BioInvasions Records (2017) Volume 6, Issue 3: 225–231
DOI: https://doi.org/10.3391/bir.2017.6.3.07

Open Access

Research Article

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

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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; 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	*Euglesa* sp.	2	J. Mouthon	46°54′09.4″N; 5°05′01.4″E
Creys-Malville	Rhone	RHCR	*Euglesa casertana* f. *ponderosa* Stelfox, 1918	2	J. Mouthon	45°46′21.1″N; 5°28′01.9″E
			Odhneripisidium tenuilineatum Stelfox, 1918	3	J. Mouthon	
Heuilley-sur-Saône	Saone	SAHE	*Euglesa* sp.	1	J. Mouthon	47°19′31.6″N; 5°27′25.1″E
Tillenay	Saone	SATIL	*Euglesa* sp.	2	J. Mouthon	47°10′45.6″N; 5°21′16.9″E
Trévoux	Saone	SATR	*Euglesa casertana* f. *ponderosa* Stelfox, 1918	2	J. Mouthon	45°56′01.5″N; 4°45′45.4″E
Vuillecin	Drugeon	DRVU	*Euglesa pulchella* 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
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) hallae Kuiper, 1983	Sydney, Australia	Lee et al. 2003	AY093520
Euglesa (Cyclocalyx) henslowana (Sheppard, 1825)	NA	Steiner 2005 (unpublished)	DQ062603
Euglesa (Cyclocalyx) hibernica Westerlund, 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 Malm, 1855	Ammerbuch, Germany	Lee et al. 2003	AY093527
Euglesa (Cyclocalyx) subtruncata Malm, 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
Odhneripisidium japonicum Pilsbry & Hirase, 1908	Nagano Prefecture, Japan	Lee et al. 2003	AY093532
Odhneripisidium moitessierianum Paladihe, 1866	NA	Steiner 2005 (unpublished)	DQ062589
Odhneripisidium parvum Mori, 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

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 °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

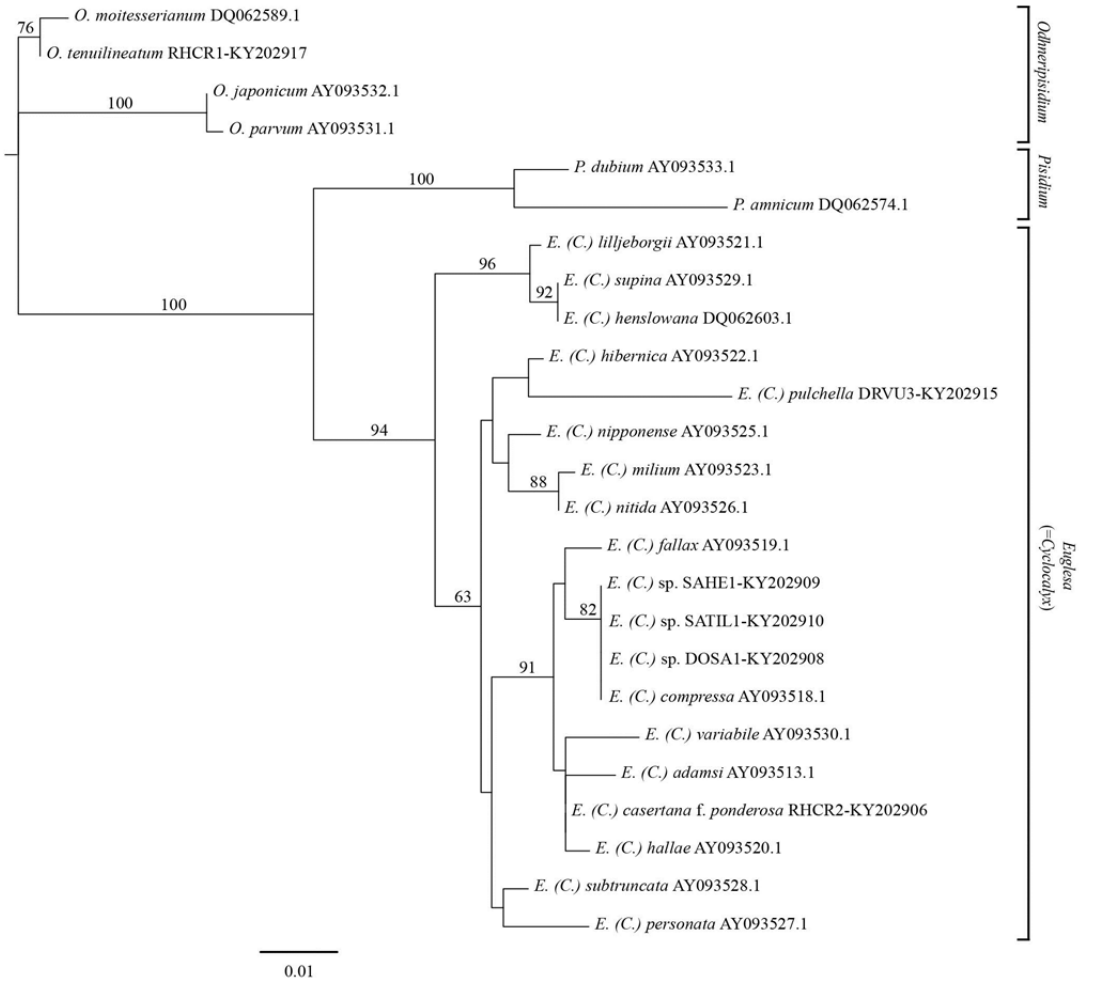

Figure 1. Maximum likelihood phylogenetic tree run with 100 bootstraps based on 593 base pairs of the ITS-1 molecular marker. Only bootstrap values up to 50% that represent moderate to high support of nodes are shown. GenBank accession numbers of sequences of *O. tenuilineatum, E. pulchella, E.* sp. 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* (= *Cyclocalyx*) 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	—					
Euglesa sp. SATIL1	0	—				
Euglesa sp. SATIL2	0.004	0.004	—			
Euglesa sp. SAHE1	0	0	0.004	—		
Euglesa sp. DOSA1	0	0	0.004	0	—	
Euglesa 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 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 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 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 $CaCO_3$, 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* Paladhilhe, 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. Lefebure, L. Konecny-Dupré (CNRS, UMR 5023 - LEHNA) and A. Chaumot (Irstea-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.

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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*.

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