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The taxonomic confusion surrounding *Pisidium* (Bivalvia, Sphaeriidae): the possible birth of a new taxon

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The presence of *Pisidium* shells with an appendiculum, presenting specific morphological characteristics not yet described has been observed for several years. Although their external shell traits appear similar the ligament-pit of these individuals differs: it is either long and narrow as in *P. supinum*, or shorter and thicker as in that of the *ponderosa* form of *P. casertanum* and would therefore belong to one or the other of these two species. They could also be the result of hybridisation and constitute a new taxon.

Key words: Bivalvia, Sphaeriidae, morphology, hybridisation, taxonomy, distribution.

INTRODUCTION

Pisidium species are small bivalves (usually <10mm) that belong to the family Sphaeriidae. They inhabit fine deposits in a variety of freshwater ecosystems. The shells of some of the species of these bivalves present considerable variation, most often linked to the hydrological and physico-chemical characteristics of the environment in which they live (Saunders & Kling, 1990; Funk & Reckendorfer, 2008). This phenomenon is particularly remarkable in *Pisidium casertanum* (Poli, 1791), the most common and most cosmopolitan of the genre, and raising one of these alleged forms or varieties to species rank often gives rise to debate (Dyduch-Falniowska, 1983; Kuiper, 1983; Piechocki, 1989; Araujo & Korniushin, 1998; Glöer & Zettler, 2005). On the contrary, polymorphism is quite limited in other species such as *Pisidium henslowanum* (Sheppard, 1823), *P. supinum* (A. Schmidt, 1851) or *P. milium* Held, 1936. For several years shells with an appendiculum (a more or less oblique fold located close to the top of each valve) and resembling *P. supinum* or forma *plicata* Zeissler, 1962, of *P. casertanum*, but presenting specific characters have been observed in rivers and canals. The aim of this article is to describe the particularities of these individuals that have not yet been mentioned.

Methods

The shells were opened and emptied of their soft contents prior to examination by microscope,. The valves were then immersed in a bath of 5% commercial Chlorox (sodium hypochlorite), in order to dissolve the remaining organic matter, washed in a solution of distilled water and dried at room temperature before being coated with platinum (5 μ m thick, 3 μ m/s) in a Balt-Tec Med 020. The observations were performed using a Quanta 250 Scanning Electron Microscope (CT μ , UCBLyon 1) at a voltage of 15 kv.



Fig. 1. Exterior of right valves. A, Pisidium spec.; B, Pisidium supinum; C, P. casertanum forma ponderosa.

Results

Description.— The shell of these individuals (called *Pisidium* spec. in the figure captions) has a subtriangular shape in the adult, ornamented with very fine striations, giving it a shiny aspect, a thick appendiculum at the top of each valve and numerous pores covering the internal surface. The hinge plate is arched but hardly or not at all thickened (Figs 1A, 2A, B, E, F, 3G, H). These bivalves also have a relatively small outer demibranch (see Korniushin & Hackenberg, 2000). Although, externally, all the age classes resemble each other, the shape of their ligament-pit is different: it is either long and narrow, as in *P. supinum*, or shorter and wider, comparable to that of *P. casertanum* forma *ponderosa*. Therefore, according to this criterion and the shape of the shell, they belong to one or the other of these two species (see e.g. Ellis, 1978).

However, the individuals with a long narrow ligamentpit analogous to that of *P. supinum* (Fig. 2A to D) differ from the latter by: (1) the absence of marked and regular striations (Fig. 1A, B), (2) a longer, wider and more projecting appendiculum (Fig. 3A to D), (3) the presence of numerous pores, absent (not visible under a binocular microscope) or rare in *P. supinum*, (4) the different morphology of the shells of neonates (Fig. 3E, F), (5) a relatively small outer demibranch whose outer edge corresponds to the 15th filament of the inner demibranch versus the 7-8th filament for the large outer demibranch of *P. supinum*.

Other particularities specific to these individuals have also been observed as has the appearance of the appendiculum manifested in the embryo by a protrusion of the shell edge that occurs for a shell length longer than in *P. supinum*: 0.6 mm vs 0.5 mm. Furthermore, the edges of the canaliculus corresponding on the inner face of each valve to the appendiculum on the outer face extend for a much greater length before joining together: 2.8 vs 1.9 mm (Fig. 3G). In most species of *Pisidium* the maximum size of the larvae before their expulsion is close to 1 mm (Holopainen & Hanski, 1986). In the Upper Rhone that of the larvae of *P. supinum* varies from 0.92 to 1.08 mm while the maximum shell length of adults ranges from 3.52 to 4.40 mm (Mouthon, 2011). On the contrary, in the Saone the size of the older larvae of these individuals frequently reaches 1.1 or 1.2 mm; the largest found measured 1.34 mm and the maximum shell length of the adults reached 4.20 mm

The aspect of individuals with a shorter and wider ligament-pit recalls the *plicata* shape of *P. casertanum* discovered in Brandeburg (northern Germany) (Zeissler, 1962; 1971) and more recently in the Rhine-Meuse delta in the Netherlands (Wallbrink, 1995). However, according to Kuiper



Fig. 2. Hinge of right and left values of *Pisidium* spec. with a long and narrow ligament-pit (**A**, **B**) like *Pisidium supinum* (**C**, **D**), or a short and broad ligament-pit (**E**, **F**) like *P. casertanum* f. *ponderosa* (**G**, **H**).

(pers. com.) these individuals differ from this morphotype. Moreover, they can be distinguished from the *ponderosa* form by: (1) the presence of an appendiculum close to the umbo, (2) less marked striations (Fig. 1A, C), (3) a narrower hingeplate (Fig. 2E, F, G, H), iv) the presence of numerous pores distributed on the inner face of the valves absents or rare in *ponderosa*. On the contrary, the outer demibranch is relatively small, as in *P. casertanum* (Korniushin, 1998).



Fig. 3. Umbonal appendiculum and shell of juveniles of *Pisidium supinum* (A, C, E) and of *Pisidium* spec. (B, D, F). G, interior of right valve of a young *Pisidium* spec. showing pores and the canaliculus (G). H, detail of internal pore of *Pisidium* spec.



Fig. 4. Localities of Pisidium spec. in eastern France.

Distribution. - Examination of the specimens of Pisidium sampled since 1977 and in my collection revealed that individuals whose external morphological characteristics correspond to the previous description have been present in the lower reaches of the Saone since 1989. They also feature in the surveys performed in 1998 and 1999 in different canals and rivers in eastern France: the canals from the Marne to the Rhine and from the Aisne to the Marne, the Meurthe, the Moselle, the Ill and the Rhone downstream of Lyon (Fig. 4). However, as Sphaeriidae and particularly Pisidium have rarely been identified at the species level, it is probable that they have also been present in other French and European watersheds. These individuals have now totally replaced P. supinum in the larger part of the river Saone. At Pontailler/S. this turnover only required two years. In the upstream part of this river at Port/S. and in the lower reaches of the river Doubs at Saunières, where they were not present until 2011, their density is already two or three times higher than that of P. supinum (for a list of species sampled in the Saone river, see Mouthon & Daufresne, 2006).

DISCUSSION

An alternative to considering that two new morphotypes have appeared recently, one a morphotype of P. supinum and the other of P. casertanum, and having similar external characteristics, differing only in the shape of their ligament pit, is to consider the emergence of a new taxon. In fact, although the conchological and anatomical differences between individuals with a rather short and wide ligament-pit and the *plicata* form of *P. casertanum* remain to be determined, those that separate them from the ponderosa form on the one hand and from the *P. supinum* individuals with a long and rather narrow ligament-pit on the other hand, are considerable and constant in the populations studied. Futhermore, we observed the presence of these individuals, which are not included in the most recent inventory of Sphaeriidae in France (Mouthon & Kuiper, 1987), throughout the eastern part of the country. The observations carried out suggest that it could result from hybridisation, a still largely underestimated speciation mechanism (Mallet, 2007), in this case between *P. supinum* and the ponderosa form of P. casertanum. The situation should be clarified by a DNA analysis of these individuals.

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