

1 **Different lineages of freshwater jellyfishes (Cnidaria, Olindiidae, *Craspedacusta*) invading**  
2 **Europe: another piece of the puzzle from Sicily, Italy**

3

4 Enrico SCHIFANI<sup>1</sup>, Arturo VIVIANO<sup>2</sup>, Roberto VIVIANO<sup>2</sup>, Luigi NASELLI-FLORES<sup>3\*</sup> &  
5 Federico MARRONE<sup>1</sup>

6

7 <sup>1</sup> Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF),  
8 Section of Zoology, University of Palermo, Via Archirafi 18, I - 90123, Palermo, Italy.

9 <sup>2</sup> Via Michele Cipolla 106, 90123, Palermo, Italy.

10 <sup>3</sup> Department of Biological, Chemical and Pharmaceutical Sciences and Technologies  
11 (STEBICEF), Section of Botany and Plant Ecology, University of Palermo, Via Archirafi 28, I -  
12 90123, Palermo, Italy.

13 \*Corresponding author, e-mail: [luigi.naselli@unipa.it](mailto:luigi.naselli@unipa.it)

14

15 **Abstract**

16 Correctly identifying and mapping the distribution patterns of passively dispersing freshwater  
17 organisms is an important task to understand the mechanisms through which these organisms move  
18 across the world and to timely counteract the introduction of invasive alien species; moreover, in  
19 the light of the growing evidences of the pervasiveness of cryptic species and cryptic biological  
20 invasions among all animal groups, the use of molecular identification tools is strongly advisable.  
21 In this context, we present the first record of the freshwater jellyfish *Craspedacusta sowerbii*  
22 Lankester, 1880 in the Mediterranean island of Sicily, Italy. This invasive species native of China  
23 was found during a routine survey campaign aimed at assessing the freshwater species richness in  
24 the Sicilian region. Individuals were collected in November 2017 and identified to species-level  
25 through the sequencing of fragments of both mitochondrial and nuclear genes, and their comparison  
26 to the sequences available on public databases. The analysis of molecular data proved that the  
27 Sicilian population belongs to *C. sowerbii* s.s., i.e. one of the two major *Craspedacusta* lineages  
28 which have been discovered to have invaded Europe and which were until recently erroneously  
29 jointly reported under the binomen *Craspedacusta sowerbii*. In order to get a clearer frame of the  
30 history and patterns of freshwater jellyfish biological invasion worldwide, the need for a molecular

identification of all the non-native *Craspedacusta* populations currently reported as *C. sowerbii* and the opportunity of monitoring human-impacted ecosystems is stressed.

**Key words:** *Craspedacusta sowerbii* (=sowerbyi), Biological Invasions, Invasive species, cryptic invasions

## Introduction

Several freshwater organisms produce resting stages which give them the potential to passively disperse across the “dry ocean” surrounding continental water bodies (Incagnone et al., 2015). Human activities have interfered in several ways to the natural dispersal and colonization patterns by altering the ecological state of inland water body, by enhancing global changes and by acting as dispersal vectors for several native and non-native species (Lockwood et al 2005; Marrone and Naselli-Flores 2015). Biological invasions, i.e., the human-mediated successful establishment of species outside their native distribution ranges, are one of the most serious threats to the conservation of the world biological diversity, and are known to be particularly harmful in inland waters (e.g. Gallardo et al. 2006; Chandra and Gerhardt 2008). Moreover, biological invasions in inland waters have been often overlooked due to the misidentification of cryptic alien taxa as native, sometimes even endemic, species (e.g. Marrone et al. 2011; Saito et al. 2018; Morais and Reichard 2018). This stresses the need for the implementation of molecular identification tools when dealing with biological invasions (Blanchet, 2012), especially for those taxa whose taxonomy is more unstable and/or morphology is poorly informative.

The olindiid freshwater jellyfishes of the genus *Craspedacusta* Lankester, 1880, native to eastern Asia (Kramp 1950), are worldwide human-spread species that have been reported to occur in all continents except Antarctica, usually under the binomen *Craspedacusta sowerbii* Lankester, 1880 (Dumont 1994; Jankowski et al. 2008). The morphology-based traditional taxonomy of the group has been largely disputed (Bouillon and Boero 2000; Jankowski 2001; He 2003) and no sound consensus has been reached to date. However, recent molecular studies (Fritz et al. 2009; Zhang et al. 2009) agree in proposing the existence of at least three different lineages of species rank within the genus, i.e. *Craspedacusta sowerbii*, *C. kiatingi* Gaw & Kung, 1939, and *C. sinensis* Gaw & Kung, 1939, plus another taxon of uncertain rank (“*C. ziguensis*” He & Xu, 1985).

Currently, *Craspedacusta sowerbii* is considered to be one of the most widespread freshwater invaders (Duggan and Eastwood 2012; Karaouzas et al. 2015) although molecular identification of most of the invasive populations is currently lacking and their actual identity

64 should be checked. Fritz et al. (2009), for instance, proved that all the freshwater jellyfish  
65 populations they investigated from Germany and Austria do actually belong to the “kiatingi cluster”  
66 of the genus *Craspedacusta* (i.e. to *Craspedacusta kiatingi*), not to *C. sowerbii* s.s. Therefore,  
67 despite different *Craspedacusta* taxa are invading water bodies throughout the world, the plurality  
68 of these biological invasions was to date overlooked due to taxonomical inadequate data, and this  
69 pattern represents a clear example of the widespread phenomenon of cryptic invasions in inland  
70 waters.

71 *Craspedacusta* jellyfishes have complex life-histories. The medusa stage is the only one easily  
72 visible as free swimming in all kind of freshwater environments (Jankowski et al. 2008).  
73 Conversely, polyps are benthic, a few millimetres long, and much less visible unless actively  
74 sought. The polyp-stage can encyst (podocysts) and produce drought-resistant resting spherical  
75 frustules, which again develop into polyps when environmental conditions improve (Dumont 1994).  
76 These diapausing stages thus facilitate human-mediated dispersal with fish stocking, relocation of  
77 aquatic plants and invertebrates, or passive dispersal through waterfowl, mammals and other  
78 animals (Incagnone et al. 2015; Failla Siquier 2017). While the polyp form dominates its life-  
79 history, the three other stages (medusa, frustule, resting body) occur in response to specific  
80 environmental conditions (Acker and Muscat 1976). The polyp stage can live for years until  
81 suitable environmental conditions make it develop to medusa (Kato and Hirabayashi 1991). Such  
82 conditions, not well understood yet, were suggested to be associated with changes in water  
83 temperatures, water level and food abundance (Acker and Muscat 1976; DeVries 1992; Michin et  
84 al. 2016).

85 As occurrence records are mostly due to random observations of medusae (which can form  
86 blooms) and polyps are usually overlooked, and due to their great habitat plasticity (DeVries 1992),  
87 *Craspedacusta* jellyfishes are expected to be even much more widely distributed than currently  
88 known (Duggan and Eastwood 2012). In summary, the factors related to their dispersal capacity are  
89 i) the ability to produce a durable, chitin-covered resting body (Kato and Hirabayashi 1991;  
90 Bouillon and Boero 2000); ii) their several strategies of vegetative reproduction (Reisinger 1957)  
91 and iii) long-term survival without sexual reproduction (Petrusek et al. 2005; Fritz et al. 2007).

92 Medusae blooms can have significant effects on zooplankton abundance and composition,  
93 thus affecting food webs (Davis 1955; Dodson and Cooper 1983; Spadinger and Maier 1999;  
94 Jankowski and Ratte 2000; Boothroyd et al. 2002; Jankowski et al. 2005; Smith and Alexander  
95 2008). Apart direct predation, this is also due to unselective killing which affects non-prey  
96 organisms alike (Smith and Alexander 2008). Moreover, native freshwater predators may have

limited impact on the medusae (Dodson and Cooper 1983; DeVries 1992). Conversely, very few information is available about the ecological impacts of polyps (DeVries 1992).

Although the genus *Craspedacusta* is widely reported throughout the West-Palearctic region, the only molecular data available to date refer to Central Europe, where only *C. kiatingi* proved to be present (Fritz et al. 2009), and Greece, where a different lineage of uncertain identity was reported based on sequences of the mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) (Karaouzas et al. 2015). In the frame of this paper, we investigated a recently-found *Craspedacusta* population from the Mediterranean island of Sicily (southern Italy) with the aim of checking which species is actually present in the Mediterranean area, and to contribute to the understanding of the history and patterns of freshwater jellyfish biological invasion in the Western Palearctic area.

## Material and methods

In the frame of an extensive sampling campaign aimed at investigating the biological diversity of Sicilian inland waters, samples were collected in 08<sup>th</sup> and 14<sup>th</sup> November 2017 in a concrete reservoir located inside the campus of the University of Palermo, Sicily, Italy (WGS84 geographical coordinates: 38°06'25.2"N, 13°21'03.1"E).

The sampled water body stores about 450 m<sup>3</sup> of water (size: 13.20 x 15.50 m, h 2.20 m) and is used to irrigate the experimental fields of the Department of Agricultural, Food and Forest Sciences (University of Palermo). The reservoir is subjected to considerable water level fluctuations and it was observed almost dry in several occasions (L. Naselli-Flores, *pers. obs.*); its re-filling occurs by pumping municipality waters with average physical and chemical characteristics as reported in the Electronic Supplementary Materials, Table S1. Water temperature was measured *in situ* with a Hanna HI9835 probe.

The presence of aquatic vertebrates was registered based on direct observations. Qualitative invertebrate samples were collected by means of three different nets: a 125 µm mesh-sized conical net was used in the open waters, a 1 mm hand net was specifically used for the visual collection of the jellyfish, and a further 200 µm hand net was used for sampling invertebrates along the walls of the reservoir. Collected samples were fixed *in situ* in 90% ethanol and sorted in the laboratory under a stereomicroscope. Crustacean samples were identified according to Alonso (1996), Dussart (1967, 1969), and Meisch (2000). A *Craspedacusta* voucher specimen was deposited in the collection of the Zoology Section, Natural History Museum, University of Florence (Italy) (MZUF), Cnidaria collection number MZUF 1501.

130 Phytoplankton for qualitative analysis was collected using a 20 µm conical net. Species were  
131 identified in living samples immediately after collection, according to the most updated taxonomic  
132 literature, using a Zeiss Axiovert microscope.

133 DNA was extracted from one of the collected jellyfish specimens on 8<sup>th</sup> November, 2017.  
134 Prior to DNA extraction, the specimen was soaked in double-distilled water for 1 h; DNA was then  
135 extracted from the whole specimen using the Ron's Tissue Mini Kit (BIORON) following the  
136 manufacturer's protocol. Fragments of the mtDNA cytochrome oxidase subunit I (COI) and of the  
137 nuDNA internal described spacers (ITS) were amplified following the protocols described in  
138 Karaouzas et al. (2015) and Fritz et al. (2009), respectively. PCR products were purified using the  
139 Exo-SAP-IT kit (Affymetrix USB) and sequenced with an ABI 3130xL (Applied Biosystems)  
140 sequencer. MEGA7 (Kumar et al. 2016) was used to translate the COI sequence to amino acids in  
141 order to check for the possible presence of frameshifts or stop codons, which would indicate the  
142 presence of sequencing errors or pseudogenes. The novel *Craspedacusta* sequences were deposited  
143 in GenBank (Accession numbers: MH230079 & MH500048); moreover, all the available  
144 *Craspedacusta* COI and ITS sequences were downloaded from GenBank to be included in the  
145 analyses (see Accession numbers in figures 2 & 3). A single sequence of *Cubaia aphrodite* Mayer,  
146 1894 (Hydrozoa, Olindiidae) was included in the mtDNA analyses to be used as an outgroup,  
147 whereas the ITS tree was rooted on *Craspedacusta sinensis*, i.e. the adelphotaxon of the clade  
148 including *C. sowerbii* s.s. and *C. kiatingi* (Zhang et al. 2009).

149 Novel and GenBank sequences were aligned in ClustalX (Thompson et al., 1997) and used  
150 in the phylogenetic analysis. Bayesian inference (BI) of phylogeny and maximum likelihood (ML)  
151 analyses were performed as implemented by MrBayes 3.2 (Ronquist et al. 2012) and PhyML v.3  
152 (Guindon & Gascuel, 2003) for both datasets. The best evolutionary model for each dataset was  
153 chosen using the software MrModeltest 2.2 (Nylander 2004) on the basis of Bayesian Information  
154 Criterion (BIC) resulting in a General Time Reversible model with gamma distributed rate variation  
155 among sites (GTR+G; nst=6) for the COI dataset, and in a Kimura 2-parameter with gamma  
156 distributed rate variation among sites (K2P+G; nst=2) for the ITS dataset. Node supports were  
157 evaluated by their posterior probabilities in the BI and by 1,000 bootstrap replicates in the ML  
158 analyses. The BI analyses were performed with two independent runs of 1,000,000 generations and  
159 four Markov chains using default heating values. Trees and parameter values were sampled every  
160 100 generations resulting in 10,000 saved trees per analysis. An initial fraction of 2,000 trees (20%)  
161 was conservatively discarded as *burn-in*. For all analyses and both datasets, standard deviation of  
162 split frequencies reached values lower than 0.018288, and values of the potential scale reduction

163 factor (PSRF) were comprised between 0.999 and 1.002 for all the partitions with frequency  $\geq 0.10$   
164 in at least one run, indicating the convergence of the runs.

165

## 166 **Results**

### 167 Samplings and species identification

168 Water temperature in the two sampling dates was 16.1 °C and 15.9 °C, respectively. In accordance  
169 to Stefani et al. (2010) and Caputo et al. (2018), jellyfish abundance appeared higher in the shaded  
170 parts of the reservoir, and *Craspedacusta* individuals were present at density lower than 1 specimen  
171 m<sup>-2</sup>. Overall ten *Craspedacusta* specimens in the medusa stage, all bearing well developed gonads,  
172 were collected in the two sampling dates. The collected specimens had a diameter ranging between  
173 1.5 and 2.0 cm (Fig. 1).

174 Co-occurring fauna and microalgae are reported in Table 1. The samples collected by  
175 scratching the walls of the reservoir did not reveal the presence of the polyp stage, which probably  
176 are more concentrated on the bottom of the reservoir, to better compel with the water level  
177 fluctuations to which this irrigation water body is subjected.

178

### 179 2. Molecular analyses

180 Upon aligning our *Craspedacusta* sequences and those downloaded from GenBank, and having  
181 trimmed the tails of the sequences which were not present in all the individuals, we obtained a 337  
182 bp-long fragment for the COI dataset, and a 607 bp-long fragment for the ITS dataset.

183 The relative shortness of the analysed COI fragment is due to the only partial overlap of the  
184 Chilean *Craspedacusta* COI fragments available on GenBank with the other available COI  
185 sequences, so that excluding the Chilean sequences from the analyses would have led to an aligned  
186 COI fragment of 574 bp. BI and ML exploratory analyses were thus also carried out based on the  
187 longer mtDNA dataset which did not include the *Craspedacusta* sequences from Chile; the  
188 clustering of the *Craspedacusta* sequences obtained proved to be consistent with the one based on  
189 the shorter mtDNA dataset presented in figure 2 (see electronic supplementary material available  
190 online, Fig. S1).

191 The BI and ML analyses of COI and ITS datasets presented consistent topologies at their  
192 major nodes (Figures 2 and 3). The tree based on the COI dataset showed the affinity of the studied  
193 Sicilian sample with those from Chile, Marathon (Greece) and Hubei (China), plus a further  
194 sequence of unclear origin (GenBank A.N.: LN901194); moreover, in addition to the two divergent  
195 *Craspedacusta* COI lineages already described by Karaouzas et al. (2015), a third lineage, currently  
196 including a single sequence (GenBank A.N.: MG000493), was observed. The ITS dataset grouped

197 the studied Sicilian *Craspedacusta* sample with Chinese samples belonging to the *Craspedacusta*  
198 *sowerbii* s.s. clade described by Zhang et al. (2009) and Fritz et al. (2009) (Fig. 3).

199

## 200 **Discussion**

### 201 Identification of European *Craspedacusta* populations

202 To date, few molecular data are available for *Craspedacusta* populations from both its native  
203 distribution ranges and invaded regions. Moreover, for some populations only ITS sequences or  
204 COI sequences are available, thus preventing from carrying on an exhaustive total evidence analysis  
205 of the molecular diversity patterns of the genus, and on the taxonomical identity of some  
206 populations. The branching pattern of the phylogenetic tree based on ITS sequences is in good  
207 accordance to those published by Fritz et al. (2009) and Zhang et al. (2009), and distinctly separates  
208 the Central European *Craspedacusta* populations, belonging to *C. kiatingi*, from the Sicilian one,  
209 belonging to the *C. sowerbii* s.s. clade. Unfortunately, no ITS sequences are available for the  
210 Chilean and Greek *Craspedacusta* populations. However, the phylogenetic tree based on COI  
211 sequences shows the existence of three clades: a first clade including a single sequence of unknown  
212 origin, a second clade, which can be ascribed to *C. kiatingi*, including German and Chinese  
213 sequences from Sichuan province, and a third clade including Sicilian, Chilean, Greek and Chinese  
214 sequences from Hubei province. The presence in this clade of the Sicilian sequence, belonging to an  
215 individual identified as *C. sowerbii* s.s. based on its ITS sequence, allows to ascribe the the Chilean  
216 populations and the Greek population from Marathon to *C. sowerbii* s.s.

217 Therefore, it can be assumed that European water bodies have been invaded at least twice by  
218 two different *Craspedacusta* species of Asian origin: *C. kiatingi*, which invaded Central European  
219 countries, and *C. sowerbii*, which invaded Sicily and Greece in Europe, and Chile, in South  
220 America. To date, the occurrence of two distinct *Craspedacusta* biological invasions in Europe was  
221 largely overlooked since different invasive species had been lumped under the binomen *C.*  
222 *sowerbii*, and the actual identity of the *Craspedacusta* populations for which no molecular data are  
223 available is still to be verified. Further efforts aimed at reviewing and defining the identity and  
224 distribution of these species and other *Craspedacusta* lineages throughout their distribution ranges  
225 will allow to better understand the history and vectors of the biological invasion of freshwater  
226 jellyfish around the globe.

227

### 228 Ecological remarks

229 Stefanelli (1948) reported for the first time the presence of *Craspedacusta sowerbii* in Italy, based  
230 on polyps and medusae “appeared” in an aquarium hosting individuals of the Asian dwarf gourami

231 *Trichogaster lalius* (F. Hamilton, 1822) fed with zooplankton collected in ponds in the vicinity of  
232 Rome. Indeed, aquaria often represents the location of first observation for this species suggesting  
233 that escape or release from aquaria is likely a frequent human-mediated facilitation to its dispersal  
234 (Gasith et al. 2011). Since its first Italian record, freshwater jellyfishes have been sporadically  
235 recorded in several water bodies in Central and Northern Italy (see e.g. Ramazotti 1962; Cotta  
236 Ramusino 1972; Stefani et al. 2010; Ciutti et al. 2017, and reference therein). In particular, the  
237 species bloomed in several small lakes on the Italian Alps, where it has been repeatedly observed  
238 since the 1990's (Morpurgo and Alber 2015), during summer 2015, a record year in all the surface  
239 temperature analyses (RealClimate, 2018), and in Ireland the first observations of *C. sowerbii*  
240 coincided with unusually high water-temperatures (Minchin et al. 2016). However, the Sicilian  
241 finding occurred in autumn, and the recorded water temperature values in the studied sites were  
242 well below 19 °C, which is considered the lower limit of temperature for optimal growth (Acker  
243 and Muscat 1976). Accordingly, this was probably the final phase of the medusa stage: their density  
244 value (around 1 individual m<sup>-2</sup>) was lower than those reported in literature (e.g. Pérez-Bote et al.  
245 2006) and could not be considered a jellyfish bloom.

246 The population discovered in Sicily belongs to the same cluster of that found in Marathon  
247 (Greece), a town located at the same latitude of Palermo, and in Mediterranean and Valdivian  
248 Chilean ecoregions; all these areas are characterized by similar Mediterranean climatic features  
249 (Karaouzas et al. 2015; Fuentes et al. 2018). Moreover, it was found in Hubei Province, located in  
250 Central China, which is characterized by a subtropical monsoon climate, with seasonal temperature  
251 excursions similar to those recorded in the Mediterranean area (Zou et al. 2012). It is possible that  
252 *Craspedacusta* species have different invasive potential under different climatic scenarios, and that  
253 *C. kiatingi* is more adapted to continental or temperate climates, while *C. sowerbii* finds its  
254 optimum in warmer climates, but this hypothesis should be tested based on a much wider sampling  
255 of the *Craspedacusta* populations occurring worldwide.

256 At a local scale, given the great ability of *C. sowerbii* to quickly spread among geographic  
257 areas, as well as its ability to remain mostly undetected due to the overlooking of the polyp form, its  
258 presence in Sicily could be wider than currently known and is likely to increase even outside urban  
259 contexts. In the studied Sicilian reservoir, the observed phytoplankton species structure was typical  
260 of those commonly found in meso-eutrophic ponds, although with a relatively low number of  
261 species, and the jellyfish co-existed with several non-native vertebrate [*Trachemys scripta*  
262 (Thunberg in Schoepff, 1792), *Gambusia holbrooki* Girard 1859, and *Carassius auratus* Linnaeus,  
263 1758] and invertebrate [*Acanthocyclops trajani* Mirabdullayev and Defaye, 2004, *Physella acuta*  
264 (Draparnaud, 1805)] species (Marrone and Naselli-Flores 2015), along with a few euryecious



crustacean autochthonous taxa already known from Sicily (Marrone et al. 2005; Pieri et al. 2006), making up quite a simplified community.

To date, insufficient data exist to predict the consequences of the presence of *C. sowerbii* on the invaded ecosystems. However, it is worth noting that in this artificial and highly disturbed ecosystem, characterized by a relatively species poor community, several allochthonous species coexisted. This highlight the importance of human-made and/or human-impacted inland water ecosystems as invasion bridgehead for several exotic species (e.g. Alfonso et al. 2010). The continuous disturbance to which these ecosystems are subjected does not allow the establishment of a “mature” community and vanish the role of those priority effects which may constitute a barrier against new colonizers. It is therefore particularly important to establish more accurate surveys of these “banal” environments in order to create an early detection observatory for exotic and invasive species.

## Acknowledgements

The authors wish to thank Fabio Stoch (Université Libre de Bruxelles, Belgium) and Valentina Pieri (Università di Parma, Italy) for their help with the identification of some crustacean taxa.

## Ethical standards

The Authors declare that this study complies with the current Italian laws.

## References

- Acker T S, Muscat A M (1976) The ecology of *Craspedacusta sowerbii* Lankester, a freshwater hydrozoan. Am Midl Naturalist 93: 323-336
- Alfonso G, Belmonte G, Marrone F, Naselli-Flores L (2010) Does lake age affect zooplankton diversity in Mediterranean lakes and reservoirs? A case study from Southern Italy. Hydrobiologia 653: 149-164
- Alonso M (1996) Crustacea, Branchiopoda. In: (M.A. Ramos et al. Eds.) Fauna Iberica, vol. 7. Museo Nacional de Ciencias Naturales. CSIC, Madrid.
- Blanchet S (2012) The use of molecular tools in invasion biology: an emphasis on freshwater ecosystems. Fish Manag Ecol 19:120-13
- Boothroyd I K, Etheredge M K, Green J D (2002) Spatial distribution, size structure, and prey of *Craspedacusta sowerbyi* Lankester in a shallow New Zealand lake. Hydrobiologia 468: 23-32
- Bouillon J, Boero F (2000). The hydrozoa: a new classification in the light of old knowledge. Thalassia Salentina 24: 3-45

- 299 Caputo L, Huovinen P, Sommaruga R, Gómez I (2018) Water transparency affects the survival of  
300 the medusa stage of the invasive freshwater jellyfish *Craspedacusta sowerbii*. *Hydrobiologia*  
301 DOI: 10.1007/s10750-018-3520-4
- 302 Chandra S, Gerhardt A (2008) Invasive species in aquatic ecosystems: issue of global concern.  
303 *Aquat Invasions* 3: 1-2
- 304 Ciutti F, Flaim G, Cappelletti C (2017) The jellyfish *Craspedacusta sowerbii* Lankester, 1880: new  
305 observations in Trentino lakes and distribution in Italy (in Italian). *Biol Amb* 31: 174-177
- 306 Cotta Ramusino M (1972) On the presence of *Craspedacusta sowerbyi* Lank. In Italian waters (in  
307 French). *Bull. Fr. Piscic* 245: 147-150
- 308 DeVries D R (1992) The freshwater jellyfish *Craspedacusta sowerbyi*: a summary of its life history,  
309 ecology, and distribution. *J Freshw Ecol* 7: 7-16
- 310 Duggan I C, Eastwood K R (2012) Detection and distribution of *Craspedacusta sowerbii*:  
311 Observations of medusae are not enough. *Aquat Invasions* 7: 271-275
- 312 Dumont H J (1994) The distribution and ecology of the fresh-and brackish-water medusae of the  
313 world. *Hydrobiologia* 272: 1-12
- 314 Dussart B (1969) Les copépodes des eaux continentales d'Europe Occidentale. II. Cyclopoïdes et  
315 biologie. Boubée et Cie, Paris
- 316 Failla Siquier M G, Serra Alanis W S, Martinez Debat C (2017) First record of *Craspedacusta*  
317 *sowerbii* Lankester, 1880 (Hydrozoa, Limnomedusae) in a natural freshwater lagoon of  
318 Uruguay, with notes on polyp stage in captivity. *Braz J Biol* 77: 665-672
- 319 Fritz G B, Schill, R O, Pfannkuchen M, Brümmer F (2007) The freshwater jellyfish *Craspedacusta*  
320 *sowerbii* Lankester, 1880 (Limnomedusa: Olindiidae) in Germany, with a brief note on its  
321 nomenclature. *J Limnol* 66: 54-59
- 322 Fritz G B, Pfannkuchen M, Reuner A, Schill R O, Brümmer F (2009) *Craspedacusta sowerbii*,  
323 Lankester 1880—population dispersal analysis using COI and ITS sequences. *J Limnol* 68: 46-  
324 52.
- 325 Fuentes R, Cárdenas L, Abarzua A, Caputo L (2018) Southward invasion of *Craspedacusta*  
326 *sowerbii* across mesotrophic lakes in Chile: geographical distribution and genetic diversity for  
327 the medusa phase. *Freshw Sci in press*
- 328 Gallardo B, Clavero M, Sánchez M I, Vilà M (2016) Global ecological impacts of invasive species  
329 in aquatic ecosystems. *Global Change Biol* 22: 151-163. Gasith A, Gafny S, Hershkovitz Y,  
330 Goldstein H, Galil B S (2011). The invasive freshwater medusa *Craspedacusta sowerbii*  
331 Lankester, 1880 (Hydrozoa: Olindiidae) in Israel. *Aquat Invasions* 6 (suppl. 1): S147-S152.
- 332 Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies  
333 by maximum likelihood. *Syst Biol* 52: 696–704
- 334 He Z W (2003) A revision of the genus *Craspedacusta* in China (Limnomedusae, Olindidae). *Acta*  
335 *Zootaxonomica Sinica* 28: 20-23.
- 336 Incagnone G, Marrone F, Robba L, Barone R, Naselli-Flores L (2015). How do aquatic organisms  
337 cross the "dry ocean" and disperse among freshwater ecosystems? A review on passive  
338 dispersal mechanisms with a special focus on temporary ponds. *Hydrobiologia* 750: 103-123.  
339 doi: <http://dx.doi.org/10.1007/s10750-014-2110-3>
- 340 Jankowski T (2001) The freshwater medusae of the world—a taxonomic and systematic literature  
341 study with some remarks on other inland water jellyfish. *Hydrobiologia* 462: 91–113

342 Jankowski T, Ratte H T (2000). On the influence of the freshwater jellyfish *Craspedacusta sowerbii*  
343 on the zooplankton community. Internationale Vereinigung für Theoretische und Angewandte  
344 Limnologie Verhandlungen 27: 3287-3290

345 Jankowski T, Strauss T, Ratte H T (2005). Trophic interactions of the freshwater jellyfish  
346 *Craspedacusta sowerbii*. J Plankt Res 27: 811-823

347 Jankowski T, Collins A G, Campbell R (2008) Global diversity of inland water cnidarians.  
348 Hydrobiologia, 595: 35-40

349 Karaouzas I, Zogaris S, Lopes-Lima M, Froufe E, Varandas S, Teixeira A. Sousa R (2015) First  
350 record of the freshwater jellyfish *Craspedacusta sowerbii* Lankester, 1880 in Greece suggests  
351 distinct European invasion events. Limnology 16: 171-177

352 Kato K I, Hirabayashi S (1991) Temperature condition initiating medusa bud formation and the  
353 mode of the appearance in a fresh water hydroid, *Craspedacusta sowerbyi*. Zool Sci 8: 1107.

354 Kramp P L (1950) Freshwater medusae in China. J Zool 120: 165-184

355 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics Analysis Version  
356 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874. doi: 10.1093/molbev/msw05

357 Lockwood J L, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species  
358 invasions. Trends Ecol Evol 20: 223-228.

359 Marrone F, Naselli-Flores L (2015) A review on the animal xenodiversity in Sicilian inland waters  
360 (Italy). Advances in Oceanography and Limnology 6: 2–12. doi:10.4081/aiol.2015.5451

361 Marrone F, Barone R, Naselli-Flores L (2005) Cladocera (Branchiopoda: Anomopoda, Ctenopoda  
362 and Onychopoda) from Sicilian inland waters: an updated inventory. Crustaceana 78: 1025-  
363 1039. doi: 10.1163/156854005775361043.

364 Marrone F, Lo Brutto S, Arculeo M (2011) Cryptic invasion in Southern Europe: the case of  
365 *Ferrissia fragilis* (Pulmonata: Ancyliidae) Mediterranean populations. Biologia 66: 484-490.  
366 doi: <http://dx.doi.org/10.2478/s11756-011-0044-z>

367 Meisch C (2000) Freshwater Ostracoda of Western and Central Europe. Spektrum Akademischer  
368 Verlag, Heidelberg, Berlin.

369 Minchin D, Caffrey J M, Haberlin D, Germaine D, Walsh C, Boelens R, Doyle T K (2016) First  
370 observations of the freshwater jellyfish *Craspedacusta sowerbii* Lankester, 1880 in Ireland  
371 coincides with unusually high water temperatures. BioInvasions Records 5: 67–74

372 Morais P, Reichard M (2018) Cryptic invasions: a review. Sci Total Env 613/614: 1438-1448.

373 Morpurgo M, Alber R (2015). First record of the freshwater jellyfish *Craspedacusta sowerbii*  
374 Lankester, 1880 (Cnidaria: Hydrozoa: Limnomedusae) in South Tyrol (Italy). Gredleriana 15:  
375 61-64

376 Nylander J A A (2004) *MrModeltest 2*. Program Distributed by the Author. Evolutionary Biology  
377 Centre, Uppsala University

378 Petrusek A, Šedivý J, Lundberg S, Svensson J E (2005) Detecting multiple invasions of  
379 *Craspedacusta sowerbyi* by identification of the sex of the medusae. INWAT: Biological  
380 invasions in inland waters, Firenze, May 5-7, 2005

381 Pieri V, Martens K, Naselli-Flores L, Marrone F, Rossetti G (2006) Distribution of recent ostracods  
382 in inland waters of Sicily (Southern Italy). J Limnol 65: 1-8. doi: 10.4081/jlimnol.2006.1

383 Ramazzotti G (1962) Finding of the freshwater jellyfish *Craspedacusta sowerbyi* in the nearby of  
384 Lake Maggiore (in Italian). Mem Ist ital Idrobiol 15: 175-181

385 RealClimate (2018) <http://www.realclimate.org/index.php/archives/2016/01/2015-temperatures/> last  
386 accessed on 20 June, 2018.

387 Reisinger E (1957) On the development history and development mechanics of *Craspedacusta*  
388 (Hydrozoa, Limnotrachelina) (in German). Zoomorphology 45: 656-698

389 Ronquist F, Teslenko M, Van der Mark P, Ayres D L, Darling A, Höhna S, Larget B, Liu L,  
390 Suchard M A, Huelsenbeck J P (2012) MrBayes v. 3.2: Efficient Bayesian phylogenetic  
391 inference and model choice across a large model space. Syst Biol 61: 539–542. doi:  
392 10.1093/sysbio/sys029.

393 Saito T, Do V T, Prozorova L, Hirano T, Fukuda H, Chiba S (2018) Endangered freshwater limpets  
394 in Japan are actually alien invasive species. Cons Gen 19: 947–958

395 Smith A S, Alexander Jr J E (2008) Potential effects of the freshwater jellyfish *Craspedacusta*  
396 *sowerbii* on zooplankton community abundance. J Plankt Res 30: 1323-1327

397 Spadinger R, Maier G (1999) Prey selection and diel feeding of the freshwater jellyfish,  
398 *Craspedacusta sowerbyi*. Freshw Biol 41: 567-573.

399 Stefani F, Leoni B, Marieni A, Garibaldi L (2010) A new record of *Craspedacusta sowerbii*,  
400 Lankester 1880 (Cnidaria, Limnomedusae) in Northern Italy. J Limnol 69: 189-192

401 Stefanelli A (1948) A freshwater jellyfish of the genus *Craspedacusta* developed in a fishtank of  
402 the Institute (in Italian). Ital J Zool 15: 41-47

403 Thompson J D, Gibson T J, Plewniak F, Jeanmougin F, Higgins D G (1997) The ClustalX windows  
404 interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.  
405 Nucleic Acids Res 24: 4876–4882

406 Zhang L Q, Wang G T, Yao W J, Li W X, Gao Q (2009) Molecular systematics of medusae in the  
407 genus *Craspedacusta* (Cnidaria: Hydrozoa: Limnomedusae) in China with the reference to the  
408 identity of species. J Plankt Res 31: 563-570

409 Zou H, Zhang J, Li W, Wu S, Wang G (2012) Mitochondrial Genome of the Freshwater Jellyfish  
410 *Craspedacusta sowerbyi* and Phylogenetics of Medusozoa. PLoS ONE 7(12): e51465. doi:  
411 10.1371/journal.pone.0051465

412

<b>Chordata</b>
<b>Reptilia</b>
<i>Trachemys scripta</i> (Thunberg in Schoepff, 1792)*
<b>Pisces</b>
<i>Gambusia holbrooki</i> Girard, 1859*
<i>Carassius auratus</i> Linnaeus, 1758*
<b>Arthropoda</b>
<b>Ostracoda</b>
<i>Cypridopsis vidua</i> (O.F. Müller, 1776),
<b>Copepoda</b>
<i>Acanthocyclops trajani</i> Mirabdullayev and Defaye, 2004*
<i>Macrocyclus albidus</i> (Jurine, 1820)
<b>Branchiopoda</b>
<i>Bosmina longirostris</i> (O. F. Müller, 1776)
<i>Coronatella rectangula</i> (Sars, 1861)
<b>Insecta</b>
Diptera <i>unident.</i>
Ephemeroptera <i>unident.</i>
<b>Mollusca</b>
<b>Gastropoda</b>
<i>Physella acuta</i> (Draparnaud, 1805)*
<b>Phytoplanktic algae</b>
<b>Bacillariophyceae</b>
<i>Ulnaria acus</i> (Kützing) Aboal
<i>Nitzschia acicularis</i> (Kützing) W.Smith
<b>Chlorophyceae</b>
<i>Gonium pectorale</i> O.F.Müller
<i>Coelastrum astroideum</i> De Notaris
<i>Monactinus simplex</i> (Meyen) Corda
<i>Pediastrum duplex</i> Meyen
<i>Tetradismus lagerheimii</i> M.J.Wynne & Guiry
<i>Desmodesmus</i> sp.
<i>Golenkinia radiata</i> Chodat
<i>Monoraphidium</i> spp.
<i>Ankistrodesmus arcuatus</i> Korshikov
<b>Zygnematophyceae</b>
<i>Staurastrum pingue</i> var. <i>planctonicum</i> (Teiling) Coesel & Meersters
<i>Mougeotia</i> sp.
<b>Euglenophyceae</b>
<i>Trachelomonas</i> sp.
<b>Chrysophyceae</b>
<i>Dinobryon</i> sp.

413

414 **Tab 1.** Taxa co-occurring with *Craspedacusta sowerbii* in the study site on November 2017 (see  
415 text). \*: non-native taxon.

416

417 **Figure Captions**

418

419 **Figure 1.** *Craspedacusta sowerbii* specimen from Palermo, Italy. Photo by Enrico Schifani.

420 **Figure 2.** Bayesian consensus phylogram based on a 337-bp-long fragment of the mitochondrial  
421 gene encoding for the cytochrome oxidase subunit I (mtDNA COI). Node support is reported as  
422 nodal posterior probabilities/ML bootstrap. Accession numbers of both novel and GenBank  
423 sequences are shown in brackets. Original Sicilian sequence is reported in bold.

424 **Figure 3.** Bayesian consensus phylogram based on a 607-bp-long fragment of the nuclear internal  
425 described spacers (nuDNA ITS). Node support is reported as nodal posterior probabilities/ML  
426 bootstrap. Accession numbers of both novel and GenBank sequences are shown in brackets.  
427 Original Sicilian sequence is reported in bold.

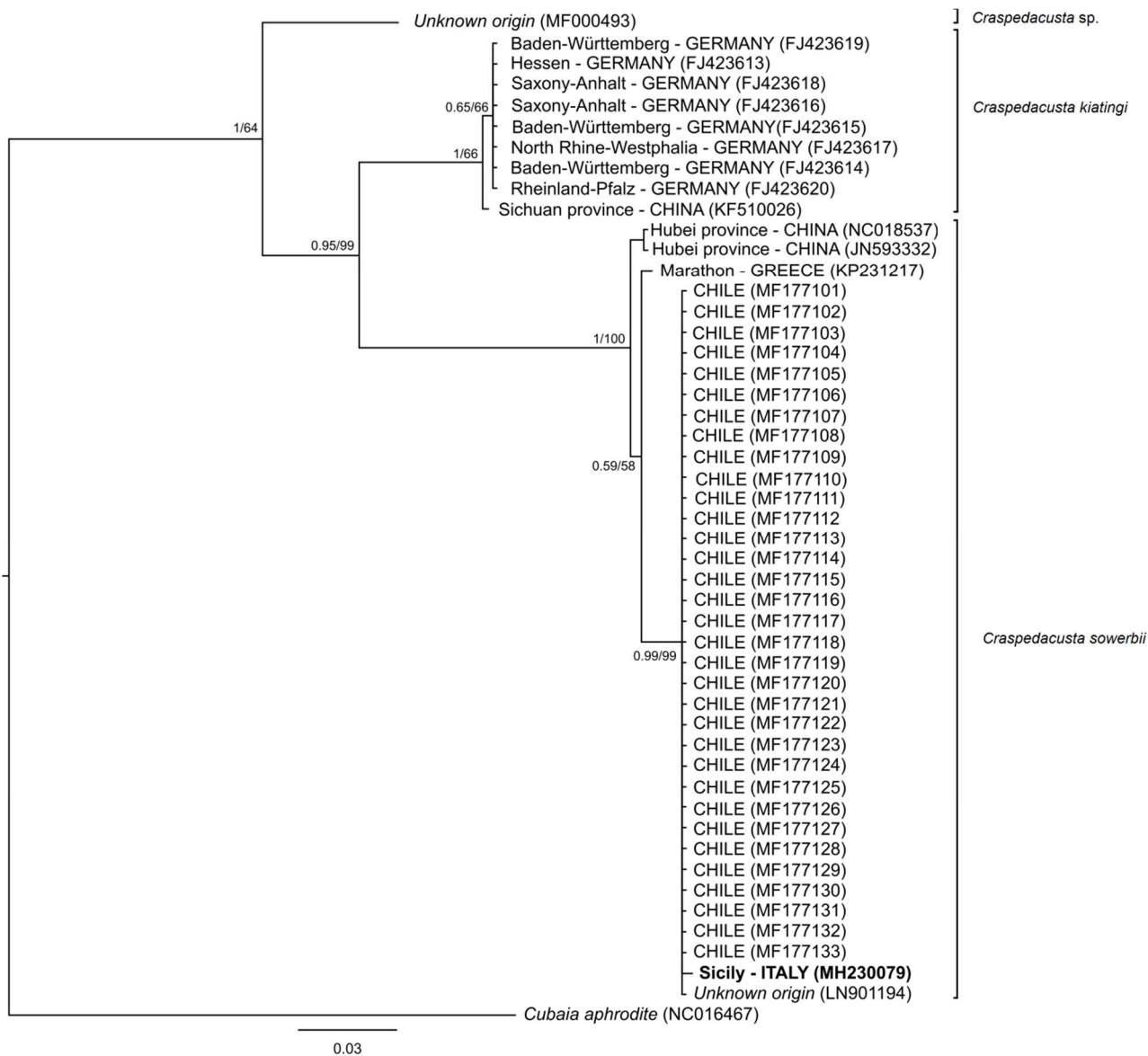
428



429

430 Figure 1

431

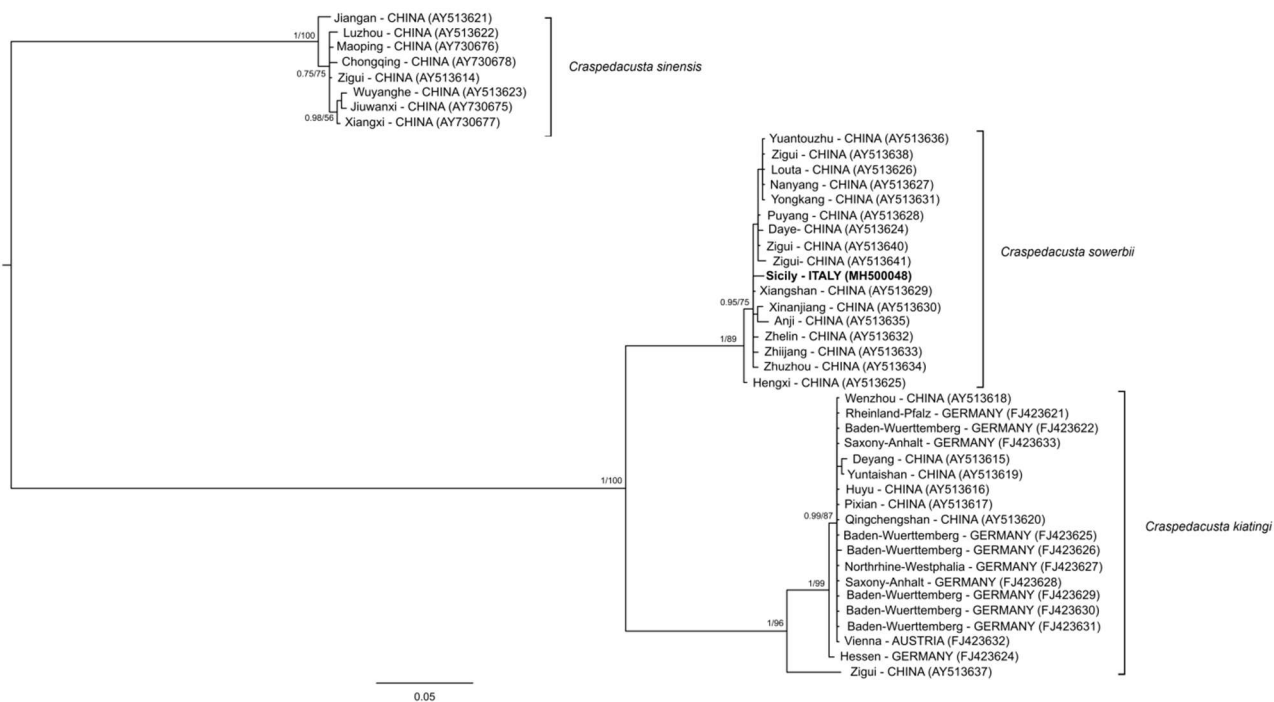


432

433 Figure 2

434





435

436 Figure 3

437

438 Electronic Supplementary Material - Different lineages of freshwater jellyfishes (Cnidaria,  
 439 Olindiidae, *Craspedacusta*) invading Europe: another piece of the puzzle from Sicily, Italy by  
 440 Schifani et al.

441

442

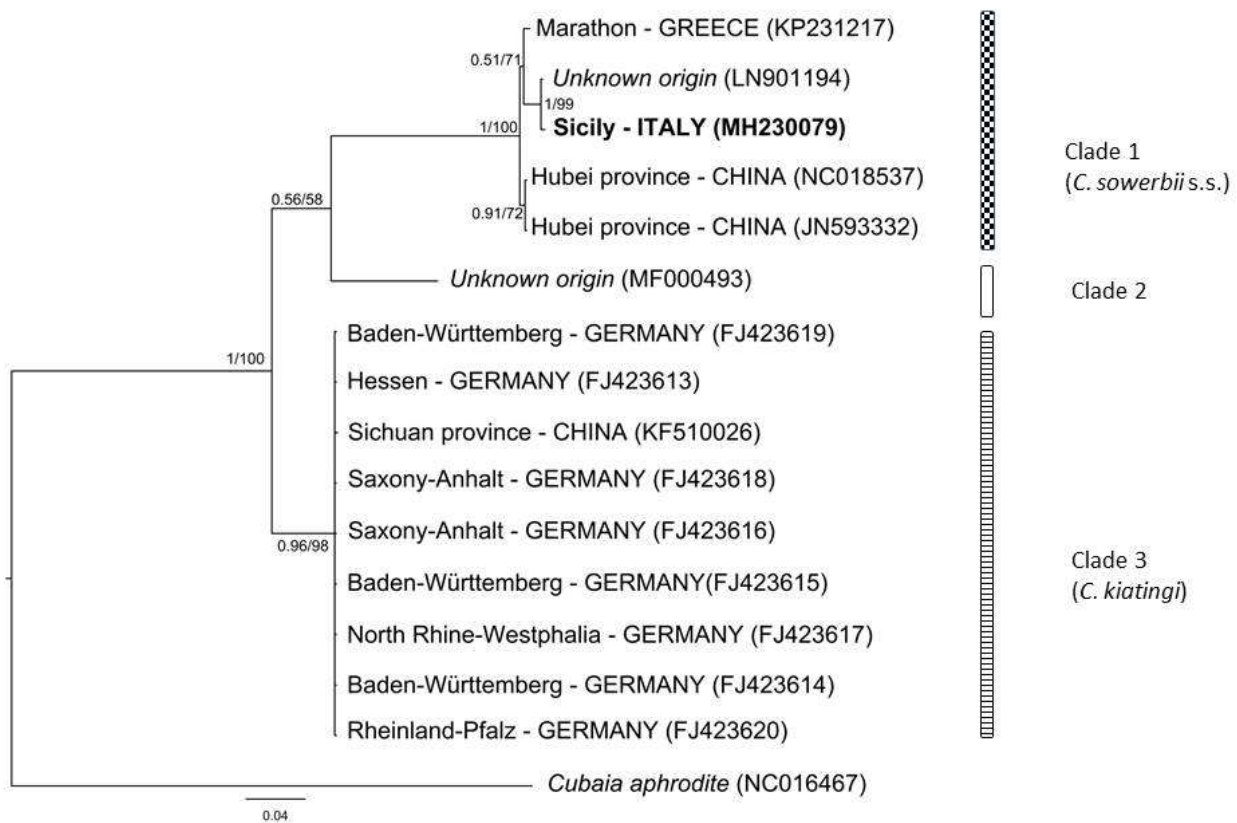
443

Variable	Unit	Value
pH		7.72
Conductivity	$\mu\text{S cm}^{-1}$	697
Hardness	$^{\circ}\text{F}$	50
Orthophosphate	$\mu\text{g L}^{-1}$	16
Nitrate	$\text{mg L}^{-1}$	7.1
Ammonium	$\text{mg L}^{-1}$	<0.05
Sulfate	$\text{mg L}^{-1}$	158
Bicarbonate	$\text{mg L}^{-1}$	172
Chlorine	$\text{mg L}^{-1}$	50.7
Sodium	$\text{mg L}^{-1}$	43.7
Total Dissolved Solids	$\text{mg L}^{-1}$	488

444

445 **Table S1.** Main chemical and physical variables of the reservoir waters (average values provided by  
 446 the Municipal Water Company, Palermo).

447



448

449 **Figure S1.** Bayesian consensus phylogram based on a 574-bp-long fragment of the mitochondrial  
 450 gene encoding for the cytochrome oxidase subunit I (mtDNA COI). Node support is reported as  
 451 nodal posterior probabilities/ML bootstrap. Accession numbers of both novel and GenBank  
 452 sequences are shown in brackets. Original Sicilian sequence is reported in bold.

453

454