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First record and biology of *Unio gibbus* Spengler, 1793 in Tunisia

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Abstract: In the framework of a recent review of the systematics and global diversity of the freshwater mussel species in Tunisia, the species *Unio gibbus* Spengler, 1793 has been recorded for the first time. This overlooked *Unio* species inhabits several rivers in North of Tunisia, Northwest Morocco and Southwest Spain. Shell characters were examined in adult and juvenile specimens from several North Tunisian rivers. The glochidia is conic, to elongate triangular, intermediate between glochidia shapes in *Unio* and *Anodonta*. The hook is triangular, with large basis. The entire internal cavity of the external demibranchs (homogeneity) acted as a marsupium (ectobranchy). The favourable water temperature at the end of fall in Tunisia involves the apparition of a high number of gravid female in winter, which minimizes the fertilization of the eggs and the larvae release. Moreover, the drying of an important part of the mussel's habitats in summer involves a mortality of a high number of specimens each year and threatens severely the populations. May and June constituted the most favourable period for reproduction.

Key words: *Unio*; hooked glochidia; life cycle; Tunisia; Northwest Africa

Introduction

There has been a need to modernise the list of non-marine Mollusca in Tunisia. Many taxonomic and nomenclature changes have appeared in the recent literature in several countries, especially in the Palearctic region (Falkner et al. 2001, 2002; Araujo et al. 2005, 2009 and references therein). In particular, a new European List, the Clecom List (Falkner et al. 2001) has now appeared and it seems appropriate to examine in more detail the constituent changes which might affect the Tunisian and northwestern Africa faunas. This has given additional urgency by the inception of a new census of the molluscs of Tunisia.

Issel (1880), Letourneux & Bourguignat (1887) and Pallary (1923) listed five species of freshwater mussels in Tunisia: *Unio delevieleusae* Hagenmüller (Letourneux et Bourguignat, 1887), *Unio doumeti* Bourguignat, 1864, *Unio durieui* Deshayes, 1847, *Unio rouirei* Letourneux et Bourguignat, 1887 and *Unio zenaticus* Letourneux et Bourguignat, 1887. Haas (1969) consided *U. rouirei* and *U. zenaticus* a synonymy of *Potomida littoralis fellmanni* (Deshayes, 1847). Two other species *Unio medjerdae* Kobelt, 1886 and *U. micelii* Kobelt, 1886 were considered also by Haas (1969) a synonymy of the only one name *Unio elongatulus durieui* Deshayes, 1847. Lately, this last name was adopted by Mandahl-Barth (1988), Van Damme (1984) and Daget (1998) for all the North African forms of the genus *Unio*. Boumaïza (1994) used the name *Unio doumeti* while indicating that it is only a very polymorphous species. Khalloufi (1998) mentioned two forms under the specific name *Unio doumeti* considered as two varieties ("minor" and major") (Figs 2 and 3, p. 116) coming from the Oued El Kébir situated in the NW Tunisia. More recently, Khalloufi & Boumaïza (2005) mentioned the species *Anodonta cygnea* (L., 1758) in the North-Center of Tunisia. Thus, the taxonomy of this polymorphic group now needs to be reconstructed as new molecular data and further biological characters, such as shell morphology, anatomy, glochidium, have come to light. Recent conchological, anatomical, reproductive and molecular data have revealed several new features of the taxonomy of West Palaearctic naiads. Thus, the two Iberian Mediterranean *Unio* discussed by Haas (1969) – *U. elongatulus penchinatianus* Bourguignat, 1865, and *U. elongatulus valentinus* Rossmässler, 1854 – are actually synonyms of *U. mancus* Lamarck, 1819 (Araujo et al. 2005) and the two Iberian *Potomida* discussed by Haas (1969) – *P. littoralis littoralis* Cuvier, 1798 and *P. littoralis umbonata* Rossmaessler, 1844 – are synonyms of *P. littoralis* Cuvier, 1798 (Reis et al. in Araujo et al. 2009). These authors have recently redescribed *Unio gibbus* Spengler, 1793, a species distributed in the north of Morocco and the south of Spain; they also speculated about the possible distribution in Algeria and Tunisia.

The present paper describes an overlooked population of *Unio gibbus* in Tunisia describing shell morphology, anatomy, and biological characters (life cycle and morphology of the larva glochidium). The species was previously considered as *Unio pictorum* L., 1758 by Ghamizi (1998).

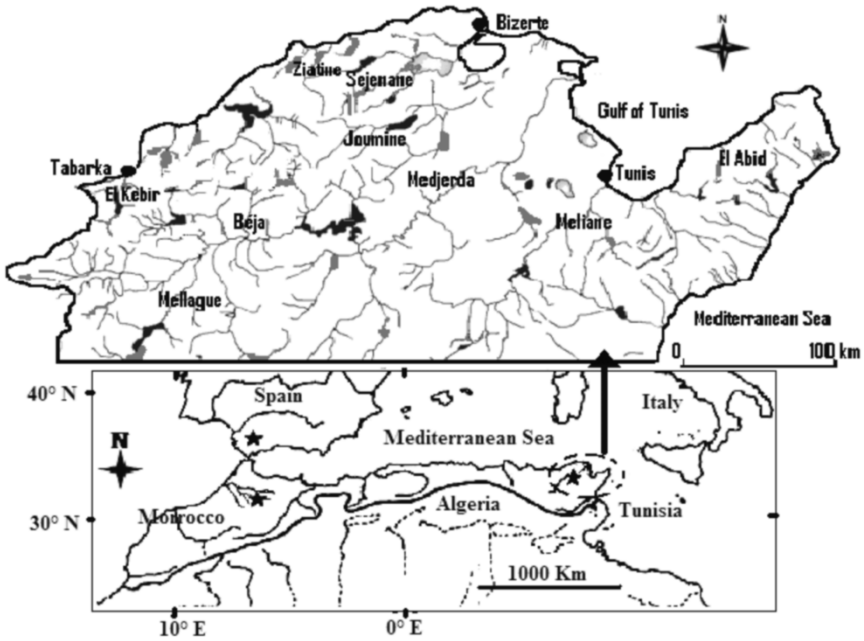

Fig. 1. Map showing the distribution of *Unio gibbus*. Black stars indicate sites where live specimens have been recently discovered.

Material and methods

Study area

Several rivers in northern Tunisia have been investigated: Oued Medjerdah, O. Mellegue, O. Béja, O. Sejenane, O. Ziatine, O. Méliane and O. Joumine (Fig. 1).

Biologic material examined

Collection of Khalloufi N. (Faculté des Sciences de Bizerte, Tunisie): several specimens coming from the Oueds: Sejenane, Habbes, Béja, El Kébir, Ziatine and Joumine.

Morphological data

The morphological characters of the specimens collected in different rivers have been examined directly and with stereomicroscope; shells were measured for total length (anteroposterior axis) and height, to the nearest 0.02 mm, using vernier caliper. The measure of the larvae is achieved by electron microscopy. The synonymy of the names used and the discussion of the taxonomic status of the studied forms were achieved on the basis of the literature and by comparison to samples of collections.

To characterize the larval stage, glochidia were collected from live mussels by their separation from gravid gills and fixed in 75% ethanol for investigation with both light and scanning electron microscopy. We washed ethanol-fixed glochidia in three changes of water, cleaned them in 5% KOH for 1.5–2 hours, washed them at least five times with distilled water, and gold-coated for scanning electron microscopy.

Study of the reproductive activity

The representatives of the genus *Unio* are with separated sexes. Their life cycle consists of five stages:

1 – The female lays unripe eggs and regrouped in their gills (marsupium) on each side a package of conglutinate which constitutes one brooded;

2 – The eggs, fertilized by spermatozoa, develop into larvae called glochidia (sing. gochidium) which are stored in the marsupium for a period of several weeks (short-term brooders) (Hoggarth 1999; Pekkarinen & Englund 1995);

3 – Outside of the female, the released larvae lead a very short pelagic life (2 to 5 days) and must attach themselves to either the gills or fins of the proper species of fish and lead a parasitic life (or commensally life for some authors) during some weeks; they undergo a metamorphosis;

4 – After the metamorphosis the larvae leave their hosts as juvenile mussel;

5 – The mussels reach the sexual maturity after some years of growth, with a size ranging between 25 and 30 mm.

For the biological survey, we chose only one river in which the species is most abundant. Monthly samplings were carried out with the hand, from September 2006 to September 2007 in a station situated at an altitude of 7 m a.s.l. in the downstream of the Sejenane River on the MC51 road, near of the Ichkeul Lake (37°11,603′ N, 09°34,764′ E). At the laboratory, all specimens of every sample have been submitted to a biopsy of the gonadic content and a microscopic exam of the content of the marsupium (gills containing the eggs or larvae).

Results

Distribution

In Tunisia we have found this species in the rivers Oued Joumine, O. Sejenane, O. Medjerda, and O. El Kébir (Fig. 1). In the Sejenane River, the species shares its habitat with *Potomida littoralis*, *Unio pictorum mancus* and *Anodonta anatina* (L. 1758).

External morphology

Shell subrectangular, thin, often rough but can be finely smooth streaky (Fig. 2). Anterior and posterior ends are rounded, but the second is usually more elevated than the first end. Colour green darkness to brown. Umbones rounded, characterized by a marked sculpture, consisting of wavy wrinkles confined at the beak. The scars of the anterior adductor and retractor muscles are confused. Left valve with two laminar pseudo-

Fig. 2. Shell morphology, external and internal of *Unio gibbus* in Tunisia. Dimensions: 49.3 × 25.8 × 16.4 mm.

Table 1. Reproductive activity of *Unio gibbus*.

	Number of the female examined	Contained of the gonad	Contained of the marsupium
Sep. 2006	36	+ (36)	–
Oct. 2006	25	+++ (19); + (6)	–
Nov. 2006	20	+++ (15); + (5)	–
Dec. 2006	5	+++ (3)	C.Œ im (2)
Jan. 2007	30	+++ (8), + (11)	C.Œ im (9); C.L.(2)
Feb. 2007	20	+++ (6)	C.Œ im(13); C.L.(1)
Mar. 2007	15	++ (4)	C.Œ (8); C.L. (3)
Apr. 2007	31	++ (7); +++ (3)	C.Œ (9); C.L. (12)
May 2007	35	+++ (9); + (5)	C.L (21)
Jun. 2007	25	+++ (5); + (3)	C.Œ (2); C.L. (15)
Jul. 2007	50	+ (43)	C.Œ (2); C.L. (5)
Aug. 2007	35	+ (35)	–
Sep. 2007	10	+ (10)	–

Explanations: Contents of the gonads: + clear, ++ dense, +++ very dense; contents of the marsupium: – empty; C.Œ im – female having conglutinate with eggs; C.Œ – female having conglutinate with embryos in different stages of development; C.L – female having conglutinate containing larvae.

cardinal teeth separated by a deep dimple; posterior tooth triangular, less stretched out and more elevated. Two laminar posterior lateral teeth; the lower tooth is always more elevated. The right valve with pseudocardinal tooth is generally beaked, hooked and very developed shape. The mean dimensions are: 66 × 36 × 21 mm and the length/height ratio vary between 1.6 and 1.75.

Anatomy

Soft parts typical of *Unio*. Generally three rows of papillae (Fig. 3) of the inhalant siphon, tapered, to bulbous basis and not bifurcated. In all the females examined, internal cavity of the external demibranchs acted as a marsupium (ectobranchy).

Larva

Glochidia conic, to elongate triangular, intermediate between glochidia shapes in *Unio* and *Anodonta* (Fig. 4A). The hook, examined by electron microscopy, is triangular, with large basis. It had about 12–15 large microstylets arranged in 2–3 longitudinal rows (Fig. 4B). Glochidial dimensions are: lenght 231.35 µm, height 232.37, width 130.88 µm

Fig. 3. Aspect of the papillae in inhalant siphon of *Unio gibbus*. Scale 500 µm.

Life cycle

The production of the gametes began at the end of the fall (Table 1, Figs 5–7). The gravid females

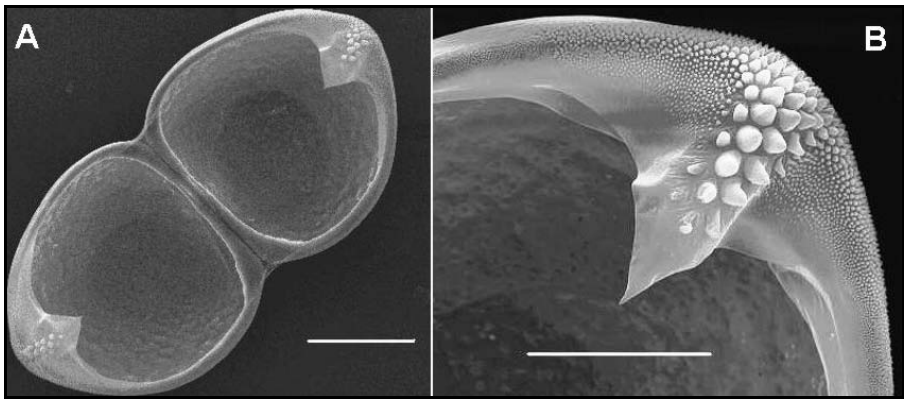

Fig. 4. Scanning electron micrographs of the glochidia of *Unio gibbus*. A – internal view (Scale 100 μm), B – the hook (Scale 50 μm).

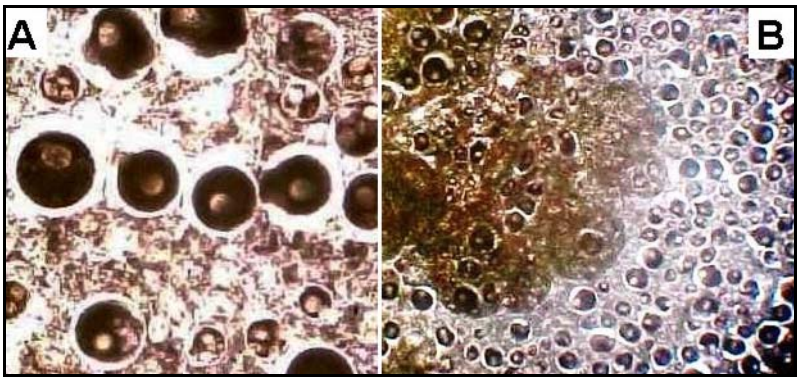

Fig. 5. Contained of *Unio gibbus* female gonads. A – September 2006, B – October, November and December 2006.

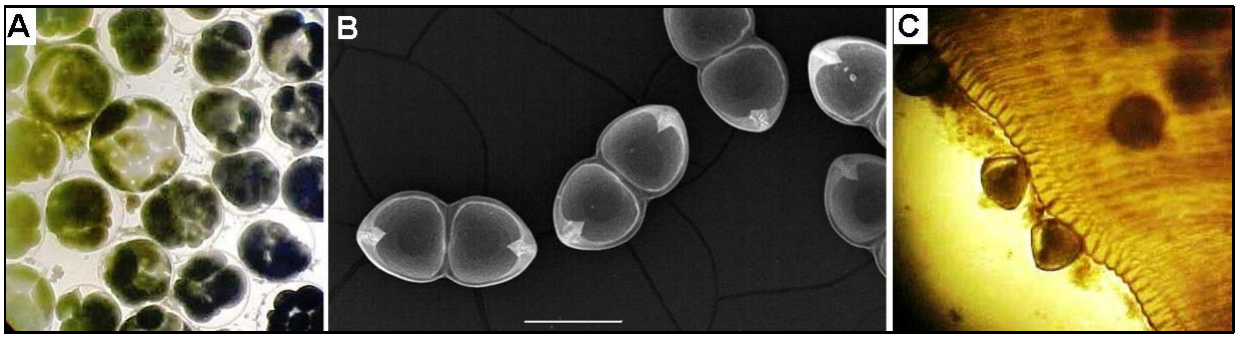

Fig. 6. Reproduction of *Unio gibbus* during the period January – July 2007. A – conglutinate containing some embryos from different stages of the embryogenesis, B – conglutinate containing larvae (Scale 250 μm), C – free larvae fixed on the gills.

(Fig. 7) appeared in the beginning of the winter, but the rate of fertilization of the eggs, laid unripe, remained lower during the whole humid season (2 females hatch some larvae / 11 gravid females in January and 1 / 14 in February). This rate increased from March (the conglutinate of the 11 gravid females contained some embryos of different stages). The reproductive activity reaches its peak during the months of May and June. The release of the larvae (Fig. 7) began at the end of winter, become very intense at the end of spring and ended in July. During the months of August and September, they had a rest (a clear gonadic content of all females examined and no gravid female were found). Several small mussels some less than five millimeters long are found at the beginning of the winter (December 2006).

Discussion

In Tunisia, *Unio gibbus* has been found for the first time in the Oued El Kébir (NW Tunisia) and listed under the name of *Unio doumeti* "minor" variety (Khalloufi 1998).

The survey of Tunisian specimens characters shows a perfect homology with the Iberian and Moroccan specimens of *U. gibbus* described by Araujo et al. (2009). Pallary (1923, 1927, p. 275–276, photos 1 and 2, pl. VII) listed this species under the name *Unio tifleticus* Pallary, 1923), then after a few years, it was listed

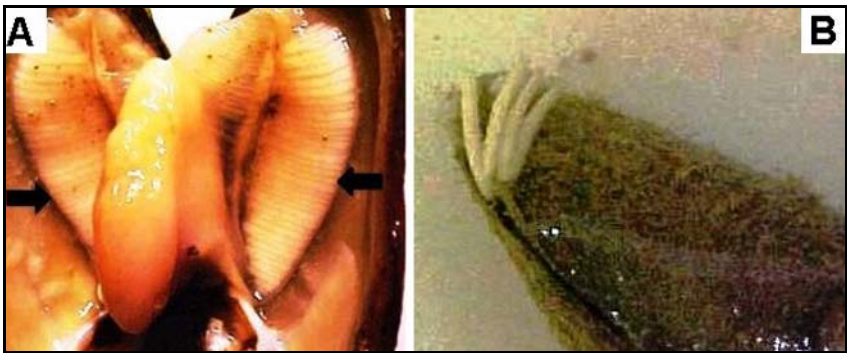

Fig. 7. Gravid female of *Unio gibbus*. A – arrows indicate gravid external gills (marsupium) with conglutinates, B – release of conglutinates.

under the name *Unio* (*Limniun*) *foucauldiana* Pallary, 1936 by the same author (Pallary 1936, p. 62–64, pl. 4, Fig. 2). We note also several similarities between our specimens and *U. gibbus* presented by Knudsen et al. (2003, Fig. 12, p. 274). Haas (1969) listed *U. turdetanus* Drouet, 1893, and *U. gibbus*, as synonyms of the SW Atlantic Iberian Peninsula *Unio pictorum delphinus* Spengler, 1793, one of the 13 taxa into which this author divided *U. pictorum*, although he listed the species *U. tifleticus* as synonyms of *Potomida littoralis fellmanni*. For Ghamizi (1998), the two species *U. tifleticus* and *U. foucauldiana* are synonymous of *U. pictorum*. This synonymy given by the two authors is to be contested because looking at the genus *Potomida* Swainson, 1840, the test is thick, rhomboidal, to robust and concave hinge, with developed teeth and hookless larva; whereas the characters given by Pallary (1927) correspond to the genus *Unio* Philipson, 1788, to test less thick, thin hinge, more or less straight, to less strong teeth and to larva provided with a hook (Fig. 4). On the other hand, the present form has very distinct characters to *U. pictorum*, the shell is subrectangular, the test often rough and thin, the beak decorated with wavy ridges and the glochidia shape is elongate triangular. In conclusion, we adopt for the NW Africa form its priority name *U. gibbus* quoted for the first time in Tunisia while waiting for a comparison on a molecular scale to European forms (in particular to Spanish form).

The two characters mentioned by Araujo et al. (2009), such as the tetrageny and hookless glochidia, and considered as the most important characters that separate *U. gibbus* from other *Unio* species, were not found in the Tunisian specimens. The glochidia are hooked and all the examined females incubate the larvae in the external gills (ectobranchy). For the tetragenous Moroccan specimen identified, until more information likely to provide invaluable indications on Unionidae phylogeny, it is more probable that is an isolated case. In addition, the presence of populations of the same species in Tunisia with a ventral hook in the larva neglects the distinctive value of the hookless glochidia indicated by the authors for *U. gibbus*. Nevertheless, this idea (the possibility of hookless glochidia in some populations of *U. gibbus*) makes it possible to rethink about the typical larva of the genus *Unio* and the others taxa (e.g., *Potomida* and *Margaritifera*) discussed in literature (e.g., Graf & Cummings 2007; Hoggarth 1999; Pekkarinen & Englund 1995; Sayenko at al. 2005).

For all Unionidae specie, the fertilization of the eggs and the obligatory parasitical phase are the two critical stages by which the glochidium larva exceeds. They especially require that the water temperature remains between 18 and 25 ℃, slow current velocity of water and an important density of fish hosts. Besides, several authors showed that this rate is of low nature (1%) even under favourable conditions (Zettler et al. 2001; Geist 2005; Corey et al. 2006; Spring Rivers 2007). During the humid season, which extends generally from October to May, the hydrodynamic conditions are very irregular, characterized by the frequency of a high current velocity of water, and turbidity. Density of host fish is low and the mean water temperature measured in Sejenane stream at the humid season is 17.01 ℃. These special conditions affect the contact of the larvae with fish hosts and drag the slowing of the mussels activity. They stop opening their apertures and decrease the movements of the papillae that attract fish, which minimizes the fertilization of the eggs and the larvae release (Corey et al. 2006). Moreover, the drying of an important part of the mussels habitats involves a mortality of a high number of specimens each year and threatens severely the populations in Tunisia. May and June month's constituted the most favourable period of reproduction, similar of European species (Blažek & Gelnar 2006). Although the data of Araujo et al. (2009) on the life cycle of *U. gibbus* in Morocco and Spain are limited in time and concerned only a reduced number of specimens, they show that the favourable reproductive season of this species extends between the end of winter and early summer. This same period with intense reproductive activity is at the origin of juvenile mussels at the end of autumn in our study site.

We know very little of the host fish utilization for metamorphosis, but we know that two species living in the Sejenane River [*Barbus callensis* Valenciennes, 1842 and *Pseudophoxinus callensis* (Guichenot, 1850)].

The instability of the hydrodynamic conditions (alternation between flood with high velocity of current water and drying) threaten the populations of mussels in Tunisia, from where *U. gibbus* should be a main pri-

ority for Tunisian invertebrate conservation measures and need measures for protection of their habitats.

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