

## First record of the North American cryptic invader *Ferrissia fragilis* (Tryon, 1863) (Mollusca: Gastropoda: Planorbidae) in the Middle East

Federico Marrone<sup>1\*</sup>, Murtada D. Naser<sup>2</sup>, Amaal Gh. Yasser<sup>2,3</sup>,  
Francesco Sacco<sup>1</sup>, and Marco Arculeo<sup>1</sup>

<sup>1</sup>Dipartimento di Scienze e Tecnologie Biologiche, Chimiche & Farmaceutiche, Università di Palermo, Palermo, Italy. <sup>2</sup>Marine Science Centre, University of Basrah, Basrah, Iraq. <sup>3</sup>College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Some gastropod specimens belonging to the planorbid genus *Ferrissia* were recently collected in Lebanon and in Iraq, where the autochthonous species *Ferrissia clessiniana* (Jickeli, 1882) is supposed to occur. The molecular identification of collected specimens proved that they belong to the allochthonous species *Ferrissia fragilis* (Tryon, 1863), the protagonist of a dramatic cryptic invasion which is of interest to the whole of Eurasia. These findings cast further doubts on the actual existence of autochthonous *Ferrissia* species in the Palaearctic. The need for a molecular characterisation of the topotypical population of *F. clessiniana*, and for a revision of the Palaearctic *Ferrissia* species, is stressed.

**Keywords:** Ancyliinae, biological invasion, cryptic species, molecular identification.

### Introduction

The planorbid gastropod genus *Ferrissia* Walker, 1903 has a worldwide distribution, but the actual number of species within the genus is unknown, and their autochthony is doubtful in several countries. The taxonomy of the genus is in fact quite uncertain, which is due to the paucity of sound diagnostic morphological characters currently known, coupled with a noteworthy conchological and environmentally-induced plasticity. The taxon has been relatively better investigated in the Nearctic, where Walther, Burch, and Ó Foighil (2010) concluded that out of the five *Ferrissia* species previously supposed to occur in North America, only two widely distributed taxa actually exist (i.e. *Ferrissia fragilis* (Tryon, 1863) and *F. rivularis* (Say, 1817)), each encompassing multiple nominal species. Conversely, the taxonomy of Palaearctic *Ferrissia* taxa is currently under debate, and only scarce data are available for Afrotropical and Australian taxa.

Although fossil records testify for an ancient occurrence of the genus *Ferrissia* in the Palaearctic region (e.g. van der Velde, 1991; Harzhauser, Neubauer, Mandic, Zuschin, & Čorić, 2012; Mienis & Rittner, 2013), its presence in the recent molluscan faunas of Europe, Africa, and Asia was completely overlooked until the description of *Ferrissia clessiniana* (Jickeli, 1882) from Egypt, and of *Ferrissia wautieri* (Mirolli, 1960) from Italy. In the last few years, an ever increasing number of records reporting the occurrence of *Ferrissia* species throughout the western Palaearctic region have been published (e.g. Marrone, Lo Brutto & Arculeo, 2011; Albrecht, Föller, Clewing, Hauffe & Wilke, *in press*, and references therein), but an agreement on the taxonomic identity of the recorded populations is currently missing, so that they have been referred to as

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\*Corresponding author. Email: [federico.marrone@unipa.it](mailto:federico.marrone@unipa.it)

either *F. wautieri*, *F. fragilis*, or *F. clessiniana*, often only on a geographic basis. Furthermore, as stressed by Walther, Lee, Burch, & Ó Foighil (2006c), each used name is charged with different biogeographic implications: *Ferrissia wautieri* was assumed to be an endemic European species, *F. clessiniana* is assumed to be a taxon with an African or Middle-East gravitation, and *F. fragilis* is the binomen used when the scored populations are assumed to be the result of a recent colonization event from North America (see Walther et al., 2010; Marrone et al., 2011, Albrecht et al., *in press*, and references therein).

Molecular studies aimed at investigating the identity of Palaearctic *Ferrissia* species showed that of a number of populations in Europe and Asia belong to the North American species *Ferrissia fragilis*, while no evidence supporting the presence of autochthonous taxa or lineages has been found to date. The mtDNA sequences of a *Ferrissia* specimen collected in Uganda and identified as "*Ferrissia clessiniana*" by Jørgensen, Kristensen, and Stothard (2004) are rather distinct from the known *F. fragilis* haplotypes, so that *F. clessiniana* is currently considered to be a *bona species* and the sister taxon of *F. fragilis* (Walther et al., 2010). However, it has to be stressed that the putative Ugandan *F. clessiniana* specimen was collected in a different biogeographic region from where the species was originally described, that no information on the sampling locality or on the identification methods used was provided, and that the putative *F. clessiniana* 16S and COI sequences available on GenBank are of rather poor quality. Accordingly, it cannot be ruled out that the *Ferrissia* specimen studied by Jørgensen et al. (2004) might in fact belong to a different, Afrotropical *Ferrissia* species or that the published sequences might be, at least partially, an artefact due to amplification or sequencing.

Recently, the possibility that both *F. wautieri* and *F. clessiniana* might actually be junior synonyms of *F. fragilis* was raised (cf. Walther, Lee, Burch, & Ó Foighil, 2006a, 2006b; Marrone et al., 2011), and the molecular characterisation of a *Ferrissia* specimen from the type locality of *F. wautieri* suggested that this last binomen is actually a junior synonym of *F. fragilis* (Albrecht et al., *in press*). If this is corroborated for both species, all the Palaearctic *Ferrissia* populations are to be considered allochthonous.

In the light of these taxonomic uncertainties, and of the practical implications related to environmental management, the aim of the present study is to determine the taxonomic identity of Middle East *Ferrissia* populations, which are currently supposed to belong to the autochthonous species *Ferrissia clessiniana*.

## Material and Methods

Two *Ferrissia* populations from the Lebanese Republic and from the Republic of Iraq were sampled. *Ferrissia* specimens were collected during field surveys on 24 August, 2008 (Lebanon, Beirut river near Al Daychounieh village: 33°50'N, 35°34'E) and on 25 October, 2012 (Iraq, Tigris river at Qurna, Dorat harriba: 31°07'N, 47°26'E). The Beirut river flows from Lebanon's mountains, passing through Al Daychounieh, a village located in the Matn District of the Mount Lebanon Governorate, to the Mediterranean Sea. The Tigris river is one of the most important rivers in Iraq, being the main water source for human use, especially for drinking water, along with the Euphrates River. It is 1850 km long, rising in the Taurus Mountainous of eastern Turkey and flowing into the Persian Gulf. Visited sites were sampled both visually and through the use of 200 µm mesh-sized hand nets passed through the vegetation and on the bottom of the studied water bodies. Collected samples were fixed *in situ* in 80% ethanol and sorted in the laboratory under a stereomicroscope. Unfortunately, within the frame of this survey no *Ferrissia* samples from Egypt, the *terra typica* of *F. clessiniana*, could be obtained.

Specimens were identified as *Ferrissia* sp. under the stereomicroscope, soaked in double-distilled water, and then processed for DNA extraction using the Qiagen “DNeasy Animal Tissue Kit” following the standard protocols. Fragments of the large ribosomal subunit 16S and the cytochrome oxidase 1 (COI) were amplified following the protocols described in Marrone et al. (2011). Chromatograms were imported and edited with ChromasLite 2.01 (Technelysium Pty. Ltd.) and aligned with BioEdit (Hall, 1999). The sequences were deposited in GenBank (16S: KF889403-KF889405; COI: KF889400-KF889402). Mega5 (Tamura, Peterson, Peterson, Stecher, Nei, & Kumar, 2011) was used to translate in amino acids the sequences of the protein-coding gene COI in order to check for the possible presence of frameshifts or premature stop codons, which would indicate the presence of sequencing errors or pseudogenes, which is a well-known and wide-spread problem in invertebrates (e.g. Marrone, Lo Brutto, Hundsdoerfer, & Arculeo, 2013).

In order to compare the *Ferrissia* sp. sequences obtained from the Lebanese and Iraqi populations with those available for *Ferrissia fragilis*, *F. rivularis*, *F. clessiniana* and *Ferrissia* sp. populations from different countries, COI and 16S *Ferrissia* species sequences were downloaded from GenBank; furthermore, 16S and COI sequences of the related planorbid *Ancylus fluviatilis* were downloaded to be used as outgroups (see the codes reported in Figures 1 and 2). The poor quality of the single available COI sequence of the putative *Ferrissia clessiniana* from Uganda (Accession Number: AY577509) (Marrone et al., 2011), does not allow it to be included in the analysis.

The molecular identification of studied specimens and the reconstruction of the phylogenetic relationships among the taxa were performed with Bayesian (BA) and Maximum Likelihood (ML) analyses as implemented in MrBayes 3.2.1 (Ronquist et al., 2012) and PhyML v.3 (Guindon & Gascuel, 2003), respectively. As a measure of branch support, bootstrap values were calculated with 1,000 replicates in the ML trees. Nodes support of BA trees were evaluated by their posterior probabilities.

For both the COI and 16S datasets, BA and ML analyses were performed using a general time-reversible model of sequence evolution for molecular data with a proportion of invariable sites (GTR+I), as selected by AIC in mrModeltest 2.2 (Nylander, 2004). For both markers, the BA analyses were performed with two independent runs of 2,000,000 generations and four Markov chains using default heating values. Trees and parameter values were sampled every 100 generations resulting in 20,000 saved trees per analysis; in both the analyses convergence was reached well before the 500,000th generation; 5,000 trees were then conservatively discarded as “burn-in”.

## Results

Both the mitochondrial markers and the analyses implemented show a well-supported clade which includes the North American *F. fragilis* populations along with all the available Eurasian *Ferrissia* sp. specimens (Figures 2–3). The other *Ferrissia* species, i.e. the single Ugandan specimen identified as “*F. clessiniana*”, the Australian *Ferrissia* sp., and the North American *F. rivularis* are separated from the “*F. fragilis* clade” by deep genetic distances and well supported nodes (Figures 1–2).

All the specimens included in the framework of this study belong to a single 16S haplotype, which is shared by the North American, Middle East and European *Ferrissia fragilis* populations, the only exceptions being the single specimen from Denmark and one out of the two specimens from Alabama (U.S.A.); these two specimens show slightly different haplotypes (differing respectively 0.3 and 0.6% of uncorrected p-distance from the commonest haplotype). Unfortunately, no 16S sequences for eastern Asian specimens are available in GenBank for comparison.

The COI sequences are slightly more diverse than 16S ones (mean p-distance among the *Ferrissia fragilis* COI sequences: 0.4%, ranging from 0 to 0.9%), and the scored haplotypes are shared among Asian, European and American populations without any clear geographic structure.

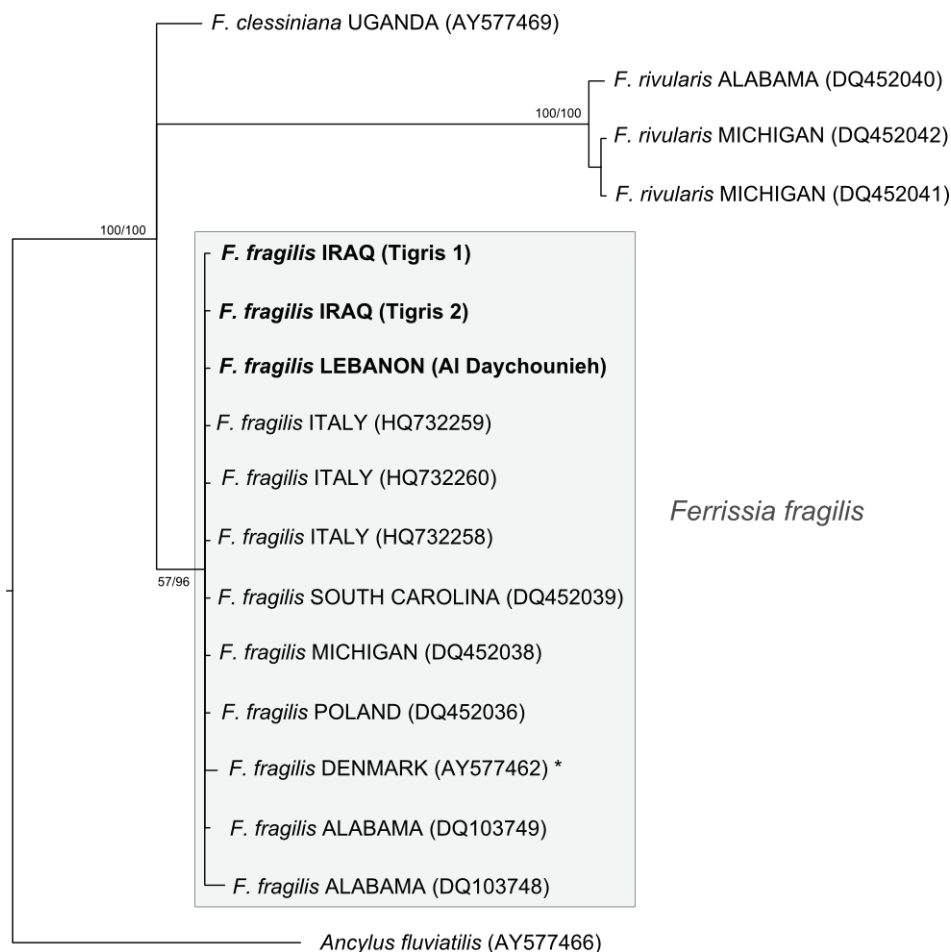


Figure 1. Bayesian phylogram (ngen = 2,000,000; nchains = 4; nrun = 2; samplefreq = 100; temp = 0.2; default priors) of the mitochondrial large subunit ribosomal DNA (mtDNA 16S) dataset composed of *Ferrissia* spp. and the outgroup *Ancyclus fluviatilis*. Node support is reported as nodal posterior probabilities/ML. GenBank accession numbers are given among brackets for non-novel haplotypes. \*: mislabelled as *Acroloxus lacustris* (see discussion in Walther et al. 2006b).

## Discussion

The topologies of the trees obtained with both 16S and COI markers stress the molecular homogeneity of the European and Asian *Ferrissia* samples, and their identity with some of the *F. fragilis* haplotypes recorded in North America, thus providing the first evidence of the presence of this invasive taxon in the Middle East, an area where the possible presence of the autochthonous *Ferrissia clessiniana* was suggested by some authors (e.g. Walther et al., 2006c; Mienis & Rittner, 2013). Conversely, no evidence for the presence of *F. clessiniana* in the whole Mediterranean area and the Middle East has been collected to date; thus, in spite of the finding of *Ferrissia* fossils from the Israeli Pleistocene (Mienis & Rittner, 2013) and from the European Miocene to Pleistocene (van der Velde 1991; Harzhauser et al., 2012), there is so far no evidence of the

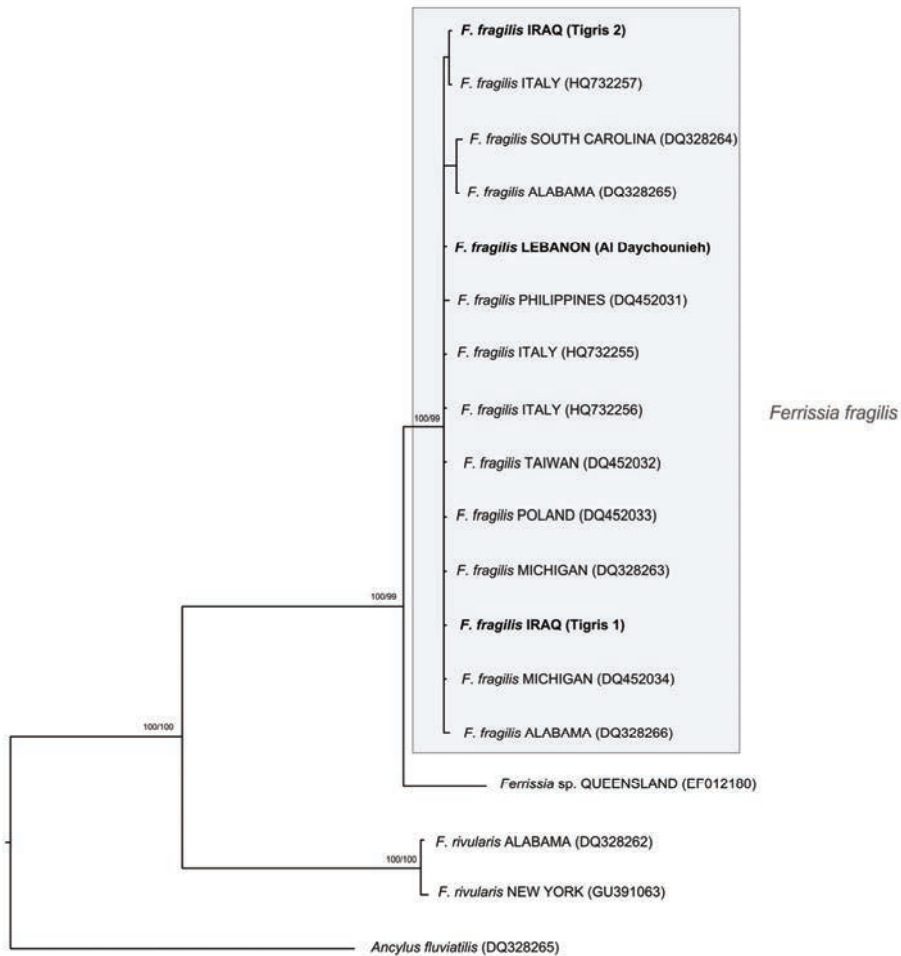


Figure 2. Bayesian phylogram (ngen = 2,000,000; nchains = 4; nrun = 2; samplefreq = 100; temp = 0.2; default priors) of the cytochrome oxidase 1 (mtDNA COI) dataset composed of *Ferrissia* spp. and the outgroup *Ancyclus fluviatilis*. Node support is reported as Nodal posterior probabilities/ML. GenBank accession numbers are given in brackets for non-novel haplotypes.

survival of ancient autochthonous lineages in the Palaearctic region, and all the European and Asian *Ferrissia* populations surveyed so far proved to belong to the invasive American species *F. fragilis*, the protagonist of an overlooked large-scale biological invasion which is currently underway and whose impact on the autochthonous freshwater biota is as yet unknown.

Obviously, based on currently available data, the alternative hypothesis that a putative autochthonous Palaearctic *Ferrissia* species might have been only recently replaced by a North-American congener cannot be completely ruled out; however, the fact that no report of *Ferrissia* sp. older than the end of the 19<sup>th</sup> century exists for the Palaearctic region, nor are there *Ferrissia* specimens stored in historical malacology collections, suggests that this is probably not the case and that no autochthonous *Ferrissia* possibly

occurred in the western Palaearctic region in the Holocene. In the light of the results presented here, the actual existence of *F. clessiniana* itself needs to be tested, and the advisability of obtaining a molecular characterisation of the topotypical population of the species is here further stressed.

The molecular characterisation of other West Palaearctic *Ferrissia* populations would enable us to trace back the colonisation route followed by this cryptic invader out of North America; however, in the light of the scarce to absent molecular structuring of the surveyed populations throughout Eurasia, it is likely that *Ferrissia fragilis* was introduced in Eurasia from a single North American source area, and that it then spread over the continents thanks to a combination of autogenous (both active and passive) and human-mediated dispersal. For example, the recent record of the hydrobiid *Ecrobia grimmeri* from Sawa Lake, south of Iraq, provides evidence of the high dispersal potential of small molluscan species by endo- or ectozoochory through avian vectors (Haase et al., 2010).

The Middle East inland water bodies are threatened by an alarming number of biological invasions threatening the autochthonous biota (e.g. Bößneck, 2011; Naser et al., 2011, 2012), which is often already significantly impaired by habitat alteration. This process is still in progress and is characterised by an accelerating pace, possibly due to ongoing globalisation processes. In the Iraqi sampling sites, as an example, *Ferrissia fragilis* is part of a molluscan community dominated by the alien gastropods *Melanoides tuberculata* (Müller, 1774) and *Physella acuta* (Draparnaud, 1805), whose impact on the indigenous taxa is well documented (e.g. Albrecht et al., 2009; Ladd & Rogowski, 2012; Naser et al., 2011). It is thus desirable that the autochthonous freshwater fauna of the Middle East is further studied, mapped and described before it is completely lost or adulterated.

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