.0	35.2	17
Temperature (°C)	6.3	23.7	18.7	5.2	10
Vegetation cover (%)	0.0	90.0	26.1	28.7	17
submersed	0.0	90.0	23.3	28.5	16
floating-leaf	0.0	20.0	1.3	5.2	15
emers	0.0	40.0	3.9	10.5	16

A comparison was made of the range of acidity (pH) and electric conductivity (EC) in European (n=13) and American (n=14) waters with *C. bevilabris* (Fig. 3). The samples were taken by the author from the surroundings of Washington DC in 2017 and 2019.

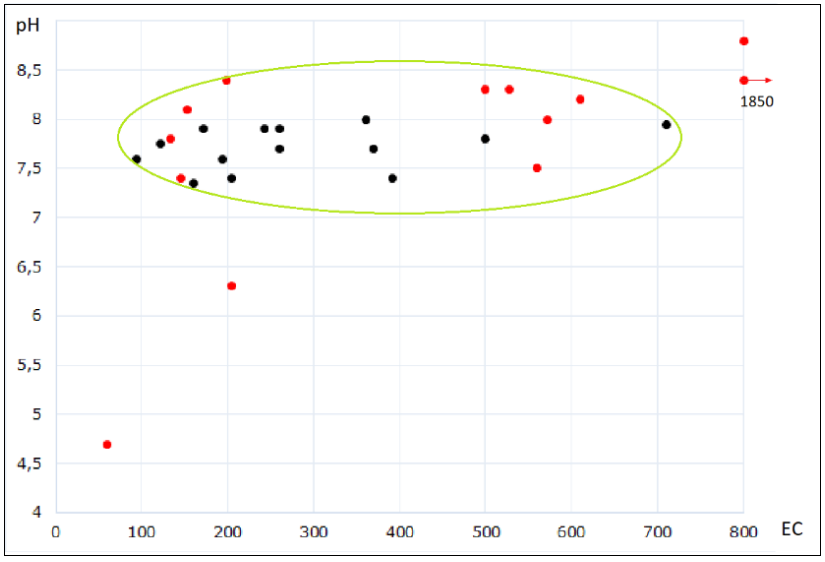

Fig 3: Acidity (pH) and conductivity (EC) in Europe (red dots) and Washington DC (black dots)

Discussion

There are 22 known cladoceran species in Luxembourg (Dohet & Hoffmann, 1995; Cauchie *et al*., 1995; Gerecke *et al*., 2005; Meisch & Massard 2015) [3-5, 1]. The Belgian fauna include 89 freshwater cladoceran species (Billiones *et al*, 2004; Louette *et al*. 2007) [6-7], and nine (sub) species are new to the fauna of Luxembourg: *Bosmina longirostris similis*, *Daphnia hyalina*, *D. pulex*, *Eubosmina berolinensis*, *Pleuroxus denticulatus*, *Pseudochydorus globulosus*, *Polyphemus pediculus*, *Sida crystalina*, and *Simocephalus vetulus*. New to the Belgian fauna are *Chydorus brevilabris*, *Disparalona leei*, and *Diaphanosoma orghidani*.

New species

Cladocerans in Luxembourg are known in Lac de la Haut-Sûre (Dohet & Hoffmann, 1995) [3] and from springs throughout Luxembourg (Gerecke *et al*., 2005) [5]. *Eubosmina berolinensis* is a remarkable new species found in the reservoir. Adult females were present (Fig. 4), leaving no doubt about the identity of this species. This *Eubosmina* species has a very broad-based, long shell spine that points backwards (Lieder, 1999) [7].

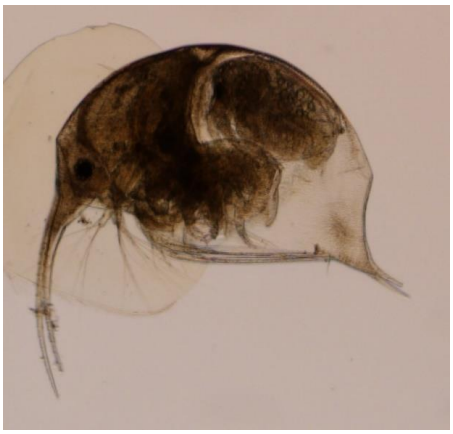

Fig 4: *E. berolinensis* from Lac de la Haut-Sûre

E. berolonensis is a rare species, with a limited distribution in Europe (Fig. 5), found mainly in Poland and around Berlin (Lieder, 1999) – hence its name. Recently, Arp and Maier (2016) [8] were responsible for the most western finding. The new record is the most southwestern in Europe (Fig. 5), and the species may be expanding its distribution to the west and south.

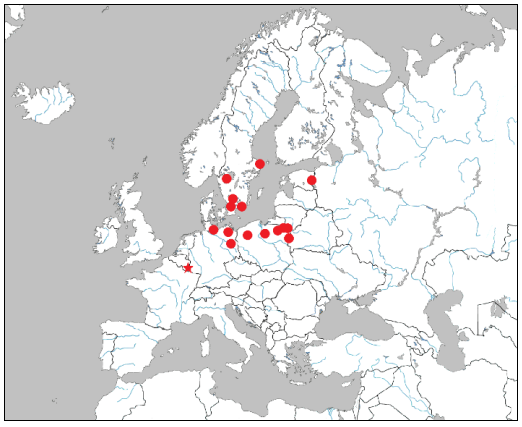

Fig 5: Distribution of *E. berolinensis* in Europe, Lac de la Haut-Sûre - red star

Other species new to Lac de la Haut-Sûre are *Daphnia hyalina, Pleuroxus denticulatus*, and *Pseudochydorus globulosus*. *Daphnia hyalina* is a species found in large, deep, oligo-mesotrophic Alpine lakes.

Polyphemus pediculus, *Sida crystallina*, and *Simocephalus vetulus* are common species with a preference for vegetation, found in a pond with floating waterlily leaves. *Daphnia pulex* was found in a temporary pond. These habitats have not been investigated in Luxembourg, and the finding of these species as new fauna is unsurprising.

Two exotic species are new to the Belgian fauna: *Disparalona leei* (Flößner, 2000; Van Damme & Cart (2012) [9-10] and *Chydorus brevilabris* (Chalkley, 2015) [11]. *Diaphanosoma orghidani* was recently found in in Germany (Weiler, 1997) [12] and the Netherlands (Soesbergen, 2010) [13]. It does not appear to be present in France (Cavrois, 2012) [14].

A *Bosmina*-species found in Martelange has hooked antennules and a very short carapace spine (Fig. 6). This is similar to *Bosmina longirostris curvirostris* or *Bosmina longirostris cornuta* (Lieder, 1999; Błędzki & Rybak, 2016) [7, 15].

Fig 6: *Bosmina cornuta*

Detailed examination revealed a variant position of the lateral head-pore, which is situated between the lines of the fornix (Fig. 7).

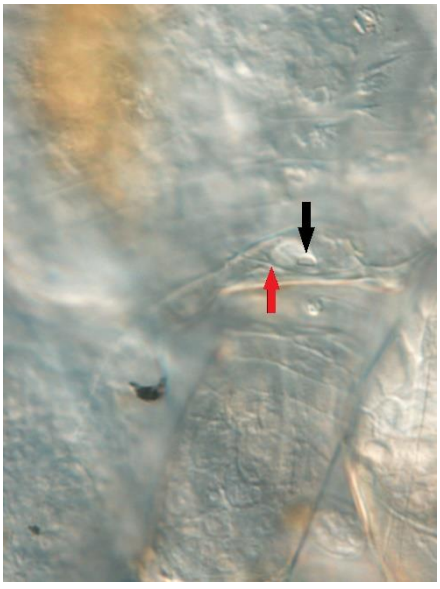

Fig 7: Position of lateral head-pore (black arrow) above lower fornix branch (red arrow)

This lateral head-pore position is known from the American species *B. liederi* and *B. freyi* (De Melo & Hebert 1994) [16]. *B. longirostris* s.s. has a lateral head pore located directly at the lower line of the fornix (Negrea, 1983; Hudec, 1989; Kořinek *et al*., 1997) [17-19]. The position of the head-pore differs from that of *B. longirostris* s.s. The deviating position of the head pore was a primary justification for splitting *B. liederi* and *B. freyi* from *B. longirostris*. This difference indicates the specific identity of *B. cornuta*. The species status of *B. cornuta* was proposed on the basis of body shape alone (Kappes & Sinch, 2002) [20]. This species is new to the Belgian fauna.

C. brevilabris in Western Europe

The first records of *C. brevilabris* in Europe come from Lac de la Haut-Sûre in Luxembourg (Dohet & Hoffmann, 1995) [3]. It was a sporadic species in 1990-1993 (Dohet & Hoffmann, 1995) [3]. Present in France (Cavrois, 2012) [14], the species was found in Auvergne, Savoie, and the river Seine by J.F. Cart (Verolet, 2009) [21]. It was first recorded in the Netherlands in 2010 and it remains a very rare species (Soesbergen, 2018) [22]. Louette *et al*. (2007) [2]do not cite any occurrences of *C. brevilabris* in Belgium; and the records from Fauvilliers (Fig. 8) and Bodange are the first to do so. The ponds are located upstream from the Lac de la Haut-C. This species may have already been present in the ponds in valley in the 1990s when it was present in the lake (Dohet & Hoffmann, 1995) [3]. It may even have originated from these isolated ponds, if it was introduced here with Rudd. The ponds overflow with high precipitation in the river Sûre and ultimately reach the reservoir. Exotic cladocerans can be spread through fish stockings (De Meester *et al*., 2002; Sharma & Kotov, 2015) [23-24].

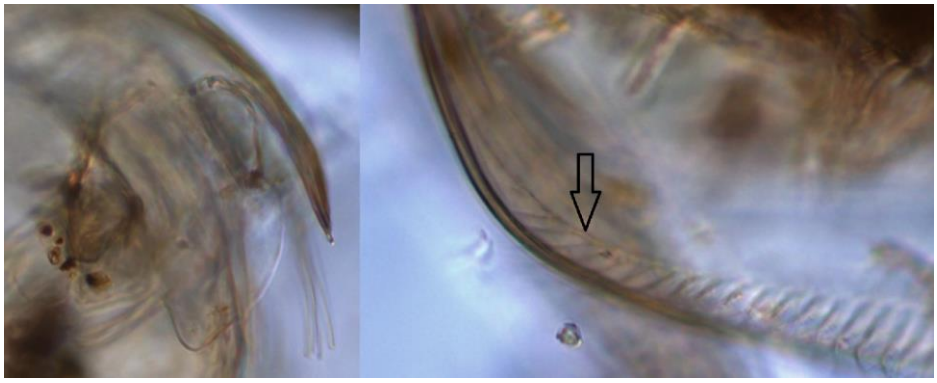

Fig 8: *C. brevilabris* from Fauvilliers labral plate (left) and setae connected with line (right)

Recognition of this species in Europe is difficult because it is only mentioned in the French key by Amoros (1984) [25]. The reason it might have gone undetected since the first record in Luxembourg is that it is easily mistaken for the very common species *C. sphaericus*. It is distinguished from *C. sphaericus* by the broad, rounded, labral plate (Fig. 8 left) and posterior setae, connected by a solid line (Fig. 8 right).

C. brevilabris is present in Belgium, France, Luxembourg, and the Netherlands (Fig. 9). There are no records from Great Britain (Chalkley, 2015) [11].

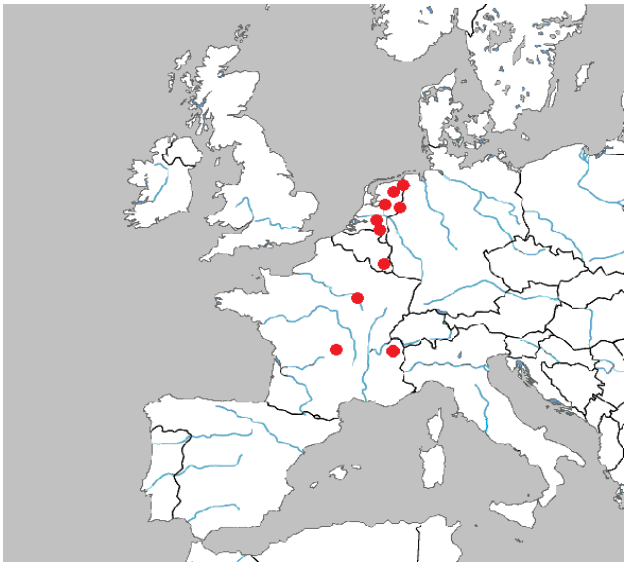

Fig 9: Distribution of *Chydorus brevilabris* in Western Europe

The species remains very rare in Western Europe, though it is widespread in the Netherlands (Fig. 10). *C. brevilabris* is a new exotic species in Western Europe, and its distribution may be severely underestimated.

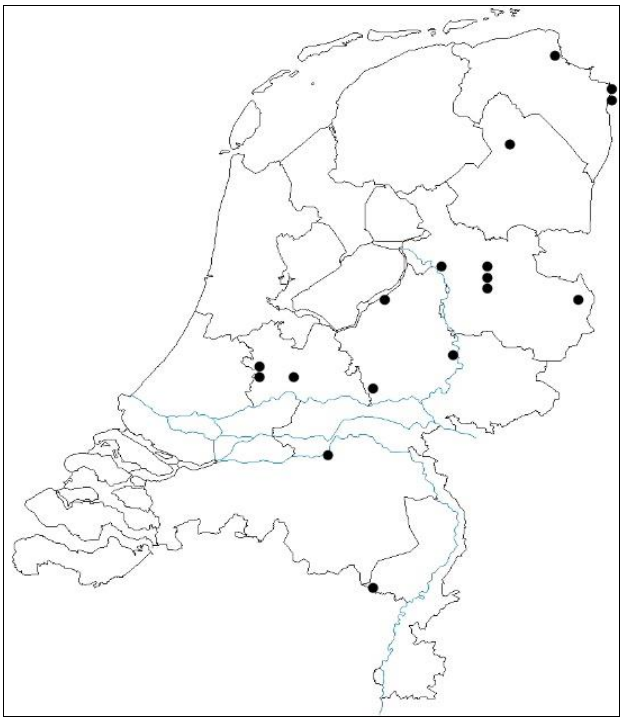

Fig 10: Distribution of *Chydorus brevilabris* in the Netherlands

Little is known of the ecology of this species. *C. brevilabris* is a eurytopic, littoral species found in mesotrophic to hypertrophic waters in the United States (Bos, 2001; Albert *et al*., 2010; Hargan, 2014) [26-28]. The habitat in Western Europe is characterized by fresh, clear water with a sandy bottom and the absence of floating-leaved water plants (Table 2). The submerged water plants present, are indicative for meso- to eutrophic conditions. It has not been found on clay soils.

C. brevilabris is found at pH 7.4-8.4 and electric conductivity of 100-700 (Fig. 3). Outliers (Fig. 3) seem to indicate tolerance to acid and saline circumstances. It is found in slightly brackish water (Hilgeman, 2019) [29] and acid freshwaters (Bakker & De Jong, 2019) [30].

C. brevilabris is found together with *C. sphaericus* (72% of the samples), *Scapholeberis mucronata* (66.7%), *Bosmina longirostris* s.l. (55.6%), *Pleuroxus truncatus* (55.6%), *Simocephalus vetulus* (50%), *Alona affinis* (44.4%), *Ceriodaphnia pulchella* (44.4%), *Pleuroxus aduncus* (44.4%), and *Acroperus harpae* (38.9%). All are species from meso-eutrophic vegetated waters. It was once found together with *Chydorus gibbus* and *Paralona pigra*, both species found in mesotrophic waters.

It is unlikely that *C. brevilabris* will become a threat to European *C. sphaericus*. The latter is an extremely tolerant species in Europe. *C. brevilabris* may become a concurrent for other indigenous chydorid species in mesotrophic habitat, such as *Chydorus gibbus*, *Chydorus latus* and *Paralona pigra*.

Conclusion

The number of known freshwater cladoceran species increased to 31 in Luxembourg and 93 in Belgium. This number includes *B. cornuta*, as a new valid species. The known range of *Eubosmina berolinensis* has widened to the southwest by 550 km, but there is still much to learn about European biodiversity.

C. brevilabris is an exotic species in Western Europe, and its distribution outside this area is unknown. Indeed, its distribution in Western Europe is only incompletely known, and this eurytopic species could ultimately become a threat to some European chydorids. Hence, vigilance is needed and the monitoring of its distribution in Europe is strongly recommended.

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