THE IDENTITY AND BIOLOGY OF *UNIO MANCUS* LAMARCK, 1819 (= *U. ELONGATULUS*) (BIVALVIA: UNIONIDAE) IN THE IBERIAN PENINSULA

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ABSTRACT

Genetic variability, shell morphology, anatomy, reproductive season, glochidium morphology and host fish of several Iberian populations of *Unio mancus* Lamarck, 1819 (= *U. elongatulus* C. Pfeiffer, 1825) were examined. The genetics of three allopatric populations were studied, including two 'races' previously described for this species on the Iberian Peninsula. All shared the same haplotype of the COI mitochondrial gene. It is proposed that all known Iberian Mediterranean populations of the genus *Unio* correspond to *Unio mancus*. All mature specimens studied were dioecious. Glochidial release occurred between March and August. The barbel *Barbus graellsii* was the natural host of the *U. mancus* glochidia in the studied area. In artificial infestations performed in aquaria, seven fish species, all native, carried the glochidia throughout their complete metamorphosis. This process occurred in 141 degree/days. Newly released juveniles were spherical and had a thin marginal rim of new shell.

INTRODUCTION

The study of *Unio* taxonomy is an ongoing task as the identity of a number of populations is still unknown. Difficulties arise primarily from the shortage of accurate biological information (glochidium morphology, reproductive strategies, larval host fish) and from the lack of molecular markers for the different populations. For these reasons shell morphology, which is very polymorphic in freshwater mussels, is still the basis for *Unio* taxonomy (Haas, 1940, 1969). Haas, an expert on Palearctic Unionoids and the author of the latest monograph of worldwide Unionacea, considered there to be a series of 'fundamental *Unio* species' comprised of different 'races' or incipient species. For his classifications, Haas (1969) largely used shell shape of the different taxa that were found in the centres of their distribution areas. Haas (1969) believed that the species *Unio elongatulus* C. Pfeiffer, 1825 (= *Unio mancus* Lamarck, 1819, see below) was made up of a group of 17 'local races', distributed mainly throughout the Mediterranean Region and Asia Minor. Two of these 17 'races' are present in the Iberian Peninsula (Haas, 1940, 1969): (1) *U. elongatulus penchinatianus* Bourguignat, 1865, which is distributed in the rivers of the northeastern Mediterranean coast, up to and including the Ebro; and (2) *U. elongatulus valentinus* Rossmaessler, 1854, which is found south of the Ebro.

Apart from shell characters, there is little available information on other characters of *Unio elongatulus s. l.* Haas (1924) found a great deal of anatomical similarity among specimens of several *U. elongatulus* populations, including specimens from the two above mentioned Spanish 'races'. Araujo, Bragado & Ramos (2000) studied the glochidial release season of one population from the Ebro basin. Various papers have been published on the species in Italy, including shell morphology (Fondi, Scala & Castagnolo, 1984), reproductive cycle (Castagnolo, 1978), host fish (Nagel & Castagnolo, 1991) and genetic variability (Badino, Celebrano & Nagel, 1991) of *Unio elongatulus* C. Pfeiffer, 1825 and *Unio mancus* Lamarck, 1819. Following Haas's classification (1940, 1969), these Italian populations would be referred to the 'races' *Unio elongatulus lawleyanus* Gentiluomo, 1868 and/or *Unio elongatulus glaucinus* Porro, 1838. Nevertheless, Falkner (1994) and Falkner, Ripken & Falkner (2002), have claimed that, following nomenclature rules, the valid name for all these Mediterranean populations is *Unio mancus* Lamarck, 1819 instead of *U. elongatulus* C. Pfeiffer, 1825 because the name *mancus* has priority over *elongatulus*.

More recently, Nagel & Badino (2001) updated the population genetics and systematics of European Unionoidea, but results on the genus *Unio* remain inconclusive. These authors considered all Mediterranean *Unio* populations, including one from the Guadiana basin (not Mediterranean) in the centre of Spain, to be *U. pictorum mancus*, a subspecies of the European species *Unio pictorum*. They did not, however, study the Spanish populations of Haas's *elongatulus* group.

The complexity of *Unio* taxonomy, combined with a general lack of knowledge needed to protect these taxa, make more in-depth studies of all the species of the genus imperative. The taxon *U. elongatulus* is protected under the two main pieces of European biodiversity conservation legislation: the Habitats Directive (Annex V) and the Bern Convention (Annex III). In Spain, it has been proposed to include *U. elongatulus* in the *Catálogo Nacional de Especies Amenazadas* under the category of species that are 'sensitive to habitat modification' (Araujo & Ramos, 2001).

In this work, we study the genetic variability of several Iberian populations of this species, their relationships with European *Unio pictorum*, and describe their main biological features: shell morphology, anatomy, distribution, reproductive season, larval stage and host fish.

MATERIAL AND METHODS

Studied material

To identify the historical distribution of the species in the Iberian Peninsula, we reviewed both the literature (Azpeitia, 1933; Haas, 1969; Soriano, Villena & Alonso, 2001) and the mollusc collection of the Museo Nacional de Ciencias Naturales (Madrid, Spain). Other specimens studied came from field samples

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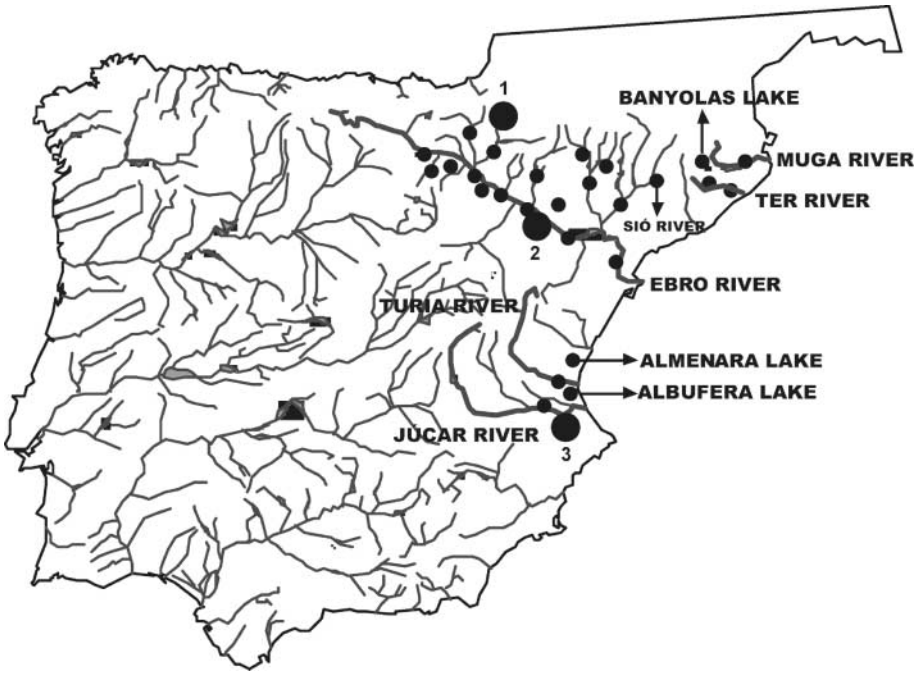

Figure 1. Sample distribution. Large circles show the provenance of the samples sequenced for the mitochondrial COI gene: 1: Irati River (Navarra). 2: Ebro River at Sástago, Canal Imperial, Acequia de Pina and Acequia de Lorés (Zaragoza). 3: Júcar River and Estany El Barranquet (Valencia).

collected from the Ebro basin during the last 10 years and from many gracious collaborators who sent additional material.

Shell characters were studied in specimens from the following rivers and lakes (Fig. 1) (Provinces in brackets). (1) Ebro River, tributaries and channels (La Rioja, Álava, Navarra, Zaragoza, Huesca, Lérida and Tarragona), Oñar River and Osor River (tributaries of the Ter River), Muga River and Banyolas Lake (Gerona). All specimens in these areas mentioned above belong to *U. e. penchinatianus* (*sensu* Haas, 1969). (2) Turia River, Júcar River and Albufera de Valencia (Valencia) and Almenara Lake (Castellón). All specimens from these regions correspond to *U. e. valentinus* (*sensu* Haas, 1969). (3) Sió River, tributary of the Ebro (Lérida); surprisingly, Haas (1969) considered these specimens to be *Potomida littoralis* (Cuvier, 1798) (see Discussion).

Anatomy

Anatomical studies of more than 20 specimens were carried out, paying close attention to characters that are taxonomically relevant in unionoids (Ortmann, 1911; Haas, 1924; Nagel, 1999). To reveal the sexual strategy of the species (hermaphroditism versus dioecy), specimens were dissected and sexed. Gonad samples and gill contents of gravid specimens were examined by optical microscopy. Anatomical studies were conducted on living and preserved specimens from the Ebro River and natural channels connected to the Ebro (*U. e. penchinatianus sensu* Haas, 1969), and on four specimens from the Júcar River (*U. e. valentinus sensu* Haas, 1969).

Molecular analyses

For molecular analyses, we extracted the genomic DNA of 12 samples by the usual phenol/chloroform technique (Sambrook, Fritsch & Maniatis, 1989), using CTAB lysis buffer (2% CTAB; 1.4 M NaCl; 0.2% β-mercaptoethanol; 20 mM EDTA; 0.1 M TRIS, pH = 8) and afterwards digested the samples with proteinase K (100 μg/ml) for 1–2 days at 50°C. The extracted DNA was amplified for a fragment of the cytochrome *c* oxidase subunit I (COI) by polymerase chain reaction, using the same primers and conditions used previously for other unionids (Machordom, Araujo, Erpenbeck & Ramos, 2003). The samples, representing different populations of the two supposed Iberian 'races' *sensu* Haas (1969), came from the following two river basins (Fig. 1): (i) Ebro basin: Sástago (UTM: 30T0722/4577, $N = 5$), Canal Imperial (UTM: 30T0653/4623, $N = 2$), Acequia de Pina (UTM: 30T0703/4599, $N = 1$) and Acequia de Lorés (UTM: 30T0653/4623, $N = 1$) (Zaragoza) and Irati River (UTM: 30T0636/4744, $N = 1$) (Navarra). (ii) Júcar basin: Júcar River (UTM: 30S0712/4328, $N = 1$) and Estany El Barranquet (UTM: 30S0737/4336, $N = 1$) (Valencia). We compared our sequences with sequences of the three available European specimens of *U. pictorum*, a species that is considered to be closely related to *U. mancus* (Nagel & Badino, 2001) (GenBank accession numbers AF156499, AF231731 and AF468684).

Life cycle

For the characterization of the larval stage, glochidia collected from both drift samples (Araujo *et al.*, 2000) and from living mussels in the aquarium were studied, using scanning electron microcopy and optical microscopy. The life cycle of the species was inferred from previously published results on glochidial release season (Araujo *et al.*, 2000), specimen dissections and experiments in aquaria. These studies were conducted using specimens collected from tributaries and channels of the Ebro River.

In order to examine the susceptibility of natural fish populations to *U. mancus* glochidia, fish were collected by electrofishing in six different campaigns. Each campaign was made during the mussel glochidial release season along 200 m stretches of the Canal Imperial de Aragón, which is fed by water from the Ebro River and is characteristically rich in *U. mancus*. The campaigns were conducted with a Robin (2200 W/220 V) DC-powered generator. All large fish captured (>10 cm total length) were identified, counted and, using a binocular magnifier (3 × power), inspected in the field for glochidial infestation. Specifically, the skin, fins and gills were examined. Only large infested specimens and all those under 10 cm (in which infestation

is not easily detected) were fixed in 10% buffered formalin and preserved in 70% ethanol for later examination; the rest were released. In the laboratory the opercula were removed, and the bodies, gill filaments and fins of each fish were examined for glochidia under a stereomicroscope.

Experimentation with host fish

Different fish species were artificially infected in the laboratory during April–June 2002 and June–July 2003 to determine their susceptibility to glochidial attachment. Experiments were performed in the Centro de Recuperación de Fauna Silvestre de La Alfranca (Zaragoza, Spain). Eight specimens of *Unio mancus* collected in April 2002 from one channel of the Ebro River in Zaragoza, and 12 specimens collected in April and May 2003 from both the Ebro and one of its channels, were maintained in one aquarium with water and natural sediment from the Canal Imperial. Ten different fish species were tested for glochidial host suitability: *Barbus graellsii* (Steindachner, 1866), *Barbus haasi* (Mertens, 1925), *Squalius pyrenaicus* (Gunther, 1868), *Squalius cephalus* (Linnaeus, 1758), *Chondrostoma miegii* (Steindachner, 1866), *Phoxinus phoxinus* (Linnaeus, 1758), *Cyprinus carpio* (Linnaeus, 1758), *Gobio gobio* (Linnaeus, 1758), *Acipenser baerii* (Brandt, 1869) and *Salaria fluviatilis* (Asso, 1801). Blennies were supplied by the Centro de Experimentación Piscícola El Palmar (Valencia, Spain) and sturgeons by a pet shop. The rest of the species were obtained in different campaigns of electrofishing in the Jalón, Mijares, Onsella and Arba de Biel Rivers (Ebro basin). Fish were inspected to ensure that they had not been previously infected with glochidia.

Once the mussels began to release glochidia, fish were added to the aquarium for 12–24 h periods until they were successfully infected. Following infestation, fish were isolated and separated by species and placed in aerated aquaria without substratum. The bottom of the aquaria was lined with a 5 mm-mesh plastic net to avoid predation of the newborn juvenile mussels. Fish were inspected for glochidium encystment on a regular basis. Aquarium temperature was recorded daily and water was periodically siphoned from the aquarium bottom through a 60 μm-mesh nylon sieve to check for possible release of glochidia or juveniles. Newborn juveniles were separated and maintained alive in semi-natural conditions (see Araujo, Quirós & Ramos, 2003) for several days.

RESULTS

Molecular analyses

The length of the partial COI sequences obtained was 657 base pairs (GenBank accession numbers AY522847 to AY522858). The empirical base frequencies were biased with a high proportion of thymine (A = 19.92, C = 15.29, G = 23.42 and T = 41.37). The average divergence found among the *U. mancus* specimens was 0.05% (from 0 to 0.15% between the Júcar basin samples and the rest of the samples). In fact, 10 of the 12 *U. mancus* specimens studied showed identical sequences, while the Júcar Basin samples had just one transition in position 592. However, this divergence rose to 3.7–4.3% when compared with *U. pictorum* (GenBank).

Shell morphology

Unio mancus in Spain (Fig. 2A) usually has a dark-brown or yellowish-green periostracum. Small specimens are usually lighter in colour. The shell is equivalve and inequilateral, with a variably oval shape. The anterior margin is rounded and the posterior elongated and truncated. The umbo is prosogirated, rounded and prominent, sometimes with tubercles. Tubercles, when present, can be simple or wavy, but are always arranged in two radiating rows, one medial and the other posterior. Length is always smaller than 10 cm, and rarely exceeds 9 cm. The inner shell is nacreous, iridescent white with a weak pallial line that is more pronounced anteriorly. The ligament is external, and the hinge plate is typical of *Unio*: left valve with two crenulate cardinal teeth, very pronounced in large specimens (sometimes both teeth appear to be fused in one laminar structure) and two large and laminar lateral posterior teeth; the lower tooth is always more elevated. In the right valve, there is one very variable cardinal tooth (varies from very crenulate, strong and hooked to laminar and delicate), and one large elongate lateral tooth. These variations can be found within specimens from the same locality.

Anatomy

Under the mantle lobes, soft parts are generally white to yellow, although the colour of the visceral mass (visible through the foot epithelium) varies depending on the sex and the season. The mantle-lobe margins are fused in only one region (the bridge between the supra-anal and the exhalant siphon), but three apertures are visible in the posterior part: the supra-anal aperture, the exhalant and the inhalant siphons (from dorsal to ventral). The inhalant is the largest of the three openings and is the only one surrounded by papillae. Nevertheless, it is not a real siphon because ventrally it is connected with the foot aperture. It has one to three rows of barrel-shaped papillae. The papillae have a distal, elongate pit and are sometimes bifurcate (Fig. 2B). The space behind the inhalant and exhalant siphons is separated by a diaphragm, not a mantle fusion, that is formed by extensions of the distal part of the gills. This diaphragm separates the mantel cavity into two chambers: the cloacal or suprabranchial cavity above, and the branchial cavity below. The exhalant opening is confined by two lateral muscular strips with an external black trimming, and is separated dorsally from the supra-anal aperture by a bridge built by the fusion of the inner mantle margins. The length of this bridge is the same as that of the exhalant siphon, and shorter than the supra-anal aperture. The entire length of the outer lamella of the lateral demibranchs is fused to the inner mantle wall. The inner gills are only fused to the visceral sac anteriorly. After that point they run unattached and finally converge and join together behind the sac to form the diaphragm. Anteriorly, the mouth is bordered on either side by two white, undulating labial palps. Except for a small distal section, each palp is completely fused to the foot epithelium. The palps have a smooth external surface and a finely canaliculated inner side.

Life cycle

Of the 18 specimens studied, eight were female and 10 male. The zygotes are incubated in the chambers formed by the water tubes (Fig. 2C). The entire internal cavity (homogeneity) of the external demibranchs acts as a marsupium (ectobranchy). The glochidia are whitish, triangular in shape (Fig. 2D) and have a ventral styliform hook (Fig. 2E) that is armed with numerous spicules (teeth). Mean glochidial sizes of the population from the Acequia de Lorés (a channel from the Ebro River) are (measured by SEM): length: 216.8 μm (SD = 7.58; $N = 15$), height: 193.3 μm (SD = 13.31; $N = 13$) and width: 162.0 (SD = 1.99; $N = 2$).

Regarding natural infestation, the only natural host of *U. mancus* glochidia in the studied area was the barbel *B. graellsii* (Table 1). Glochidia attached primarily to the gill filaments (90%), although some glochidia attached to the fins (10%).

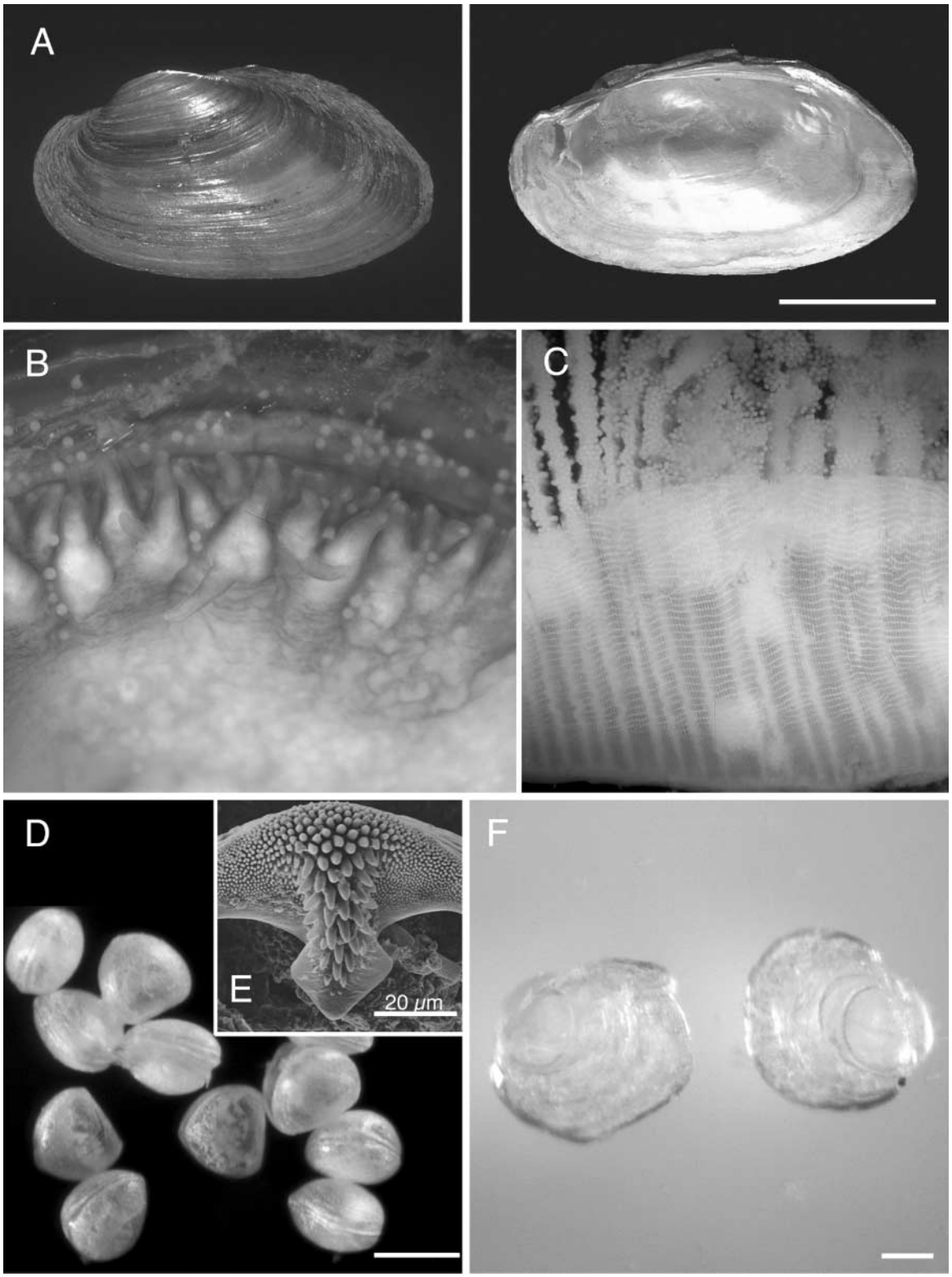

Figure 2. *Unio mancus*. **A.** Specimen from the Ebro River. Scale bar = 2 cm. **B.** Detail of the inhalant siphon papillae in a live specimen. **C.** Ova in the water tubes of a female specimen. **D.** Glochidia of Spanish *U. mancus*. Scale bar = 200 μm. **E.** Detail of the styliform hook. **F.** One-week-old *U. mancus* juveniles. Scale bar = 200 μm.

Experimentation with host fish

Release of mature *U. mancus* glochidia in aquaria occurred between the end of April and August. They were expelled in fine, long threads from the exhalant opening. All fish species tested (10) were successfully infested, but in only seven of these did the glochidia carry out complete metamorphosis: *Barbus graellsii*, *Barbus haasi*, *Squalius pyrenaicus*, *Squalius cephalus*, *Chondrostoma*

Table 1. Species and number of specimens of fish collected, preserved and infested with *Unio mancus* glochidia

Date	Species	Collected specimens	Preserved specimens	% Infected
21/05/02	*Barbus graellsii*	25	9	28
	Alburnus alburnus	12	1	0
	Cyprinus carpio	2	0	0
	Gobio gobio	2	0	0
	Chondrostoma miegii	1	0	0
30/05/02	*Barbus graellsii*	21	14	66
	Alburnus alburnus	7	1	0
	Cyprinus carpio	3	0	0
	Gobio gobio	2	2	0
	Chondrostoma miegii	5	0	0
5/06/02	*Barbus graellsii*	54	12	22
	Alburnus alburnus	10	0	0
	Cyprinus carpio	1	0	0
	Micropterus salmoides	1	0	0
	Carassius auratus	1	0	0
	Chondrostoma miegii	5	1	0
18/06/02	*Barbus graellsii*	28	17	61
	Alburnus alburnus	8	5	0
	Cyprinus carpio	1	0	0
	Gobio gobio	4	0	0
	Chondrostoma miegii	9	0	0
31/07/02	*Barbus graellsii*	37	18	49
	Alburnus alburnus	6	0	0
	Chondrostoma miegii	1	0	0
27/08/02	*Barbus graellsii*	26	3	8
	Alburnus alburnus	51	0	0
	Cyprinus carpio	1	0	0
	Micropterus salmoides	6	0	0
	Carassius auratus	1	0	0
	Chondrostoma miegii	12	0	0

miegii, *Phoxinus phoxinus* and *Salaria fluviatilis*. Experimental conditions and number of juveniles obtained are shown in Table 2. Metamorphosis took place in the seven fish species on the fin and gill filaments. In two replicate experiments with carp (*Cyprinus carpio*), gobio (*Gobio gobio*) and sturgeon (*Acipenser baeri*), no successful metamorphosis occurred, since attached glochidia (to fins and gill filaments) were sloughed off during the first 8 days.

Recently released juveniles of *U. mancus* were spherical with a thin marginal rim of new shell. Measurements (made by light microscope) were: length ($N = 2$): 262 μm and 277.5 μm; heigth ($N = 2$): 187.3 μm and 187 μm and width ($N = 3$): 225 μm, 225 mm and 232.5 μm. During the first days of culture, growth was asymmetrical and the rear region of the juvenile was larger than the front (Fig. 2F).

Table 2. Results of the infestation experiments with *Unio mancus* glochidia

Fish species	*N*	Place of infestation	Mean temperature °C	Degree-days	Number of juveniles
Barbus graellsii[*]	4–2	Gills and fins†	19–28	278–141	42–170
Barbus haasi	5‡	Gills and fins†	26	287	115
Squalius pyrenaicus	3	Gills and fins†	19	254	100
Squalius cephalus	2	Gills and fins	23.5	235	181
Chondrostoma miegii	5‡	Gills and fins†	23.5	235	110
Phoxinus phoxinus	4	Gills and fins	26	197	43
Cyprinus carpio	2	–	19	–	–
Gobio gobio	2	–	19	–	–
Acipenser baerii	3	–	26	–	–
Salaria fluviatilis	8	Gills and fins‡	19	272	379

[*]With this species two different experiments were made.
[†]When overinfested, the nose was also covered by glochidia.
[‡]Two fish died during the experiment.

DISCUSSION

Molecular analyses show that the Iberian populations studied, including the two different 'races' previously considered by Haas (1969), could not be different species. Although Haas (1940, 1969) used the name *U. elongatulus* C. Pfeiffer, 1825 for this Mediterranean species, Falkner (1994) and Falkner *et al.* (2002) have demonstrated that the valid name of the species is *U. mancus* Lamarck, 1819. In addition, Falkner (1994) designated a lectotype of the species. Our data also demonstrate that *U. mancus* found in Spain is not the same species as the European *U. pictorum* from GenBank (4% of divergence among sequences). In fact, values around 4% are common between congeneric mollusc species (Hebert, Ratnasingham & de Waard, 2003) and mitochondrial intraspecific divergences are rarely greater than 2% (Avise, 2000). Similar analyses would be neccessary to clarify the identity of the other 15 European 'races' of *U. mancus* (*U. elongatulus sensu* Haas, 1969) and their possible relationship with the species *U. pictorum* (Nagel & Badino, 2001).

Studying other variable gene regions and using larger samples may yield different results, but until more marked differences can be found our data leads us to reject the idea that two different 'races', *U. e. penchinatianus* and *U. e. valentinus*, exist in Spain (*sensu* Haas, 1940, 1969). One third 'race', *U. e. aleroni* Companyó & Massot, 1865, reported by Haas (1969) and found only in Central Southern France, has been cited in different rivers of Cataluña (North East Spain) (Altaba, 1991; Ordeix, Camprodón, Comas, Molist & Barniol, 1998; Boix, Sala & Feo, 2001). Nevertheless, the brief description of this taxon by Altaba (1991) concentrates only on shell characters, and fits perfectly within the shell variability described here for *U. mancus*. Molecular analyses would be able to resolve both this uncertainty as well as the questions surrounding the possible existence of an endemic species of *Unio* at the Banyolas Lake (Girona, Spain), as was also proposed by Altaba (1991). However, in an in-depth study of the naiads of Banyolas Lake, Haas (1916) considered the specimens of *Unio* found here to be *U. elongatulus*, that is the same species found in Spanish and French Mediterranean rivers. Indeed, several years later, Haas (1924) demonstrated that the unios from the Banyolas Lake were anatomically identical to other Spanish Mediterranean *U. elongatulus* populations. Finally, in his seminal book *Superfamilia Unionacea* (Haas, 1969), he included this population in the synonymy of *U. e. penchinatianus*.

Thus, on the basis of the current data, we propose that all the Iberian Mediterranean *Unio* populations studied here belong to only one species, *Unio mancus*. Only one population from the Ebro Basin, found in the Sió River, could be questioned. Haas himself was puzzled by this population and as the corresponding labels at the Senckenberg Museum (Lot SMF 003048) demonstrate, he first considered the species to be *U. crassus*, and later called it *Potomida littoralis*. Although the shell shape of these specimens is unusual they certainly do not belong to the genus *Potomida*. In any event, we have shown in this paper that *Unio* populations, far removed from the Ebro basin including specimens with unusual shell shape (i.e. Irati River), are genetically identical to the typical *U. mancus* from the middle Ebro River (Fig. 2A). It may be possible, however, that sibling *Unio* populations live in Spanish Mediterranean rivers or lakes, although there is no data to support this conjecture.

The typical papillae of both *Unio* and *Anodonta* are acute and conic (Type 3 of Nagel, 1999), but other morphologies may exist (Nagel, 1999). However, the shape and colour of the inhalant siphon papillae are not useful in distinguishing *U. mancus* from other Spanish species (personal observation). The number of rows in which the papillae are arranged seems to differ among Iberian species, and specimens of *U. mancus* have the fewest rows of papillae (personal observation). Nevertheless, it would be necessary to study and describe the papillae character in all the European *Unio* species to determine the taxonomic value of this feature.

The first modern description of the *U. elongatulus* glochidium came from Giusti (1973), although it lacked the extremely important size character. Following Haas (1924), the measurements of *U. elongatulus* glochidium from the Ebro are 0.224 mm in length and 0.172 mm in height. Hoggarth (1999) gives the following measurements for an Italian *U. elongatulus* population: 218–232 μm (length) and 210–218 μm (height). Measurements of glochidial size in our study are similar to those cited by these two authors, while shape and characters of the hook are identical. Although the small size of *Unio* glochidia distinguishes this genus from *Anodonta*, this size character does not differentiate one *Unio* species from another (Pekkarinen & Englund, 1995).

Data on the reproductive season of Spanish *U. mancus* come from Araujo *et al.* (2000), who, using a drift net, found that glochidial release occurred between March and September. This paper, however, did not present data on the host fish of this species, because electrofishing was only used during the reproductive season of *Margaritifera auricularia* (February–March). Data from Italian populations show that gametogenesis of *U. mancus* begins in September–October (Castagnolo, 1978). Glochidial incubation takes place during February and October, and fish infestation occurs during April and October (Castagnolo, 1978; Nagel & Castagnolo, 1991). This is a summer-breeding (tachytictic) species.

With regard to the glochidial host fish, *U. mancus* is a species with a very wide host range (this paper; Nagel & Castagnolo, 1991), unlike species of *Margaritifera*, which have very few suitable hosts. This wide spectrum of suitable host fish may be a common feature that the genus *Unio* shares with *Anodonta* (Nagel & Castagnolo, 1991; Bauer, 2001). Although results from electrofishing points to the barbel as the only host in natural conditions, further studies are necessary to determine if fish species which are successfuly infected in captivity (i. e. *Squalius pyrenaicus*, *S. cephalus*, *Salaria fluviatilis*, *Chondrostoma miegii*, *Phoxinus phoxinus* and *Barbus haasi*) are capable of acting as natural hosts in other localities.

As has been demonstrated in other species of freshwater mussels (Araujo, Cámara & Ramos, 2002), our results with *Unio mancus* also suggest that temperature plays a critical role in the metamorphosis of the glochidium in the fish. Thus, this process can be accelerated from 14 to 5 days by raising the temperature by 9°C (see Table 2). The glochidium shell growth that we observed during *Unio mancus* metamorphosis is thought to be unusual in unionids (Wächtler, Dreher-Mansur & Richter, 2001), while it commonly occurs in margaritiferids (Araujo *et al.*, 2002). The apparent height reduction of the glochidial valve during infectation (from 193.25 μm to 187 μm) may have been a result of the different measurement methods used (SEM versus light microscope).

Data in the present paper has improved our knowledge of the taxon *U. mancus s. l.* Nevertheless, new sampling and molecular studies must be carried out on all populations of this species in order to determine the validity of the taxonomic units proposed by Haas (1969). Indeed, these kinds of studies should also be expanded to include the rest of the European *Unio* populations.

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