Title

First record of Temnosewellia minor (Platyhelminthes, Temnocephalidae) in Sicily, with a plea for a re-examination of the identity of the publicly available molecular sequences of the genus

Journal
Biogeographia – The Journal of Integrative Biogeography, 36(0)

ISSN
1594-7629

Authors
Vecchioni, Luca
Chirco, Pietro
Bazan, Giuseppe
et al.

Publication Date
2021

DOI
10.21426/B636051182

Biogeographia – The Journal of Integrative Biogeography 36 (2021): a003
https://doi.org/10.21426/B636051182

First record of *Temnosewellia minor* (Platyhelminthes, Temnocephalidae) in Sicily, with a plea for a re-examination of the identity of the publicly available molecular sequences of the genus

LUCA VECCHIONI[1,*], PIETRO CHIRCO[1], GIUSEPPE BAZAN[1], FEDERICO MARRONE[1], VINCENZO ARIZZA[1], MARCO ARCULEO[1]

[1] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 18, 90123 Palermo (Italy)*

* *corresponding author, email: luca.vecchioni@unipa.it*

Keywords: 28S rDNA, Alien species, *Cherax destructor*, Ectosymbionts.

SUMMARY

Ectosymbiotic temnocephalan flatworms belonging to the genus *Temnosewellia* were collected on *Cherax destructor* in an aquaculture farm in Sicily, Italy. This represents the first record of a temnocephalan species for the fauna of the island. Morphological and molecular identification of the collected specimens proved that they belong to the allochthonous species *Temnosewellia minor*, which was introduced along with crayfishes bred in aquaculture farms. The phylogenetic analyses carried out for the molecular identification of the Sicilian population highlighted some inconsistencies in the grouping of the *Temnosewellia* sequences available online, thus stressing the opportunity of a careful re-examination of the voucher samples and their identifications. The risks of its unwary introduction in the wild and the need of monitoring its possible impacts on native biota are briefly discussed.

INTRODUCTION

The occurrence of non-indigenous species (NIS) is known to have a significant impact on native freshwater fauna, directly and indirectly altering the composition and structure of native communities and leading to dramatic losses of freshwater biological diversity (e.g., Peeler et al. 2011; Ricciardi and MacIsaac 2011; Arias and Burrial 2021). Introductions of allochthonous parasites and their hosts can lead to infection of native host species by the introduced parasites ("spill-over") and/or infection of the introduced hosts by the native parasite species. In the latter case, the introduced host acts as an additional reservoir for infection of native hosts, increasing disease impact on native fauna ("parasite spillback"). Both phenomena require a lot more study, as

Figure 1. The temnocephalan flatworm *Temnosewellia minor* recorded on *Cherax destructor* in a crayfish farm, in Sicily (Italy).

the extent of the impact on both introduced and native hosts is often poorly known (Kelly et al. 2009). In the last decades, several alien crayfish species have invaded European inland waters (Aquiloni et al. 2010; Weiperth et al. 2020, and references therein), often outcompeting native species. The most recent introduction in Italian inland water is that of *Cherax destructor* Clark, 1936 (Scalici et al. 2009; Deidun et al. 2018), whereas the congeneric *Cherax quadricarinatus* (von Martens, 1868), to date confined to aquaculture farms in Italy (Chiesa et al. 2015), is already reported for natural water bodies in the neighbouring Malta (Deidun et al. 2018). Although it is well-known that parastacid species are often hosts of ectosymbiotic temnocephalan flatworms (Damborenea and Brusa 2009), to date only little evidence of co-introductions of these turbellarians in European waters along with their hosts is available, both in aquaculture facilities (Quaglio et al. 1999; Chiesa et al. 2015) and in the wild (Mazza et al. 2018).

In the present study, we report the first record of *Temnosewellia minor* Haswell, 1888 (Platyhelminthes, Temnocephalida) in Sicily, occurring on *Cherax destructor* individuals cultured in a commercial aquaculture farm.

MATERIALS AND METHODS

Overall, more than 50 temnocephalan individuals were collected from the yabby *Cherax destructor* (Fig. 1) in an aquaculture

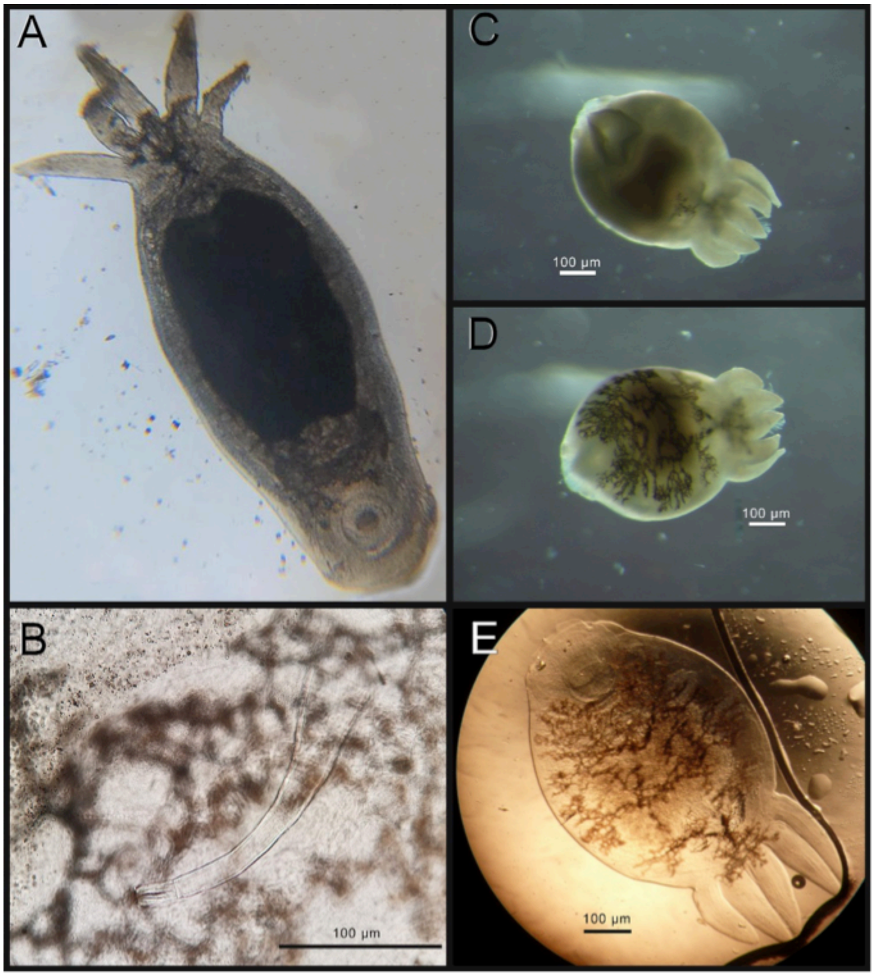

Figure 2. Stereomicroscope photographs of *Temnosewellia minor*. A, ventral view of a in vivo specimen; B, cirrus structure; C, dorsal view; D, ventral view; E, ventral view of a specimen fixed in glycerine solution.

farm located in Fiumefreddo di Sicilia (Contrada Vignagrande, province of Catania, Sicily). Specimens were identified based on the morphology of the introvert of the cirrus (Haswell 1888; Sewell et al. 2006; Damborenea and Cannon 2001), and then preserved in 90% ethanol. Twenty specimens of the collected temnocephalans were deposited at the Museo di Storia Naturale, Sezione di Zoologia "La Specola", Università di Firenze, Italy (MZUF) under the collection number MZUF PC/386; thirty further specimens are stored in the authors' collection at the University of Palermo, Italy, and are available for loan on request.

To confirm the morphology-based identification of the collected specimens, a fragment of the nuclear rDNA 28S was amplified from a single individual following the procedures described in Vecchioni et al. (2021), sequenced by Macrogen inc. (SPAIN), and uploaded to the public database GenBank (Accession Number, A.N.: MW314801). Moreover, in order to compare the new sequence with those publicly available, a selection of 35 further 28S rDNA sequences were downloaded from GenBank and included in the analyses (see Fig. 3 for their GenBank A.N.). In addition, a sequence of *Didymorchis* sp. was downloaded from GenBank and included in the analyses to be used as outgroup (GenBank A.N. AY157163). Novel and GenBank sequences were aligned with MEGAX (Kumar et al. 2018), and the inference about the phylogenetic relationships among the taxa, based on neighbour-joining (NJ) and bayesian inference (BI), was performed following the protocol described by Vecchioni et al. (2019). Unfortunately, some of the publicly available 28S GenBank sequences belonging to *Temnosewellia* spp. could not be included in present dataset due to the amplification of different, poorly overlapping 28S fragments in different studies (e.g., Chiesa et al. 2015; Hoyal Cuthill et al. 2016).

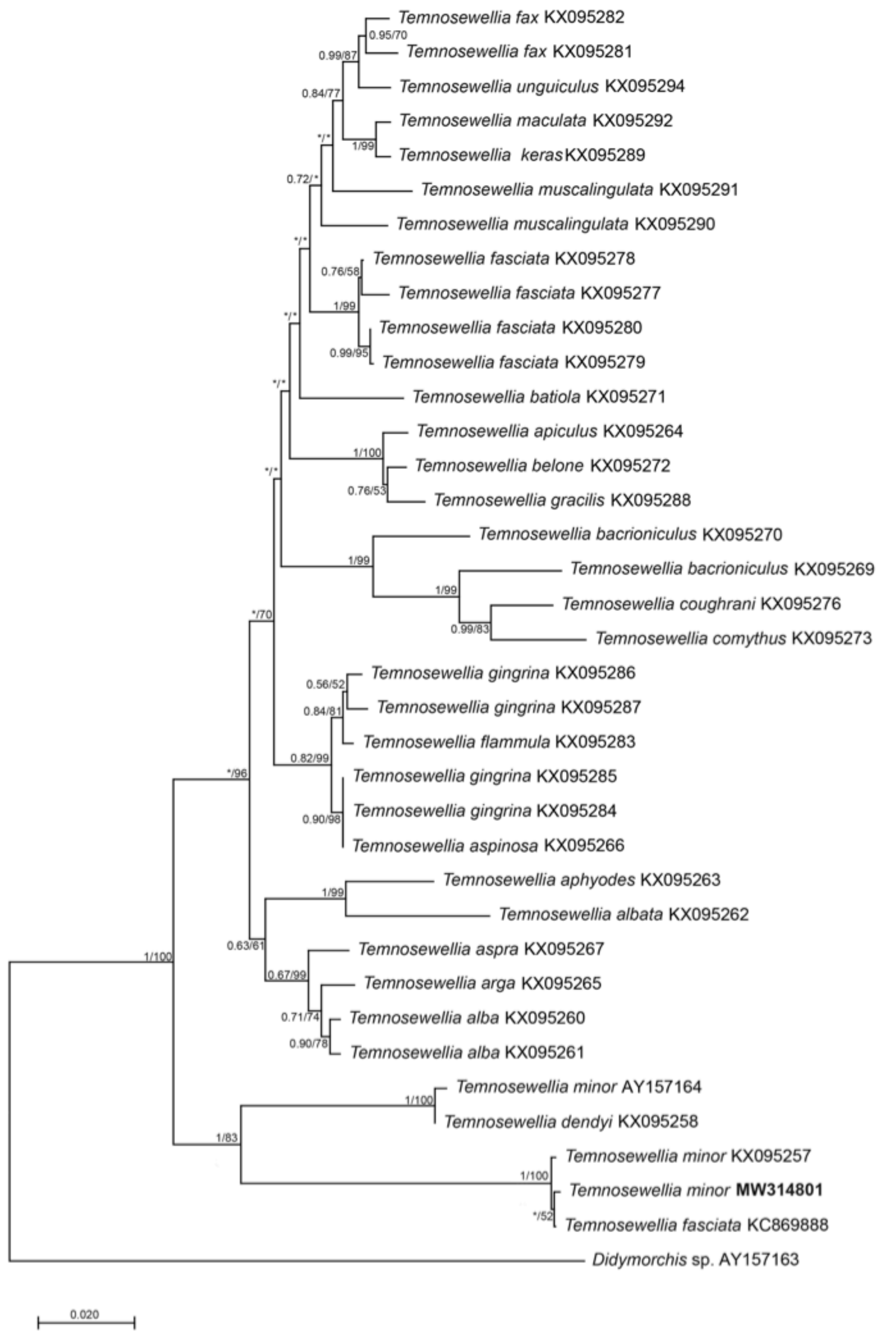

Figure 3. Neighbor-Joining tree of the *Temnosewellia* species based on a 731-bp-long fragment of the nuclear 28S rDNA gene, obtained using Kimura-2-parameter distance model. *Didymorchis* sp. (GenBank A.N. AY157163) was used as outgroup. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI) and bootstrap values (neighbour-joining, NJ). *, Nodal statistical supports <0.50. Our novel sequence is reported in bold.

RESULTS

The morphological identification of the collected specimens allowed us to identify the collected temnocephalans as *Temnosewellia minor* based on the introvert of the cirrus (i.e., the male copulatory organ - Fig. 2B), considered one of the diagnostic characters (Cannon and Sewell 2001; Damborenea and Cannon 2001; Sewell et al. 2006) (Figs. 1 & 2).

The inferred NJ and BI phylogenetic trees showed a congruent clustering of the analysed sequences, which are grouped in monophyletic clades mostly in accordance with their alleged identifications (Fig. 3). Nevertheless, some exceptions were observed. First, our novel sequence falls within a clade that includes sequences labelled on GenBank as *T. minor* and *T. fasciata* (Haswell, 1888) (GenBank A.N.: KC869888). The other four sequences of *T. fasciata* form an unrelated, well-supported clade. Moreover, the single sequence identified as *T. minor* by Lockyer et al. (2003) (GenBank A.N. AY157164) clusters together with the single available sequence for *T. dendyi* (Haswell, 1893). Finally, *T. gingrina* Sewell, Cannon and Blair, 2006 seems paraphyletic because of the position of *T. aspinosa* Sewell, Cannon and Blair, 2006 and *T. flammula* Sewell, Cannon and Blair, 2006 (Fig. 3).

DISCUSSION

1. Identification

Present morphological and molecular results suggest that the observed Sicilian temnocephalan population belongs to *Temnosewellia minor* (Figs. 2 & 3). However, the phylogenetic position of two sequences ascribed to *T. fasciata* and *T. minor* (GenBank A.N. KC869888 and AY157164, respectively) raised doubts of possible misidentification of the specimens used to produce both of them. We here thus propose that these last sequences have to be considered as mislabelled, and likely to be actually ascribed to *T. minor* and *T. dendyi*, respectively. However, the alternative hypothesis that the current pattern might be at least partly ascribable to hybridization or introgressive phenomena might also be considered. In addition, the phylogenetic relationships between sequences ascribed to *T. gingrina*, *T. aspinosa* and *T. flammula* (Fig. 3) seem to be in contrast with the results shown by Hoyal Cuthill et al. (2016), although this decoupling might be due to the conservative nature of the single marker used in the frame of the present study.

In light of the possible misidentification of some samples, a comprehensive revision of the taxonomy and identification of the temnocephalans for which sequences are available on public databases is of paramount importance. This should be done implementing an approach combining a careful morphological re-examination of the specimens along with their molecular characterization using standard primer pairs (e.g., “Ltem180” and “Ltem1000R” used by Hoyal Cuthill et al. (2016)), and the deposit of voucher specimens in public repositories.

2. Conservation issues

To date, the life cycle of most temnocephalid species is poorly known, and there is no clear evidence on actual negative effects associated with the presence of these ectosymbiotic organisms on their host species. Nevertheless, for the precautionary principle, the possible spreading in Sicilian natural water bodies of *Temnosewellia minor* should be carefully prevented. In fact, it would be a new alien species for Sicilian inland water fauna (Marrone and Naselli-Flores 2015), and it might affect the autochthonous decapods occurring on the islands, as the freshwater crab *Potamon fluviatile* (Herbst, 1785) (Vecchioni et al. 2017; Marrone et al. 2020). Once in the wild, *T. minor* could possibly also take advantage of the spreading occurrence on the island of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Faraone et al. 2017), which is known to be a suitable host for *T. minor* (Mazza et al. 2018), and might thus act as a dispersal vector and biological reservoir for the temnocephalid. The monitoring of all feral and cultured non-native crayfish populations in Sicily is crucial in order to be able to promptly intervene when needed, thus managing the possible biological invasions of alien crayfish and their symbionts or parasites at an early stage, reducing their negative effect on the local fauna (e.g., Quaglio et al. 1999; Marino et al. 2014; Mrugała et al. 2016; Madzivanzira et al. 2020).

ACKNOWLEDGEMENTS

S. Cianfanelli (Museum of Natural History, University of Florence, Italy) and A. Milano (Dipartimento Pesca Mediterranea – Regione Siciliana, Italy) are acknowledged for the support provided during the present research activity. G. Badalamenti (Palermo, Italy) is acknowledged for having provided the *T. minor* on *C. destructor* photograph included in the present manuscript. This research was supported by the fund "POFEAMP 02/INA/17S" of the Sicily Region.

AUTHOR CONTRIBUTIONS

MA planned and headed the project. PC carried out the field work. LV carried out the laboratory work and the analyses. LV and FM wrote a first draft of the manuscript, which was discussed and improved by all of the authors.

REFERENCES

Aquiloni, L., Tricarico, E. & Gherardi, F. (2010) Crayfish in Italy: distribution, threats and management. International Aquatic Research, 2, 1–14.

Arias, A. & Torralba-Burrial, A. (2021) First record of the redclaw crayfish *Cherax quadricarinatus* (Von Martens, 1868) on the Iberian Peninsula. Limnetica, 40, 33–42. DOI: 10.23818/limn.40.03

Cannon, L.R.G. & Sewell, K.B. (2001) A review of *Temnosewellia* (Platyhelminthes) ectosymbionts of *Cherax* (Crustacea: Parastacidae) in Australia. Memoirs of the Queensland Museum, 46(2), 385–399.

Chiesa, S., Scalici, M., Lucentini, L. & Marzano, F.N. (2015) Molecular identification of an alien temnocephalan crayfish parasite in Italian freshwaters. Aquatic Invasions, 10, 209–216. DOI: 10.3391/ai.2015.10.2.09

Damborenea, C. & Brusa, F. (2009) A new species of *Temnosewellia* (Platyhelminthes, Temnocephalida) ectosymbiont on *Villopotamon thaii* (Crustacea, Decapoda, Potamidae) from Vietnam. Zoosystema, 31(2), 321–332. DOI: 10.5252/z2009n2a5

Damborenea, M.C. & Cannon, L.R.G. (2001) On neotropical *Temnocephala* (Platyhelminthes). Journal of Natural History, 35, 1103–1118. DOI: 10.1080/00222930152434454

Deidun, A., Sciberras, A., Formosa, J., Zava, B., Insacco, G., Corsini-Foka, M. & Crandall, K.A. (2018) Invasion by non-indigenous freshwater decapods of Malta and Sicily, central Mediterranean Sea. Journal of Crustacean Biology, 38(6), 748–753. DOI: 10.1093/jcbiol/ruy076

Faraone, F.P, Giacalone, G., Canale, D.E, D'Angelo, S., Favaccio, G., Garozzo, V., et al. (2017) Tracking the invasion of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda Cambaridae) in Sicily: a "citizen science" approach. Biogeographia – The Journal of Integrative Biogeography, 32, 25–29. DOI: 10.21426/B632135512.

Haswell, W.A. (1888) Temnocephala, an aberrant monogenetic Trematode. Quarterly Journal of Microscopical Science, 28, 279–302.

Hoyal Cuthill, J.F., Sewell, K.B., Cannon, L.R.G., Charleston, M.A., Lawler, S., Littlewood, D.T.J., Olson, P.D. & Blair, D. (2016) Australian spiny mountain crayfish and their temnocephalan ectosymbionts: an ancient association on the edge of coextinction? Proceedings of the Royal Society B: Biological Sciences, 283, 20160585. DOI: 10.1098/rspb.2016.0585

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R. & Tompkins, D.M. (2009) Parasite spillback: a neglected concept in invasion ecology? Ecology, 90(8), 2047–2056. DOI: 10.1890/08-1085.1

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35(6), 1547–1549. DOI: 10.1093/molbev/msy096

Lockyer, A.E., Olson, P.D. & Littlewood, D.T.J (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biological Journal of the Linnean Society, 78, 155–171. DOI: 10.1046/j.1095-8312.2003.00141.x

Madzivanzira, T.C., South, J., Wood, L.E., Nunes, A.L. & Weyl, O.L.F. (2020) A review of freshwater crayfish introductions in Africa. Reviews in Fisheries Science & Aquaculture, DOI: 10.1080/23308249.2020.1802405

Marino, F., Pretto, T., Tosi, F., Monaco, S., De Stefano, C., Manfrin, A. & Quaglio, F. (2014) Mass mortality of *Cherax quadricarinatus* (von Martens, 1868) reared in Sicily (Italy): crayfish plague introduced in an intensive farming. Freshwater Crayfish, 20, 93–96. DOI: 10.5869/fc.2014.v20-1.93

Marrone, F., Vecchioni, L., Deidun, A., Mabrouki, Y., Abdeslam, A. & Arculeo, M. (2020) DNA taxonomy of the potamid freshwater crabs from Northern Africa (Decapoda, Potamidae). Zoologica Scripta, 49, 473–487. DOI: 10.1111/zsc.12415

Marrone, F., & Naselli-Flores, L. (2015) A review on the animal xenodiversity in Sicilian inland waters (Italy). Advances in Oceanography and Limnology, 6(1-2). DOI: 10.4081/aiol.2015.5451

Mazza, G., Scalici, M., Inghilesi, A.F., Aquiloni, L., Pretto, T., Monaco, A. & Tricarico, E. (2018) The Red Alien vs. the Blue Destructor: The eradication of *Cherax destructor* by *Procambarus clarkii* in Latium (Central Italy). Diversity, 10, 126. DOI: 10.3390/d10040126

Mrugała, A. Lukáš Veselý, L., Petrusek, A., Viljamaa-Dirks, S. & Kouba, A. (2016) May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe? Aquatic Invasions, 11(4), 459–468. DOI: 10.3391/ai.2016.11.4.10

Peeler, E.J., Oidtmann, B.C., Midtlyng, P.J., et al. (2011) Non-native aquatic animals introductions have driven disease emergence in Europe. Biological Invasions, 13, 1291–1303. DOI: 10.1007/s10530-010-9890-9

Quaglio, F., Trentini, M., Mazzoni, D. & Nobile, L. (1999) Observations on Temnocephalid (Platyhelminthes) ectosymbionts in Australian Freshwater crayfish (*Cherax destructor* Clark) introduced in Italy. Freshwater Crayfish, 12(1), 335–342.

Ricciardi, A. & MacIsaac, H.J. (2011) Impacts of biological invasions on freshwater ecosystems. In: Fifty years of invasion ecology: the legacy of Charles Elton. (ed. by D.M. Richardson), pp. 211–224. Wiley-Blackwell, Oxford, UK.

Scalici, M., Chiesa, S., Gherardi, F., Ruffini, M., Gibertini, G. & Nonnis Marzano, F. (2009) The new threat to Italian inland waters from the alien crayfish "gang": the Australian *Cherax destructor* Clark, 1936. Hydrobiologia, 632, 341–345. DOI:10.1007/s10750-009-9839-0

Sewell, K.B., Cannon, L.R.G. & Blair, D. (2006) A review of *Temnohaswellia* and *Temnosewellia* (Platyhelminthes: Temnocephalida: Temnocephalidae) ectosymbionts from Australian crayfish *Euastacus* (Parastacidae). Memoirs of the Queensland Museum, 52, 199–279.

Vecchioni, L., Arculeo, M., Cottarelli, V. & Marrone, F. (2021) Range-wide phylogeography and taxonomy of the marine rock pools dweller *Tigriopus fulvus* (Fischer, 1860) (Copepoda, Harpacticoida). Journal of Zoological Systematics and Evolutionary Research, *in press*. DOI: 10.1111/jzs.12457

Vecchioni, L., Deidun, A., Sciberras, J., Sciberras, A., Marrone, F. & Arculeo, M. (2017) The late Pleistocene origin of the Italian and Maltese populations of *Potamon fluviatile* (Malacostraca: Decapoda): insights from an expanded sampling of molecular data. The

European Zoological Journal, 84, 575–582. DOI: 10.1080/24750263.2017.1405084

Vecchioni, L., Marrone, F., Belaiba, E., Tiralongo, F., Bahri-Sfar, L. & Arculeo, M. (2019) The DNA barcoding of Mediterranean combtooth blennies suggests the paraphyly of some taxa (Perciformes, Blenniidae). Journal of Fish Biology, 94, 339–344. DOI: 10.1111/jfb.13897.

Weiperth, A., Bláha, M., Szajbert, B., Seprős, R., Bányai, Z., Patoka, J. & Kouba, A. (2020) Hungary: a European hotspot of non-native crayfish biodiversity. Knowledge & Management of Aquatic Ecosystems, 421, 43. DOI: 10.1051/kmae/2020035

Submitted: 15 December 2020

First decision: 6 March 2021

Accepted: 8 March 2021

Edited by Diego Fontaneto