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 ovo-viviparous. 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 Taïr-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; Schulttheiß et al. 2008; Stunženas 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,
95% ethanol and conserved at 1°C. All specimens were immediately transferred into −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 H2O, 3 µL of Standard Buffer 10X (Biolabs B9014S), 0.9 µL of MgCl2 at 50mM, 0.6 µL of each primer at 10 µM, 0.26 µL of Taq polymerase at 5 U/µL, 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°C/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

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

| City                | River | Code station | Taxon                                      | ITS-1 | Collector | Geographic coordinates |
|---------------------|-------|--------------|--------------------------------------------|-------|-----------|------------------------|
| Saumières           | Doubs | DOSA         | Euglesa sp.                                | 2     | J. Mouton | 46°54′09.4″N; 5°05′01.4″E |
| Creys-Malville      | Rhone | RHCR         | Euglesa casertana f. ponderosa Stelfox, 1918| 2     | J. Mouton | 45°46′21.1″N; 5°28′01.9″E |
| Odneripisidium tenuilineatum Stelfox, 1918 | 3     | J. Mouton |                                             |       |           |                        |
| Heulliley-sur-Saône | Saone | SAHE         | Euglesa sp.                                | 1     | J. Mouton | 47°19′31.6″N; 5°27′25.1″E |
| Tillyenay           | Saone | SATIL        | Euglesa sp.                                | 2     | J. Mouton | 47°10′45.6″N; 5°21′16.9″E |
| Trévoux             | Saone | SATR         | Euglesa casertana f. ponderosa Stelfox, 1918| 2     | J. Mouton | 45°56′01.5″N; 4°45′54.4″E |
| Vuillecin           | Drugeon | DRVU       | Euglesa pulchella Jenyns, 1832              | 4     | J. Mouton | 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) |
|--------------------------------------|-------------------|-----------------|-------------------------|
| Euglesa (Cyclocalyx) adamsi Stimpson, 1851 | Michigan, USA     | Lee et al. 2003 | AY093513                |
| Euglesa (Cyclocalyx) compressa Prime, 1852 | Michigan, USA     | Lee et al. 2003 | AY093518                |
| Euglesa (Cyclocalyx) fallax Sterki, 1896 | Michigan, USA     | Lee et al. 2003 | AY093519                |
| Euglesa (Cyclocalyx) halliae Kuiper, 1983 | Sydney, Australia | Lee et al. 2003 | AY093520                |
| Euglesa (Cyclocalyx) henslowana (Sheppard, 1825) | NA               | Steiner 2005 (unpublished) | DQ062603                |
| Euglesa (Cyclocalyx) hibernica Westerland, 1894 | HeiligesMeer, Germany | Lee et al. 2003 | AY093522                |
| Euglesa (Cyclocalyx) lilljeborgii Clessin, 1886 | Kurilislands, Russia | Lee et al. 2003 | AY093521                |
| Euglesa (Cyclocalyx) milium Held, 1836 | HeiligesMeer, Germany | Lee et al. 2003 | AY093523                |
| Euglesa (Cyclocalyx) nipponense Kuroda, 1930 | GunmaPrefecture, Japan | Lee et al. 2003 | AY093525                |
| Euglesa (Cyclocalyx) nitida Jenyns, 1832 | HeiligesMeer, Germany | Lee et al. 2003 | AY093526                |
| Euglesa (Cyclocalyx) personata Malms, 1855 | Ammerbuch, Germany | Lee et al. 2003 | AY093527                |
| Euglesa (Cyclocalyx) subtruncata Malms, 1855 | HeiligesMeer, Germany | Lee et al. 2003 | AY093528                |
| Euglesa (Cyclocalyx) supina Schmidt, 1850 | HopstenerAch., Germany | Lee et al. 2003 | AY093529                |
| Euglesa (Cyclocalyx) variabile Prime, 1852 | Michigan, USA     | Lee et al. 2003 | AY093530                |
| Odneripisidium japonicum Pilshy & Hirase, 1908 | Nagano Prefecture, Japan | Lee et al. 2003 | AY093532                |
| Odneripisidium moitessierianum Paladihe, 1866 | NA               | Steiner 2005 (unpublished) | DQ062589                |
| Odneripisidium purvum Mort, 1938 | Ehime Prefecture, Japan | Lee et al. 2003 | AY093531                |
| Pisidium amnicum (O.F. Müller, 1774) | NA               | Steiner 2005 (unpublished) | DQ062574                |
| Pisidium dubium (Say, 1817) | Michigan, USA     | Lee et al. 2003 | AY093533                |

Doubts and Rhone rivers. In addition 7 individuals of Odneripisidium 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.
Genetic evidence of the presence in France of *Euglesa compressa*

**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.

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 (=Cylcalyx)* 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.

|              | *E. compressa* Ay093518 | *Euglesa* sp. SATIL1 | *Euglesa* sp. SATIL2 | *Euglesa* sp. SAHE1 | *Euglesa* sp. DOSA1 | *Euglesa* sp. DOSA2 |
|--------------|-------------------------|----------------------|----------------------|---------------------|---------------------|---------------------|
| *E. compressa* Ay093518 | 0                       | —                    | —                    | —                   | —                   | —                   |
| *Euglesa* sp. SATIL1 | 0.004                   | 0.004                | —                    | —                   | —                   | —                   |
| *Euglesa* sp. SATIL2 | 0                      | 0                    | 0.004                | 0.004              | 0                   | —                   |
| *Euglesa* sp. SAHE1 | 0                      | 0                    | 0                    | 0                   | 0                   | —                   |
| *Euglesa* sp. DOSA1 | 0                      | 0                    | 0.004                | 0                   | 0                   | —                   |
| *Euglesa* sp. DOSA2 | 0                      | 0                    | 0.004                | 0                   | 0                   | —                   |

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 pisidiid 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 pisidiid clams are in good agreement with the phylogenetic structure of Sphaeriniinae proposed by Lee and ÓFoighil (2003). Our study included six supplementary species which were not taken into account in Lee and ÓFoighil’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 pisidiid 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* Zeeslser, 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) (Zeeslser 1962, 1971). Following the extension of its geographical area 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 Maclsaac (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 Saône 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
Acknowledgements

The authors thank C. Donaudy, T. Lefebure, L. Konecny-Dupré (CNRS, UMR 5023 - LEHNA) and A. Chaumont (Irstea-Lyon) for their advices about molecular analysis, P. Clevenot and J. Loiseau (Hydrosphere) for the Seine river material, G. Le Goff for the photographs of E. compressa and K. Hudson 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, De Lange HJ, De Jonge J, Den Besten PJ, Oosterbaan J, Peeters ETH (2007a) Effects of habitat restoration on the productivity of benthic macroinvertebrate communities 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. Ecosurveys 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: Pelecypoda). Acta Genetica Sinica 33: 702–710, https://doi.org/10.1016/S0379-4172(06)60102-9

Clarke AH (1981) The freshwater mollusks 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-Rodriguez JL, Méndez UR, Alba Ávila JA (2014) Inland dunes fauna and flora from Paloe lake Irtilita 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/10.1899/0087-3592(2004)023<0557:SPAPAS>2.0.CO;2

Dousery EP, Blankart S, Criscuolo A, Delsec F, Doudou C, Lartilot N, Philippe H, Ranwez V (2010) Phylogénie moléculaire. In: F. Thomas, T. Leffèvre, 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 Oudvijk CDA, Bailey SA, Jenkins PT, Limén H, Maatsaak 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 zoologische Gesellschaft Braunschweig 10(1): 27–56

Freire R, Arias J, Mendez J, Insua A (2010) Sequence variation of the internal transcribed spacer (ITS) region of ribosomal DNA of Ceraestoderma species (Bivalvia: Cardiidae). Journal of Molluscan Studies 76: 77–86, https://doi.org/10.1093/mollus/epy047

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.1969.14.1.1010

Gittenberger E, Janssen AW (1998) De Nederlandse zoetwater mollusken. Recent 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

Grigorov IA, Kornishun AV, Maatsaak HJ (2000) Mestessier’s pea clam Pisidium moitessierianum (Bivalvia, Sphaeriidae): a cryptogenic mollusc in the Great Lakes. Hydrobiologia 435: 153–165, https://doi.org/10.1023/A:1004066904445

Grigorov IA, Colautti RI, Mills ED, Holeck K, Ballert AG, Maatsaak 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

Hedrick WB (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

Holeck K, Mills EL, Maatsaak 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/10.1641/0006-3508(2004)054[0919:BTW]2.0.CO;2

Kenney MP (1976) Atlas of the non-marine Mollusca of the British Isles. London, 208 pp

Kuiper GJ (1981) Atten en zijn Pisidiums [The eaten Pisidium]. Correspondentieblad van de Nederlandse Malacologische Vereniging 198: 1118–1121

Lee T, Ø 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: 245–269, https://doi.org/10.1046/j.1096-3642.2003.00047.x

Lopez GR, Holopainen JI (1987) Intersitial 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.0409371102

Mackie GL (1978) Are sphaerid clam ooviviparous or viviparous? The Nautilus 92: 145–147

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.
Genetic evidence of the presence in France of *Euglesa compressa*

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 mussels 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 mussels in relation to calcium content of the water. *Journal of Molluscan Studies* 49: 204–212

Meier-Brook C (1977) Intramarsupial 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/10.1016/0380-1330(93)71197-1

Mouthon J, Taïr-Abbaci K (2012) The taxonomic confusion surrounding *Pisidium* (Bivalvia, Sphaeriidae): the possible birth of a new taxon. *Bacteria* 7(4–6): 126–130

Mouthon J, Loiseau J (2000) *Musculium transversum* (Say, 1829): a species new to the fauna of France (Bivalvia, Sphaeriidae). *Bacteria* 64: 71–77

Mouthon J, Tair-Abbaci K (2012) The taxonomic confusion surrounding *Pisidium* (Bivalvia, Sphaeriidae): the possible birth of a new taxon. *Bacteria* (7)(4–6): 126–130

Mouthon J, Daufresne M (2015) Resilience of mollusc communities 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

Piri V, Bichain JM (2009) Phylogenetic relationships and description of a new stygobite species of *Bythiella* (Mollusca, Gastropoda, Caenogastropoda, Annicolliidae) from southern France. *Zookeys* 31: 987–1000, https://doi.org/10.3897/zookeys.31.675

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 “invasion 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, Bolthneck U, Wilke T (2008) The neglected side of speciation in ancient lakes: phyleogeography of an inconspicuous mussel taxon in lakes Ohrid and Prespa. *Hydrobiology* 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

Stunzenas V, Petkevičaitė R, Stankevičiūtė 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/crjeb-ej-2010-0018

Thomas GJ (1959) Self-fertilization and production of young in a sphaerid 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, Haguchi 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 variabile* (Prime) and *Pisidium compressum* (Prime) (Bivalvia: Sphaeriidae). *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) Bestimmungstabellen für die Mitteleuropäischen Sphaeriaceae. *Linzologica* 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