



HAL
open science

**Genetic evidence of the presence in France of the North
American species *Euglesa compressa* Prime, 1852
(*Bivalvia*, *Sphaeriidae*)**

J. Mouthon, M. Forcellini

► **To cite this version:**

J. Mouthon, M. Forcellini. Genetic evidence of the presence in France of the North American species *Euglesa compressa* Prime, 1852 (*Bivalvia*, *Sphaeriidae*). *BioInvasions Records*, 2017, 6 (3), pp.225-231. 10.3391/bir.2017.6.3.07 . hal-01741621

HAL Id: hal-01741621

<https://hal.science/hal-01741621>

Submitted on 23 Mar 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Research Article

Genetic evidence of the presence in France of the North American species *Euglesa compressa* Prime, 1852 (Bivalvia, Sphaeriidae)

Jacques Mouthon and Maxence Forcellini*

Irstea, UR MALY, centre de Lyon-Villeurbanne, 5 rue de la Doua, BP 32108, 69616 Villeurbanne Cedex, France

Author e-mails: maxence.forcellini@irstea.fr (MF), jmouthon@free.fr (JM)

*Corresponding author

Received: 14 November 2016 / Accepted: 14 February 2017 / Published online: 8 March 2017

Handling editor: Kit Magellan

Abstract

The first internal transcribed spacer (ITS-1) of ribosomal RNA of 5 individuals of unknown pisidiid clams collected over several years in the Rhone river basin was extracted to specify their taxonomic position. The phylogenetic tree obtained from the comparison of these sequences to those of 19 species available in the NCBI genetic database is in good agreement with the phylogenetic structure of Sphaeriinae and revealed that the unknown bivalves belong to the North American species *Euglesa compressa*. This invasive mollusk has now colonized the major French river basins and could be responsible for the major decline of *E. supina* in the areas where it has settled. The main features of *E. compressa* are also presented.

Key words: invasive species, taxonomy, phylogeny, *Euglesa* (*Cyclocalyx*), *Pisidium*, *Odhneripisidium*

Introduction

Pisidiid clams are very small bivalves (adult size range from 1.5 to 11 mm) in the family Sphaeriidae and are distributed all over the world except in Antarctica. These bivalves inhabit fine deposits in a wide range of freshwater ecosystems. They are hermaphroditic, capable of self-fertilization and ovoviparous. The young develop inside a brood sac in the inner demibranchs of the gills (Thomas 1959; Heard 1965; Meier-Brook 1977; Mackie 1978). Pisidiid clams are interstitial suspension feeders (Lopez and Holopainen 1987). A nuclear and mitochondrial genome based phylogenetic tree of the Sphaeriinae, a Sphaeriidae subfamily in which the genera *Pisidium*, *Odhneripisidium* and *Cyclocalyx* are replaced by *Euglesa* according to the principle of anteriority (Falkner et al. 2002; Gargominy et al. 2011) among others, was proposed by Lee and Ó Foighil (2003).

In 1989, specimens of pisidiid clams were discovered in the lower part of the Saone river (east France) that did not correspond to known European species;

moreover the bibliographical and iconographic research carried out on the pisidiid fauna of other continents remained indecisive. These bivalves, which present intermediate characteristics between *Euglesa supina* Schmidt, 1851 and *E. casertana* forma *ponderosa*, led to the hypothesis of hybridization or the possible emergence of a new taxon (Mouthon and Tair-Abbaci 2012). The aim of this article is to clarify the taxonomic position of these specimens from the first internal transcribed spacer (ITS-1) of ribosomal RNA, a relevant marker for species level identification (Freire et al. 2010) widely used in phylogenetic studies on bivalves (Cheng et al. 2006; Prié and Bichain 2009) and specifically on Sphaeriidae (Lee and Ó Foighil 2003; Schultheiß et al. 2008; Stunžėnas et al. 2011).

Material and methods

Material studied

Five individuals of pisidiid species (called *Euglesa* sp. in the text) and 4 individuals of *E. casertana* forma *ponderosa* were collected in 2015 and 2016 in Saone,

Table 1. Details of sampled individuals giving locality, river, collector and geographic coordinates

City	River	Code station	Taxon	ITS-1	Collector	Geographic coordinates
Saunières	Doubs	DOSA	<i>Euglesa</i> sp.	2	J. Mouthon	46°54'09.4"N; 5°05'01.4"E
Creys-Malville	Rhone	RHCR	<i>Euglesa casertana</i> f. <i>ponderosa</i> Stelfox, 1918	2	J. Mouthon	45°46'21.1"N; 5°28'01.9"E
			<i>Odhneripisidium tenuilineatum</i> Stelfox, 1918	3	J. Mouthon	
Heuilley-sur-Saône	Saone	SAHE	<i>Euglesa</i> sp.	1	J. Mouthon	47°19'31.6"N; 5°27'25.1"E
Tillenay	Saone	SATIL	<i>Euglesa</i> sp.	2	J. Mouthon	47°10'45.6"N; 5°21'16.9"E
Trévoux	Saone	SATR	<i>Euglesa casertana</i> f. <i>ponderosa</i> Stelfox, 1918	2	J. Mouthon	45°56'01.5"N; 4°45'45.4"E
Vuillecin	Druegon	DRVU	<i>Euglesa pulchella</i> Jenyns, 1832	4	J. Mouthon	46°56'10.5"N; 6°19'32.6"E

Table 2. GenBank accession numbers of sequences of pisidiid clams used in this study

Species	Locality	Author	Accession number	
			(NCBI)	ITS-1
<i>Euglesa (Cyclocalyx) adamsi</i> Stimpson, 1851	Michigan, USA	Lee et al. 2003	AY093513	
<i>Euglesa (Cyclocalyx) compressa</i> Prime, 1852	Michigan, USA	Lee et al. 2003	AY093518	
<i>Euglesa (Cyclocalyx) fallax</i> Sterki, 1896	Michigan, USA	Lee et al. 2003	AY093519	
<i>Euglesa (Cyclocalyx) hallae</i> Kuiper, 1983	Sydney, Australia	Lee et al. 2003	AY093520	
<i>Euglesa (Cyclocalyx) henslowana</i> (Sheppard, 1825)	NA	Steiner 2005 (unpublished)	DQ062603	
<i>Euglesa (Cyclocalyx) hibernica</i> Westerlund, 1894	Heiliges Meer, Germany	Lee et al. 2003	AY093522	
<i>Euglesa (Cyclocalyx) lilljeborgii</i> Clessin, 1886	Kuril Islands, Russia	Lee et al. 2003	AY093521	
<i>Euglesa (Cyclocalyx) milium</i> Held, 1836	Heiliges Meer, Germany	Lee et al. 2003	AY093523	
<i>Euglesa (Cyclocalyx) nipponense</i> Kuroda, 1930	Gunma Prefecture, Japan	Lee et al. 2003	AY093525	
<i>Euglesa (Cyclocalyx) nitida</i> Jenyns, 1832	Heiliges Meer, Germany	Lee et al. 2003	AY093526	
<i>Euglesa (Cyclocalyx) personata</i> Malm, 1855	Ammerbuch, Germany	Lee et al. 2003	AY093527	
<i>Euglesa (Cyclocalyx) subtruncata</i> Malm, 1855	Heiliges Meer, Germany	Lee et al. 2003	AY093528	
<i>Euglesa (Cyclocalyx) supina</i> Schmidt, 1850	Hopstener Ach., Germany	Lee et al. 2003	AY093529	
<i>Euglesa (Cyclocalyx) variabile</i> Prime, 1852	Michigan, USA	Lee et al. 2003	AY093530	
<i>Odhneripisidium japonicum</i> Pilsbry & Hirase, 1908	Nagano Prefecture, Japan	Lee et al. 2003	AY093532	
<i>Odhneripisidium moitessierianum</i> Paladihe, 1866	NA	Steiner 2005 (unpublished)	DQ062589	
<i>Odhneripisidium parvum</i> Mori, 1938	Ehime Prefecture, Japan	Lee et al. 2003	AY093531	
<i>Pisidium amnicum</i> (O.F. Müller, 1774)	NA	Steiner 2005 (unpublished)	DQ062574	
<i>Pisidium dubium</i> (Say, 1817)	Michigan, USA	Lee et al. 2003	AY093533	

Doubs and Rhone rivers. In addition 7 individuals of *Odhneripisidium tenuilineatum* Stelfox, 1918 and *Euglesa pulchella* Jenyns, 1832 for which ITS-1 sequences are not available were also sampled (Table 1). All specimens were immediately transferred into 95% ethanol and conserved at -18°C .

Molecular analysis

Genomic RNA was extracted from ethanol preserved tissue. A piece of mantle tissue per individual (or the whole animal for the smallest specimens) was added to 150 μL of Chelex 7% (Walsh et al. 1991) and to 10 μL of proteinase K. The tissue fragments were then incubated according to the following cycle: 2 hours at 50°C , 15 minutes at 90°C and then 5 min at 15°C .

An approximately 760 nucleotide fragment of the nuclear ribosomal first Internal Transcribed Spacer (ITS-1) was amplified using primers annealing to flanking regions of 18S and 5.8S genes (White et al. 1996). A standard PCR mix included per sample an

amount of 2 μL genomic DNA, 22.1 μL of H_2O , 3 μL of Standard Buffer 10X (Biolabs B9014S), 0.9 μL of MgCl_2 at 50mM, 0.6 μL of each primer at 10 μM , 0.3 μL of BSA 100X at 10 mg/mL, 0.26 μL of dNTP at 20 mM and 0.26 μL of Taq polymerase at 5 U/ μL (EUROBIOTAQ). The polymerase chain reaction conditions were as follows. After an initial step of 94°C for 5 min, an initial annealing temperature of 65°C was decreased by $2^{\circ}\text{C}/\text{cycle}$ until the final annealing temperature of 55°C . Then followed 37 cycles of 30 s at 94°C , 30 s at 51°C and 45 s at 72°C . The reaction was terminated after a final step at 72°C for 8 min.

PCR results were checked on a 1.3% agarose gel with TAE 1X. The forward strand was sequenced by using the Sanger method (Sanger et al. 1977) by Biofidal. Sequence chromatograms were edited manually for all individuals using FinchTV (version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; <http://www.geospiza.com>). ITS-1 fragments alignment was done using Prank (Löytynoja and Goldman 2005). In order

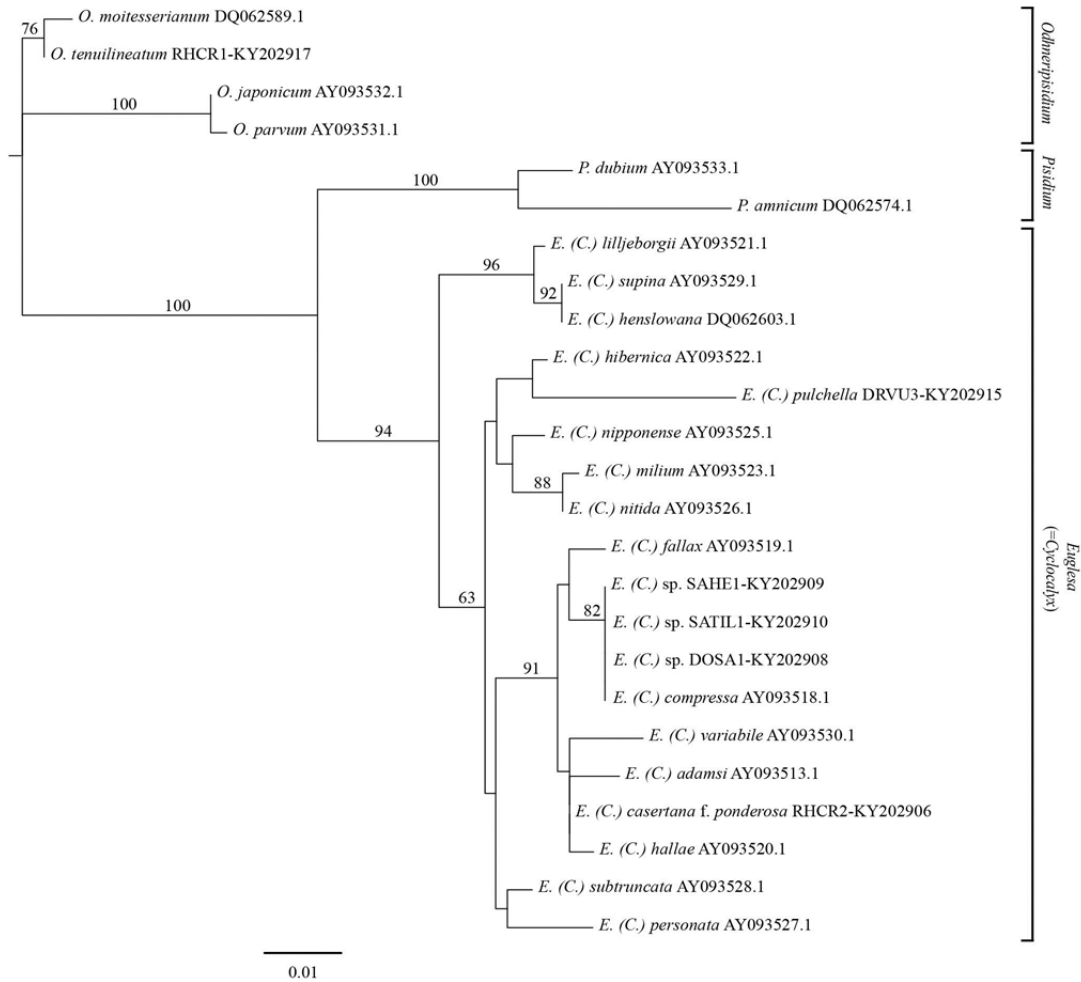


Figure 1. Maximum likelihood phylogenetic tree run with 100 bootstraps based on 593 base pairs of the ITS-1 molecular marker. Only bootstrap values up to 50% that represent moderate to high support of nodes are shown. GenBank accession numbers of sequences of *O. tenuilineatum*, *E. pulchella*, *E. sp.* and *E. casertana* f. *ponderosa* are indicated after the code of species.

to compare these sequences with our pisidiid clams, the nuclear ITS-1 ribosomal locus of 19 species was extracted from the genetic database NCBI (Table 2). The tree building was processed in PhyML with the Neighbor Joining method and the robustness of nodes was assessed by a non-parametric bootstrap test (100 cycles) (Felsenstein 1985).

Results

The first ITS-1 sequence analysis showed no differences between several individuals of the same species. Accordingly, only one individual per species and one individual per locality for *Euglesa* sp., *E. casertana* f. *ponderosa*, *E. pulchella* and *O. tenuilineatum* were represented. The final ITS-1

alignment consisted of 593 base pair fragments and the maximum likelihood (ML) phylogenetic tree is depicted in Figure 1.

The ML phylogenetic tree produced three robustly supported monophyletic lineages: 1) *Odhneripisidium* contained two Japanese bivalves *O. japonicum* and *O. parvum* on the one hand and the two Eurasian species *O. moitessierianum* and *O. tenuilineatum* on the other hand; 2) *Pisidium* s.s. which comprises the North American *P. dubium* and the Eurasian *P. amnicum*; 3) *Euglesa* (= *Cycloclalyx*) containing all the other species including *E. pulchella*. Within this lineage we noted a robust clade including four North American species, namely *E. adamsi*, *E. compressa*, *E. fallax* and *E. variabile*, the Australian species *E. hallae*, and the cosmopolitan species *E. casertana*.

Table 3. Matrix of pairwise distance between sequences of *Euglesa* sp. individuals and the registered *E. compressa*. Calculations were produce using R (R Core Team 2016), ape 4.0 library (Paradis et al. 2004) and the dist.dna function without model of DNA evolution.

	<i>E. compressa</i> AY093518	<i>Euglesa</i> sp. SATIL1	<i>Euglesa</i> sp. SATIL2	<i>Euglesa</i> sp. SAHE1	<i>Euglesa</i> sp. DOSA1	<i>Euglesa</i> sp. DOSA2
<i>E. compressa</i> AY093518	—					
<i>Euglesa</i> sp. SATIL1	0	—				
<i>Euglesa</i> sp. SATIL2	0.004	0.004	—			
<i>Euglesa</i> sp. SAHE1	0	0	0.004	—		
<i>Euglesa</i> sp. DOSA1	0	0	0.004	0	—	
<i>Euglesa</i> sp. DOSA2	0	0	0.004	0	0	—



Figure 2. Shells of *Euglesa compressa*, Doubs river: old, adult and young individuals (Note the different scales). Photographs by © 2016 G. Le Goff (Irstea).

The ML tree also indicated that French individuals of *Euglesa* sp. and the American individual of *E. compressa* belonged to a shared monophyletic clade. This is supported by a bootstrap value of 82%, representing solid support of the node (Hillis and Bull 1993; Douzery et al. 2010). Moreover, the matrix of pairwise distances between sequences of *Euglesa* sp. individuals and the registered *E. compressa* (Table 3) showed no difference between individuals except for one (*Euglesa* sp. SATIL2), which had two substitutions at positions 90 and 108 respectively (see Appendix 1 for alignment details). This result showed that all the specimens of *Euglesa* sp. investigated belong to the species *E. compressa* and confirmed the presence of this North-American pisiidiid in the major French river basins (Figure 2). A previous analysis based on the 16S mitochondrial RNA sequences gave similar results.

Discussion

The results obtained for the three lineages of pisiidiid clams are in good agreement with the phylogenetic structure of Sphaeriinae proposed by Lee and Ó Foighil (2003). Our study included six supplementary species which were not taken into account in Lee and Ó Foighil's (2003) ITS-1 study. These are: 1) *Odhneripisidium moitessierianum* and *O. tenuilineatum*,

well-grouped with the two other *Odhneripisidium*, i.e. *O. japonicum* and *O. parvum*; 2) *P. amnicum*, well-grouped with the other *Pisidium* s.s., i.e. *P. dubium*; 3) *Euglesa lilljeborgii*, *Euglesa henslowana* and *E. pulchella* also well-grouped with all the *Euglesa* (= *Cyclocalyx*) species.

The main outcome of this study is that the unknown individuals described in a previous article (Mouthon and Taïr-Abbaci 2012) were neither hybrids of *Euglesa supina* and *E. casertana* forma *ponderosa* nor a new species, but belonged to *E. compressa*, a highly polymorphous North American species (Sterki 1905, 1916).

After *E. casertana* Poli, 1791, *E. compressa* (vernacular name: ridge beak pea clam) is the most common pisiidiid clam in the North American continent (Sterki 1905; Herrington 1962), but it is rarer in the Atlantic Coastal zone (Mackie 2007). Fossil specimens from the Middle Pliocene have been found in the United States (Herrington 1962), and from the late Quaternary in northern Mexico (Czaja et al. 2014).

The main features of *E. compressa* are: a more or less triangular shell, colour whitish to grayish, rather stout, prominent umbones, typically with a well-developed appendiculum (a more or less oblique fold or ridge located close to the top of each valve), periostracum dull to silky not glossy, striae ranging

from fine to coarse, regularly to irregularly spaced, rather stout hinge (Sterki 1905; Herrington 1962; Clarke 1981; Mackie 2007). The maximal shell length ranged from 3.8 mm (Herrington 1962) to about 5.5 mm for Canadian specimens (Clarke 1981). French specimens reached 4.4 mm (Mouthon and Taïr-Abbaci 2012). About ten varieties of this polymorphous species were described by Sterki (1905, 1916). According to Sterki (1905, 1916; see also Herrington 1962; Mackie et al. 1980) “the common river and creek form whose surface is rather coarse, sharp, regular, having concentric striae with microscopic wrinkles” is considered as typical. In France on the contrary, all the *E. compressa* collected in rivers and canals are very finely and regularly striated (see also the figures in Mouthon and Taïr-Abbaci 2012).

Euglesa compressa inhabits only perennial and alkaline waters (>50 mg CaCO₃, Mackie and Flippance 1983) of creeks, rivers, ponds and lakes down to 20 meters depth (Herrington 1962; Mackie 2007). It lives at an altitude of over 2000 m (Frank 2010), its longevity varies from one to two years and it produces one or two cohorts a year (Heard 1965; Gillespie 1969; Way and Wissing 1982). The number of shelled larvae/parent (brood size) reaches at least 42 (Clarke 1981). Nevertheless the biology of this euryecious species is still not well-known.

Recorded in Canada, America and Mexico in the region of Mexico City, *E. compressa* has been considered as a Nearctic species up to now. In France, this bivalve has been discovered in the catchment basins of the Seine, the Rhone and the Rhine, then more recently in that of the Loire (Mouthon and Taïr-Abbaci 2012). Unfortunately, no data for the catchment basin of the Garonne are available as yet. In the Netherlands, it sounds likely that *E. compressa* was confused with *E. casertana* forma *plicata* Zeissler, 1962 recorded in the Rhine-Meuse delta (Wallbrink 1995; De Lange et al. 2004; Bij de Vaate et al. 2007a, b) when considering the series of photos published by Van Haaren (2015). Unfortunately, there is no such available information concerning specimens found in the catchment basin of the Elbe (Germany) (Zeissler 1962, 1971). Following the extension of its geographical area to the European continent, *E. compressa* now has a Holarctic distribution by invasion.

Of the Sphaeriidae only *Sphaerium* (*Musculium*) *transversum* Say, 1829, a native of North America, successfully colonized Europe where it was discovered in 1856 in the British Isles (Kerney 1976). More recently it was also recorded from the Netherlands (Kuiper 1981; Gittenberger and Janssen 1998) then in France (Mouthon and Loiseau 2000)

but it remained specifically located in these countries. Thus the arrival of *Euglesa compressa* in France is the first observation of the introduction of a pisidiid bivalve of North American origin in Europe. On the contrary, five species *Sphaerium corneum* Linnaeus, 1758, *P. amnicum* (Müller, 1774), *E. henslowana*, *E. supina* and *O. moitessierianum* Paladilhé, 1866 are considered as having been introduced in America, all in the region of the Great Lakes (Herrington 1962; Burch 1975; Mackie et al. 1980; Clarke 1981; Grigorovich et al. 2000). However the “exotic” nature of *E. supina* in America is questionable because fossil specimens were found in Upper Pliocene and Early Pleistocene deposits of Idaho (western North America) (Herrington 1962). The discovery of live individuals since approximately 1959 in Lake Ontario (Herrington 1962) suggests a possible recent introduction in the Great Lakes region.

The modalities of the introduction of *Euglesa compressa* in France are unknown and the meagre interest of freshwater biologists in these small bivalves provides few clues as to its origin. However, its presence in the lower Seine suggests an introduction via the discharge of ballast water by transoceanic commercial ships as the most likely mode of introduction. Indeed, shipping activities are considered as the principal vector in the introduction of numerous species (about 100) observed in the region of the Great Lakes, probably from Eurasia (which acts as a potential donor region), during the 20th century (Mills et al. 1993; Mackie 1999; Grigorovich et al. 2000, 2003; Ricciardi 2001; Holeck et al. 2004; Duggan et al. 2005; Drake and Lodge 2007). Thus it is reasonable to assume that this vector is also responsible for the migration of American species towards Europe even if the phenomenon is apparently less marked in this direction with regard to bivalve mollusks. The connection of major catchment basins through canals has very probably favored its spread within France (Bij de Vaate et al. 2002).

Euglesa compressa has spread rapidly and has quickly become dominant in the colonized habitats (i.e. fine sediments), and corresponds to the criteria characterizing an invasive species according to the framework proposed by Colautti and MacIsaac (2004). Furthermore this bivalve appears to have a negative impact on the populations of *E. supina* which have sometimes disappeared only two years after the arrival of *E. compressa* (Mouthon and Taïr-Abbaci 2012). This turnover was observed in the Saone and Doubs rivers (catchment basin of the Rhone) which were subjected to long term monitoring (Mouthon and Daufresne 2015), and also in the Loire river. However in the Great Lakes region

and the rivers of Quebec, where the two species are present, it is likely that they cohabit. More research is required to identify the cause of the disappearance of *E. supina* populations observed in several rivers of France after their colonization by *E. compressa*.

Acknowledgements

The authors thank C. Douady, T. Lefebvre, L. Konecny-Dupré (CNRS, UMR 5023 - LEHNA) and A. Chaumot (Irrstea-Lyon) for their advices about molecular analysis, P. Clevenot and J. Loiseau (Hydrosphère) for the Seine river material, G. Le Goff for the photographs of *E. compressa* and K. Hodson for improving the language. We are also grateful to the two anonymous reviewers for their helpful comments.

References

- Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1159–1174, <https://doi.org/10.1139/f02-098>
- Bij de Vaate A, Klink AG, Greijdanus-Klaas M, Jans LH, Oosterbaan J, Kok F (2007a) Effects of habitat restoration on the macroinvertebrate fauna in a foreland along the river Waal, the main distributary in the Rhine delta. *River Research and Applications* 23: 171–183, <https://doi.org/10.1002/rra.972>
- Bij de Vaate A, Klink AG, Paalrast P (2007b) Macrozoobenthos in the Lower Seine: a survey from the perspective of the European Water Framework Directive. *Ecoconsult report*: 200703, 138 pp
- Burch JB (1975) Freshwater sphaeriacean clams (Mollusca: Pelecypoda) of North America. Malacological Publications, Hamburg, Michigan, 96 pp
- Cheng HL, Xia DQ, Wu TT, Meng XP, Ji HJ, Dong ZG (2006) Study on sequences of ribosomal DNA internal transcribed spacers of clams belonging to the Veneridae family (Mollusca: Bivalvia). *Acta Genetica Sinica* 33: 702–710, [https://doi.org/10.1016/S0379-4172\(06\)60102-9](https://doi.org/10.1016/S0379-4172(06)60102-9)
- Clarke AH (1981) The freshwater molluscs of Canada. National Museum of Natural Sciences, Ottawa, Canada, 446 pp
- Colautti RI, MacIsaac HJ (2004) A neutral terminology to define ‘invasive’ species. *Diversity and Distributions* 10: 135–141, <https://doi.org/10.1111/j.1366-9516.2004.00061.x>
- Czaja A, Palacios-Fest MR, Estrada-Rodríguez JL, Méndez UR, Alba Ávila JA (2014) Inland dunes fauna and flora from Paleo lake Irritila in the Comarca Lagunera, Coahuila, northern Mexico. *Boletín de la Sociedad Geológica Mexicana* 66(3): 541–551
- De Lange HJ, De Jonge J, Den Besten PJ, Oosterbaan J, Peeters ETH (2004) Sediment pollution and predation affect structure and production of benthic macroinvertebrate communities in the Rhine-Meuse delta, The Netherlands. *Journal of the North American Benthological Society* 23: 557–579, [https://doi.org/10.1899/0887-3593\(2004\)023<0557:SPAPAS>2.0.CO;2](https://doi.org/10.1899/0887-3593(2004)023<0557:SPAPAS>2.0.CO;2)
- Douzery EJP, Blanquart S, Criscuolo A, Delsuc F, Douady C, Lartillot N, Philippe H, Ranwez V (2010) Phylogénie moléculaire. In: F. Thomas, T. Lefèvre, M. Raymond (eds) “Biologie Evolutive”: collection LMD. De Boeck, Bruxelles, pp 183–243
- Drake JM, Lodge DM (2007) Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. *Aquatic Invasions* 2: 121–131, <https://doi.org/10.3391/ai.2007.2.2.7>
- Duggan IC, van Overdijk CDA, Bailey SA, Jenkins PT, Limén H, MacIsaac HJ (2005) Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2463–2474, <https://doi.org/10.1139/f05-160>
- Falkner G, Ripken TEJ, Falkner M (2002) Mollusques continentaux de la France: liste de référence annotée et bibliographie. *Patrimoines naturels* 52: 1–350
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791, <https://doi.org/10.2307/2408678>
- Frank C (2010) Sphaeriidae (Mollusca: Bivalvia) aus den westlichen USA. *Mitteilungen der zoologischen Gesellschaft Braunau* 10(1): 27–56
- Freire R, Arias A, Mendez J, Insua A (2010) Sequence variation of the internal transcribed spacer (ITS) region of ribosomal DNA in *Cerastoderma* species (Bivalvia: Cardiidae). *Journal of Molluscan Studies* 76: 77–86, <https://doi.org/10.1093/mollus/eyp047>
- Gargominy O, Prié V, Bichain JM, Cucherat X, Fontaine B (2011) liste de référence annotée des mollusques continentaux de France. *MalaCo* 7: 307–382
- Gillespie DM (1969) Population studies of four species of molluscs in the Madison River, Yellowstone National Park. *Limnology and Oceanography* 14: 101–114, <https://doi.org/10.4319/lo.1969.14.1.0101>
- Gittenberger E, Janssen AW (1998) De Nederlandse zoetwater mollusken. Recente en fossiele weekdieren uit zoet en brak water [Dutch freshwater mollusks. Recent and fossil mollusks of fresh and brackish waters of The Netherlands], Leiden, 288 pp
- Grigorovich AI, Korniushin AV, MacIsaac HJ (2000) Moitessier’s pea clam *Pisidium moitessierianum* (Bivalvia, Sphaeriidae): a cryptogenic mollusc in the Great Lakes. *Hydrobiologia* 435: 153–165, <https://doi.org/10.1023/A:1004066609445>
- Grigorovich IA, Colautti RI, Mills ED, Holec K, Ballert AG, MacIsaac HJ (2003) Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 740–756, <https://doi.org/10.1139/f03-053>
- Heard WH (1965) Comparative life histories of North American pill clams (Sphaeriidae: *Pisidium*). *Malacologia* 2: 381–411
- Herrington HB (1962) A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). *Miscellaneous Publications Museum of Zoology, University of Michigan* 118: 1–74
- Hillis DM, Bull JJ (1993) An Empirical-Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. *Systematic Biology* 42: 182–192, <https://doi.org/10.1093/sysbio/42.2.182>
- Holec K, Mills EL, MacIsaac HJ, Dochoda M, Colautti RI, Ricciardi A (2004) Bridging troubled waters: understanding links between biological invasions, transoceanic shipping, and other entry vectors in the Laurentian Great Lakes. *BioScience* 10: 919–929, [https://doi.org/10.1641/0006-3568\(2004\)054\[0919:BTW\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0919:BTW]2.0.CO;2)
- Kerney MP (1976) Atlas of the non-marine Mollusca of the British Isles. London, 208 pp
- Kuiper JGJ (1981) Aten en zijn Pisidiiums [The eaten Pisidium]. *Correspondentieblad van de Nederlandse Malacologische Vereniging* 198: 1118–1121
- Lee T, Ó Foighil D (2003) Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve molluscs, inferred from nuclear (ITS-1) and mitochondrial (16S) ribosomal gene sequences. *Zoological Journal of the Linnean Society* 137: 245–260, <https://doi.org/10.1046/j.1096-3642.2003.00047.x>
- Lopez GR, Holopainen IJ (1987) Interstitial suspension-feeding by *Pisidium* spp. (Pisidiidae: Bivalvia): a new guild in the lentic benthos? *American Malacological Bulletin* 5: 21–30
- Löytynoja A, Goldman N (2005) An algorithm for progressive multiple alignment of sequences with insertions. *Proceedings of the National Academy of Sciences USA* 102: 10557–10562, <https://doi.org/10.1073/pnas.0409137102>
- Mackie GL (1978) Are sphaeriid clams ovoviviparous or viviparous? *The Nautilus* 92: 145–147

- Mackie GL (1999) Ballast water introductions of Mollusca. In: Claudi R, Leach JH (eds), Nonindigenous freshwater organisms: vectors, biology and impacts. CRC Press LLC, Boca Raton, Florida, pp 219–254
- Mackie GL (2007) Biology of freshwater Corbiculid and Sphaeriid clams of North America. *Ohio biological Survey, new series* 15(3): 1–436
- Mackie GL, White DS, Zdeba TW (1980) A guide to freshwater mollusks of the Laurentian Great Lakes with special emphasis on the genus *Pisidium*. U.S. Environmental Protection Agency, Duluth, Minnesota, 144 pp
- Mackie GL, Flippance LA (1983) Intra- and interspecific variations in calcium content in freshwater mollusca in relation to calcium content of the water. *Journal of Molluscan Studies* 49: 204–212
- Meier-Brook C (1977) Intramarisupial suppression of fetal development in Sphaeriid clams. *Malacological Review* 10: 53–58
- Mills EL, Leach JH, Carlton JT, Secor CL (1993) Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. *Journal of the Great Lakes Research* 19: 1–54, [https://doi.org/10.1016/S0380-1330\(93\)71197-1](https://doi.org/10.1016/S0380-1330(93)71197-1)
- Mouthon J, Loiseau J (2000) *Musculium transversum* (Say, 1829): a species new to the fauna of France (Bivalvia, Sphaeriidae). *Basteria* 64: 71–77
- Mouthon J, Tair-Abbaci K (2012) The taxonomic confusion surrounding *Pisidium* (Bivalvia, Sphaeriidae): the possible birth of a new taxon. *Basteria* 76(4–6): 126–130
- Mouthon J, Daufresne M (2015) Resilience of mollusc communities of the river Saone (eastern France) and its two main tributaries after the 2003 heatwave. *Freshwater Biology* 60: 2571–2583, <https://doi.org/10.1111/fwb.12540>
- Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290, <https://doi.org/10.1093/bioinformatics/btg412>
- Prié V, Bichain JM (2009) Phylogenetic relationships and description of a new stygobite species of *Bythinella* (Mollusca, Gastropoda, Caenogastropoda, Amnicolidae) from southern France. *Zoosystema* 31: 987–1000, <https://doi.org/10.5252/z2009n4a12>
- R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>
- Ricciardi A (2001) Facilitative interactions among aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2513–2525, <https://doi.org/10.1139/f01-178>
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* 74: 5463–5467, <https://doi.org/10.1073/pnas.74.12.5463>
- Schultheiß R, Albrecht C, Bößneck U, Wilke T (2008) The neglected side of speciation in ancient lakes: phylogeography of an inconspicuous mollusc taxon in lakes Ohrid and Prespa. *Hydrobiologia* 615: 141–156, <https://doi.org/10.1007/s10750-008-9553-3>
- Sterki V (1905) New varieties of North American *Pisidia*. *The Nautilus* 19(7): 80–84
- Sterki V (1916) A preliminary catalogue of the North American Sphaeriidae. *Annals of the Carnegie Museum* 10 (3–4): 429–477
- Stunžėnas V, Petkevičiūtė R, Stanevičiūtė G (2011) Phylogeny of *Sphaerium solidum* (Bivalvia) based on karyotype and sequences of 16S and ITS1 rDNA. *Central European Journal of Biology* 6: 105–117, <https://doi.org/10.2478/s11535-010-0101-6>
- Thomas GJ (1959) Self-fertilization and production of young in a sphaeriid clam. *The Nautilus* 72: 131–140
- Van Haaren T (2015) *Pisidium casertanum* forma *plicatum*, een afwijkende erwtenmossel [*Pisidium casertanum* forma *plicatum*, a different pea mussel] *Zoekbeeld* 5(2): 15–17
- Wallbrink H (1995) Een opmerkelijke vondst in de Nieuwe Merwede: *Pisidium casertanum plicatum* Zeissler, 1962 [A remarkable discovery in the new Merwede: *Pisidium casertanum plicatum* Zeissler, 1962]. *Correspondentieblad van de Nederlandse Malacologische Vereniging* 284: 61–65
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10(4): 506–513
- Way CM, Wissing TE (1982) Environmental heterogeneity and life history variability in the freshwater clams, *Pisidium variable* (Prime) and *Pisidium compressum* (Prime) (Bivalvia: Pisidiidae). *Canadian Journal of Zoology* 60: 2841–2851, <https://doi.org/10.1139/z82-363>
- White LR, McPherson BA, Stauffer JR (1996) Molecular genetic identification tools for the unionids of French Creek, Pennsylvania. *Malacologia* 38: 181–202
- Zeissler H (1962) Die Mollusken aus der zentralen Torfschicht des Köpenicker Burggrabens. In: Hermann J, “Köpenick”, *Ergebnisse der Archäologischen Stadtkernforschung in Berlin* 12(4): 103–106
- Zeissler H (1971) Bestimmungstabelle für die Mitteleuropäischen Sphaeriaceae. *Limnologica* 8(2): 453–503

Supplementary material

The following supplementary material is available for this article:

Appendix 1. Sequences alignment of 18S ribosomal RNA gene (partial sequences), internal transcribed spacer 1 (complete sequences) and 5.8S ribosomal RNA gene (partial sequences) for *Euglesa* sp. individuals and the registered *E. compressa*.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2017/Supplements/BIR_2017_Mouthon_Forcellini_Appendix_1.pdf