Evidence of an established population of Poecilia reticulata and Neocaridina davidi in metropolitan France

Article *in* Cybium: International Journal of Ichthyology · January 2025

DOI: 10.26028/cybium/2024-034

5 authors, including:

Tom Jamonneau
Université Toulouse III - Paul Sabatier
6 PUBLICATIONS **4** CITATIONS
SEE PROFILE

Andrea C. Reyes Camargo
Université de Montpellier
1 PUBLICATION **0** CITATIONS
SEE PROFILE

Ilhan Ben Halima
Université de Montpellier
1 PUBLICATION **0** CITATIONS
SEE PROFILE

Sebastian Prati
University of Duisburg-Essen
36 PUBLICATIONS **200** CITATIONS
SEE PROFILE

Evidence of an established population of *Poecilia reticulata* and *Neocaridina davidi* in metropolitan France

by

Tom JAMONNEAU* (1, 2), Andrea C. REYES CAMARGO (1, 3), Ilhan BEN HALIMA (1), Sebastian PRATI (4, 5) & Gaël P.J. DENYS (6, 7)

Submitted: 21 May 2024
Accepted: 26 Sep. 2024
Pub. Online:
Editor: N. Poulet

Key words
Poeciliidae
Atyidae
Biological invasions
Exotic species
Ornamental trade
DNA barcoding

Abstract. – An established population of Trinidadian guppies (*Poecilia reticulata*) and Cherry shrimp (*Neocaridina davidi*) has been observed in thermal waters in the Parc des Thermes of Juvignac, near Montpellier (Southern France). This is the first record of these two species in metropolitan France. Their identity has been morphologically and molecularly confirmed. According to locals, individuals of *P. reticulata* have been thriving in the study site at least since 2019, and those of *N. davidi* since 2021. The relatively long presence of both species, the high proportion of ovigerous females, and the presence of juveniles suggest favourable conditions for the reproduction of both species. Morphological trait heterogeneity in *P. reticulata* and *N. davidi* individuals indicates either a single release of different pools of individuals or multiple releases via aquarium dumping. The thermal water body may be temporarily connected to the nearby Mosson River, favouring the spread of both species. *P. reticulata* may only survive in the Mosson River during the summer months, while the more thermally adaptable *N. davidi* may survive throughout the year. These species are known to have multidimensional impacts on ecosystems and are vectors for pathogens that might impact native biota; therefore, close monitoring of the site is urged.

Résumé. – Mise en évidence d'une population établie de *Poecilia reticulata* et de *Neocaridina davidi* en France métropolitaine

Une population établie de guppy commun (*Poecilia reticulata*) et de néocaridine de David (*Neocaridina davidi*) a été observée dans un plan d'eau et un canal alimentés par une source thermale dans le Parc des Thermes de Juvignac, situé dans le sud de la France, près de Montpellier. Il s'agit du premier signalement de ces deux espèces en France métropolitaine. Leur identité a été confirmée par des approches morphologiques et moléculaires. Selon les habitants locaux, des individus de *P. reticulata* vivent sur le site depuis au moins 2019 et on y retrouve des individus de *N. davidi* depuis au moins 2021. La présence relativement longue de ces deux espèces sur le site, la forte proportion de femelles ovigères et la présence de juvéniles, suggèrent des conditions favorables à la reproduction de ces deux espèces. L'hétérogénéité des traits morphologiques des individus de *P. reticulata* comme de *N. davidi* plaide en faveur d'un unique relâché avec des individus d'origines différentes, ou de multiples relâchés, probablement à partir d'aquariums de particuliers. Comme la masse d'eau du site peut être temporairement connectée à la rivière Mosson, située à proximité, la propagation de ces deux espèces pourrait être possible pendant les mois les plus chauds. *P. reticulata* pourrait ne survivre dans la Mosson que durant cette période, alors que *N. davidi*, qui a une meilleure capacité d'adaptation thermique, pourrait y persister toute l'année. Ces deux espèces sont connues pour leurs multiples impacts écosystémiques et sont par exemple des vecteurs de pathogènes. Un suivi étroit du site est donc nécessaire et urgent.

INTRODUCTION

The release of non-native and potentially invasive species in freshwater ecosystems often alters ecosystem functioning, for instance, negatively impacting the abundance and diversity of native species (Cucherousset and Olden, 2011; Weber and Traunspurger, 2016; Britton *et al*., 2023). Such decline is generally linked to biotic interactions (*e.g.*, competition and predation), pathogen transmission, genetic introgression and changes in habitat structure, nutrients, and organic matter (Cucherousset and Olden, 2011; Gallardo *et al*., 2016; Britton *et al*., 2023; Lambea-Camblor *et al*., 2023). The increased popularity of the aquarium hobby, including metropolitan France, makes of pet trading an emerging path-

(1) Université de Montpellier II, Place Eugène Bataillon, 34095 Montpellier, France. tommalraux@gmail.com, andreacaro2616@gmail.com, ilhanbh@gmail.com
(2) Association Guibétois, 211 chemin du Triol, 34343 Viols-le-Fort, France.
(3) Reserva: The Youth Land Trust, P.O. Box 57277, Washington, D.C. 20037, USA.
(4) Department of Aquatic Ecology, University of Duisburg-Essen, Universitätstr. 5, 45141 Essen, Germany. sebastian.prati@uni-due.de
(5) Centre for Water and Environmental Research, University of Duisburg-Essen, Universitätsstr. 2, 45141 Essen, Germany.
(6) UAR Patrimoine Naturel – Centre d'expertise et de données (2006 OFB – CNRS – MNHN – IRD), Muséum national d'Histoire naturelle, 36 rue Geoffroy-Saint-Hilaire CP 41, 75005 Paris, France. gael.denys@mnhn.fr
(7) UMR Biologie des organismes et écosystèmes aquatiques (BOREA 8067), MNHN, CNRS, IRD, SU, UCN, UA, 57 rue Cuvier CP26, 75005 Paris, France.
* Corresponding author

way for the introduction of non-native species (van Kleef *et al.*, 2008; Patoka *et al.*, 2018).

Aquarium dumping refers to releasing aquatic pets into natural water bodies. This practice tends to be biased towards the cheapest and most successful pets, which are also generally the most prolific and environmentally adaptable species (Gippet and Bertelsmeier, 2021). Among the most common and successful aquarium pets, there are fish such as the Trinidadian (or common) guppy *Poecilia reticulata* Peters, 1859 (Teleostei, Cyprinodontiformes) and crustaceans such as the cherry shrimp *Neocaridina davidi* Bouvier, 1904 (Malacostraca, Decapoda). Their success lies in their low prices, vibrant colours and easy maintenance and reproduction, which make them the ideal candidates for both experienced and novice aquarium hobbyists (Maceda-Veiga *et al.*, 2013; Magalhães and Jacobi 2013; Maciaszek *et al.*, 2018).

Poecilia reticulata originates from South America, namely Venezuela, Guyana and the island of Trinidad and Tobago (de Bragança *et al.*, 2020). However, over the last 150 years, it has been introduced worldwide due to aquarium releases and its use as biological control of mosquito larvae, establishing self-sustaining populations in at least 70 countries (Deacon, 2010; FAO, 2024); sometimes negatively impacting the native fauna (Deacon, 2010; Holitzki *et al.*, 2013; de Bragança *et al.*, 2020). In continental Europe, established populations of *P. reticulata* have been reported from Albania, Czech Republic, Germany, Hungary, Slovakia, Netherlands, Romania, Poland, Slovakia, Spain, UK and Ukraine (Elvira and Almodóvar, 2001; Nowak *et al.*, 2008; Deacon *et al.*, 2011; Nekrasova *et al.*, 2023). Except for a population in southern Spain, *P. reticulata* has failed to establish outside geothermal springs or thermally polluted water bodies due to the relatively low winter temperatures of continental Europe (Elvira and Almodóvar, 2001).

Neocaridina davidi, a shrimp of Southeast Asian origins, is more tolerant to colder temperatures than *P. reticulata*, as water in its natural habitat (mainland of China and Taiwan) ranges from 6°C in winter to 30°C during summer (Klotz *et al.*, 2013). It has been introduced elsewhere in Asia, Europe and North America through aquarium releases and its use as fishing bait (Onuki and Fuke, 2022). In continental Europe, self-sustaining populations of pet trade origin are known from thermal waters of Germany, Hungary, Poland and Slovakia (Klotz *et al.*, 2013; Jabłońska *et al.*, 2018; Weiperth *et al.*, 2019; Prati *et al.*, 2024b). However, recent findings indicate their spreading toward cooler waters (Schneider *et al.*, 2022; Prati *et al.*, 2024b). Such expansion may be due to their adaptation to cooler conditions and/or increased temperatures resulting from global warming.

In the foreseeable future, global warming will likely enhance habitat availability for *N. davidi* and *P. reticulata* in Europe (Nekrasova *et al.*, 2021a; Prati *et al.*, 2024b). Therefore, monitoring and reporting newly established populations is crucial to prevent possible adverse impacts on native biota. On the 7th of October 2023, some Poeciliid fish individuals similar to *Poecilia* sp. and shrimp likely to be *Neocaridina* sp. were observed in a thermal spring in southern France (Herault department). In this article, we report the first established population of *P. reticulata* and *N. davidi* in metropolitan France.

MATERIAL AND METHODS

Study site

The study site is located in the "Parc des thermes" (*i.e.*, thermal park) of the municipality of Juvignac (Herault, France; 43.627707, 3.811910). The earliest records mentioning the beginning of the park date back to the Gallo-Roman era, when it was surrounded by agricultural fields (Juvignac municipality, 2023). In 1986, it became municipal property and a free source of water. Despite the opening of a thermal bath in 2014 and its subsequent closure in 2021 due to the COVID-19 pandemic, the park remains very popular among locals.

In the thermal park, there is a small circular pond (up to 40 cm water depth) fed by thermal waters. The pond provides an ideal breeding ground for warm-adapted exotic species, as temperature seems to remain above 20°C all year round. Pond waters flow in a canal (Fig. 1) which in turn flows towards the Mosson River for around 70 m, before culminating in a T-shape, with left and right branches, each progressing for 20-30 m parallel to the river. In this last section, the water becomes progressively shallower on both sides, likely seeping underground and joining the Mosson. However, during high water events, the water of the canal may flow through temporary overflow ditches, which shortly end in the Mosson River (Fig. 1), favouring the spread of exotic species.

Sampling

Sampling was conducted five times between December 20th, 2023 and March 4th, 2024 using one 2L baited bottle per sampling event, according to the departmental French Fishing Act. The bottle was baited with earthworms and submerged. Fish and shrimp were euthanised using ice submersion (Wallace *et al.*, 2018) and photographed, some of them being ultimately fixed in 96% ethanol for later morphological and molecular identification. Poeciliid fish and *Neocaridina* sp. shrimp photographs were taken with an Olympus Tough TG-7 camera and edited using Adobe Photoshop (2020) and Illustrator (2021). The photograph of the poecilid gonopodium was taken using an Olympus SZX7 stereomicroscope equipped with an MC170 Leica camera. Temperature and pH were measured at the sampling sites using a Lutron PH-222.

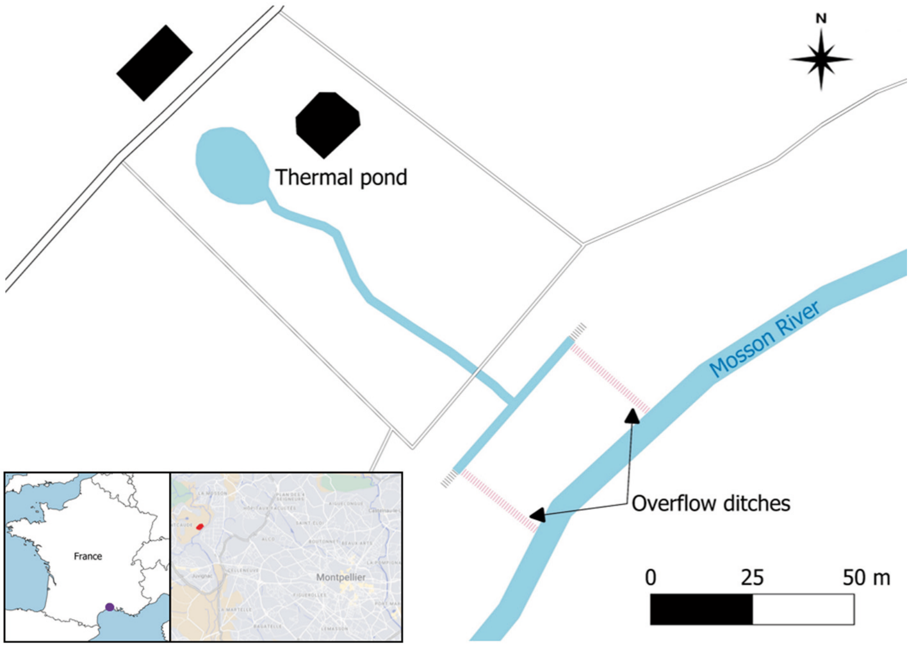

Figure 1. – Map of the study site. Gray dashed lines represent areas where the water seems to seep underground while rose dashed lines represent overflow ditches temporarily connecting the thermal water body with the Mosson River during high water events. Water flows from right to left in the Mosson. On the bottom-left corner, the map on the left represents the location of Montpellier (violet dot); and the map on the right represents the exact location of the study site (red polygon) within Montpellier and its suburbs.

Identification

Preserved guppy and shrimp specimens were identified based on morphological and molecular data. Guppies' morphological identification followed Poeser *et al.* (2005), including male's gonopodium morphology which is required for poecilid identification (*e.g.*, Ghedotti, 2000). Shrimps identification was made according to the morphological keys of Englund and Cai (1998) and Klotz *et al.* (2013). Eleven guppy specimens were conserved in the ichthyological collections of the Muséum national d'Histoire naturelle (MNHN, Paris) with the catalogue number MNHN-IC-2024-0095. DNA barcoding analyses of fish and shrimp were based on the mitochondrial marker cytochrome c-oxidase subunit 1 (COI) (Hebert *et al.*, 2003).

DNA extraction, PCR, sequencing and sequence editing followed Denys *et al.* (2014) and Prati *et al.* (2023). The generated sequences were submitted to NCBI GenBank (accession number PP593877 to PP593878 and PQ483117 to PQ483123). The sequences obtained were compared against NCBI GenBank records using BLASTN (blast.ncbi.nlm.nih.gov). Sequences were aligned with a molecular DataFrame of reference (Annex 1 and Annex 2) using the MAFFT v7.490 algorithm with standard settings (Katoh *et al.*, 2019). A Maximum likelihood phylogenetic tree with bootstrap support values (1000 replicates) was then produced in IQ-Tree 2.2.2.6 (Minh *et al.*, 2020) using the HKY+F+G4 (fish) and TPM2u+F+G4 (shrimp) substitution models based on Bayesian Information Criterion. The outgroup sequences used were *Poecilia vivipara* Bloch & Schneider, 1801 (GenBank accession number GU701911) and *Neocaridina iriomotensis* Naruse, Shokita & Cai, 2006 (GenBank accession number LC659943), respectively. The names of *N. davidi* clades are based on the work of Onuki and Fuke (2022) and the names of haplotypes on the work of Prati *et al.* (2024b).

RESULTS

The Poeciliid sample consisted of four males, four females and three immature individuals (Fig. 2), while that of shrimp consisted of 19 females, eight of which were ovigerous, 17 males and four immature individuals (Fig. 3).

The fish size ranged between 8.3 and 19.8 mm standard length, with females (14.9-19.8 mm) tending to be larger than males (14.7-17.7 mm). The caudal fin of male poecilid presented various shapes, lengths and colouration patterns. The body presented two or three black spots (behind the opercles, before the insertion of the caudal fin and sometimes on the belly, at the level of the anal fin), and an iridescent blue colouration on the jaws as well as orange marks on the caudal peduncle. Females had nine anal and six/seven dorsal fin rays. Male's gonopodium presented an absence of retrorse hook on the tip of gonopodial ray No. 3 which has also a series of ventral serrae and an eleven retrorse hook on the tip of gonopodial ray No. 5 (Fig. 4). These characteristics led to the identification of *P. reticulata*, allowing to discriminate this species from other poecilids sold on the pet trade or occurring in France, *e.g.*, *Poecilia wingei* Poeser, Kempkes & Isbrücker, 2005 (pers. obs.) or *Gambusia holbrooki* Girard, 1859 (Keith *et al.*, 2020). Similarly, shrimp size ranged from 1.76 to 6.51 mm carapax length, with females (5.32 ± 0.55 mm) tending to be larger than males (4.36 ±

Figure 2. – Freshly euthanised male (**A**, **B**, **C**) and female (**D**, **E**, **F**) specimens of *Poecilia* caught in the thermal park of Juvignac on April 16th, 2024; showing their morphological variability. Within them, (D) is a gravid female.

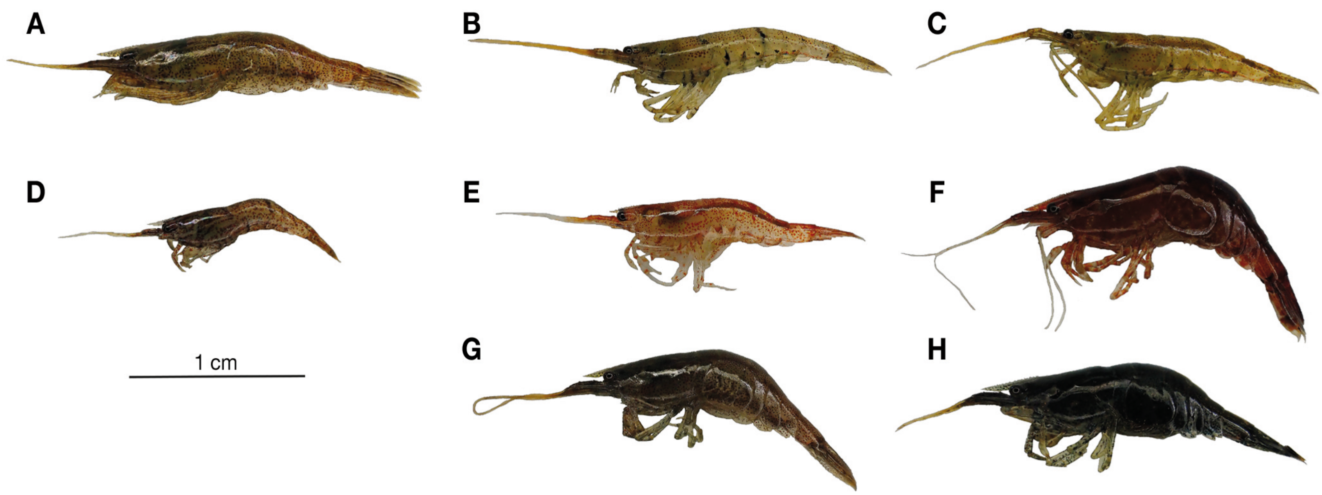

Figure 3. – Freshly euthanised green-brownish (**A**, **B**, **C**), reddish (**D**, **E**, **F**) and blackish (**G**, **H**) specimens of *Neocaridina davidi* caught in the thermal park of Juvignac on April 16th, 2024. Within them females (A, G, F, H), males (B, C) and an immature individual (D).

0.61 mm). The colouration of shrimps varied widely, including transparent brownish, greenish, yellowish, blackish and red individuals. The presence of sexual dimorphism in the third pereiopods and a shorter appendix interna of the male second pleopod, identified the shrimp as *Neocardina davidi* according to Shih *et al*. (2024).

Morphological identifications were corroborated by molecular identifications. The ML phylogenetic tree with the 34 COI sequences (644 bp) puts the guppy individuals from the thermal park of Juvignac within the *Poecilia reticulata* clade (Fig. 5). Our seven specimens are grouped into four haplotypes: two single ones, a third one shared with specimens introduced in Brazil, China, India, Indonesia, Malaysia and Uganda, and a last one shared with introduced specimens in Germany. Shrimps were molecularly identified as *Neocaridina davidi* (syn. *N. denticulata sinensis* and *N. heteropoda*) and belonged to two different haplotypes (Fig. 6). *N. davidi* Ndh6 (n = 8) showed 99.3% similarity and 99.7% coverage, and *N. davidi* Ndh7 (n = 12) showed 99.4% similarity and 100% coverage to *N. davidi* collected in China (NCBI GenBank accession number NC_023823). Different haplotypes did not reflect differences in colourations. All identified individuals belonged to the B1 clade *sensu* Onuki and Fuke (2022).

A large number of guppies was sighted in the warmest area of the water system, specifically in the pond directly fed by thermal waters and the nearby section of the canal. In this

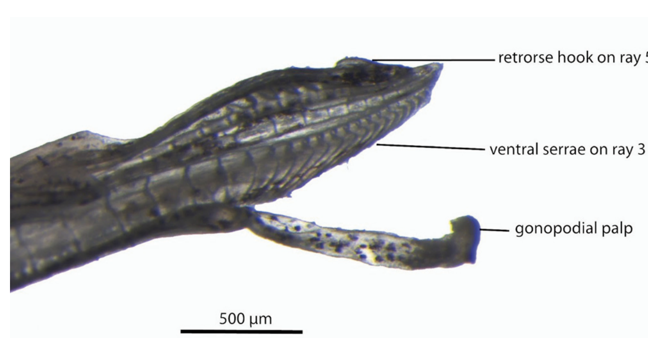

Figure 4. – Gonopodium of a male specimen of *Poecilia reticulata* (14.7 mm standard length; MNHN-IC-2024-0095) caught in the thermal park of Juvignac on January 1st, 2024; photo credits M. Hautecoeur.

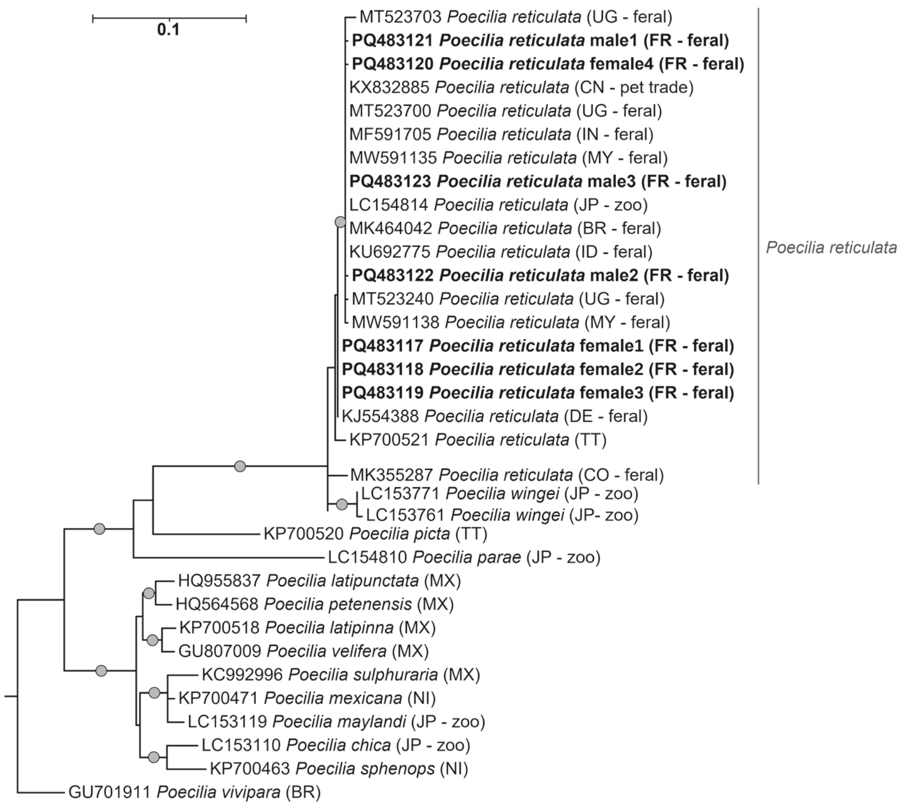

Figure 5. – Maximum likelihood phylogenetic tree of *Poecilia reticulata*. The tree was obtained using IQ-Tree 2.2.2.6 and with the HKY+F+G4 substitution model. Gray dots represent bootstrap support values above 80%. Sequences obtained in this study are indicated in bold and the outgroup sequence is *Poecilia vivipara* (GU701911). Abbreviations: Brazil (BR), China (CN), Columbia (CO), Germany (DE), France (FR), Indonesia (ID), India (IN), Japan (JP), Malaysia (MY), Mexico (MX), Nicaragua (NI), Trinidad and Tobago (TT) and Uganda (UG).

area, we recorded temperatures ranging from 16 to 22.5°C. However, these fish were also observed further downstream, where temperatures were as low as 13.5°C. Shrimp were found throughout the circular pond and along the canal. Nonetheless, they seemed to show a higher tolerance to colder waters, as they were found in the most distant parts of the canal where temperatures were as low as 11°C.

DISCUSSION

Most animals sold in pet trading shops spend their entire life in captivity; however, due to the increasing popularity of the hobby, the release of aquatic pets is becoming more frequent (Duggan, 2010; Lockwood, *et al*., 2019; Cuinet *et al*., 2024; Denys and Manné, 2024). The release of exotic pets is often motivated by the owners' inability to provide adequate care for large, old, aggressive, sick, or highly prolific animals such as Poeciliid fish and *Neocaridina* sp. shrimps (Duggan, 2010; Lockwood *et al*., 2019; Prati *et al*., 2024b). Once released in new ecosystems, exotic pets face multiple challenges. Therefore, a great level of tolerance to different biotic and abiotic conditions is necessary for their successful establishment (Havel *et al*., 2015). Besides tolerance to abiotic conditions (*e.g*., temperature, dissolved oxygen or nutrients), pivotal aspects favouring colonisation of new environments comprise a high reproductive output and a generalist diet (Gippet and Bertelsmeier, 2021). This is the case for many established species of pet trade origins, including *P. reticulata* and *N. davidi*, which are nowadays found in several European locations (Nekrasova *et al*., 2023; Prati *et al*., 2024b).

This study provides the first documented record of *Poecilia reticulata* and *Neocaridina davidi* from metropolitan France. *Poecilia reticulata* has already been introduced in many overseas territories such as French Guiana (Brosse *et al*., 2021), Martinique and Guadeloupe (Lim *et al*., 2002; Monti *et al*., 2010), La Réunion and Mayotte islands (Keith *et al*., 2006), New Caledonia (Marquet *et al*., 2003) and French Polynesia (Keith *et al*., 2002; Lim *et al*., 2002). Similarly, *N. davidi* has been introduced in La Réunion Island (Prati *et al*., 2024a).

According to local residents, guppies seem to have been present in the warm waters of the thermal park of Juvignac at least since 2019, while shrimp at least since 2021. The relatively long presence of these two species in the study site, the high proportion of ovigerous females and the presence of juveniles, suggest favourable conditions for reproduction. Thus, both species can be considered as locally established. The presence of heterogeneous morphological traits and different haplotypes within individuals of each species suggests that either a single release of a different pool of individu-

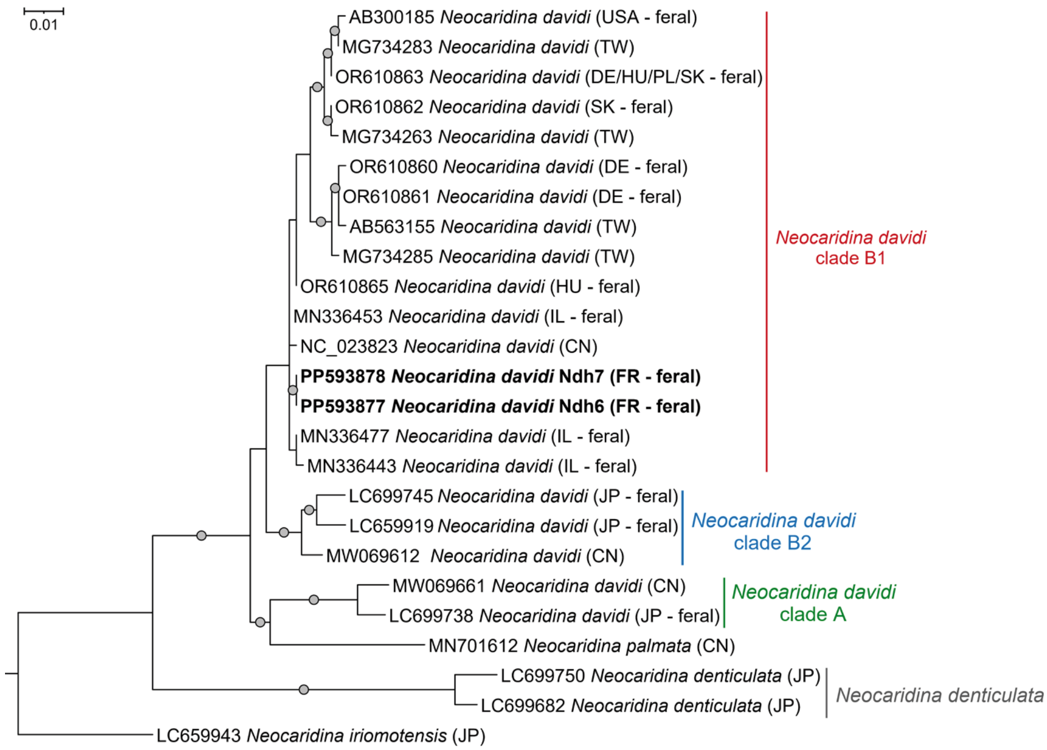

Figure 6. – Maximum likelihood phylogenetic tree of *Neocaridina davidi*. The tree was obtained using IQ-Tree 2.2.2.6 and with the TPM2u+F+G4 substitution model. The names and circumscriptions of *N. davidi* clades are based on Onuki and Fuke (2022). Gray dots represent bootstrap support values above 80%. Sequences obtained in this study are indicated in bold, and the outgroup sequence is *Neocaridina iriomotensis* (LC659943). Abbreviations: China (CN), Germany (DE), France (FR), Hungary (HU), Israel (IL), Japan (JP), Slovakia (SK), Poland (PL), Taiwan (TW) and United States of America (USA).

als or multiple releases might have occurred. However, the relatively long presence on-site and the short reproduction time of both species point towards multiple releases of captive-bred individuals. Feral populations of *P. reticulata* and *N. davidi* without captive-bred individuals would generally display lighter and more homogeneous colourations as the bright colouration of captive-bred individuals is likely to be lost within a few generations through reproduction and predation (Rodd *et al.,* 1997; Godin and McDonough, 2003; Millar *et al*., 2006; Prati *et al*., 2024b). Accordingly, *P. reticulata* and the gray wagtail *Motacilla cinerea* Tunstall, 1771, have been observed on-site to prey upon *N. davidi*; and the common kingfisher *Alcedo atthis* Linnaeus, 1758, and the grey heron *Ardea cinerea* Linnaeus, 1758 to prey upon *P. reticulata*. Such selective pressure will likely eliminate the most eye-catching individuals from the population resulting in less coloured individuals reaching maturity and reproduction.

P. reticulata and *N. davidi* are known to prefer relatively high water temperatures, *a priori* preventing them from settling in most European rivers (Weiperth *et al*., 2019; Nekrasova *et al*., 2021a). However, climate change and warmer periods could have favoured their current establishment thus facilitating a possible future spread to nearby waterways (Nekrasova *et al*., 2021b; Prati *et al*., 2024b). For instance, in the Palearctic area, *P. reticulata*, whose optimal temperature ranges from 14-28°C (Nekrasova *et al*., 2021a), has been established several times in thermal waters. This is notably the case in the thermally altered Gillbach-Erft river system near Cologne, Germany and in the thermal springs near Bad Saulgau (Lukas *et al*., 2017). Likewise, *N. davidi* have established viable populations in several thermally polluted waters across Europe, sometimes co-occurring with guppies as in Germany in the Erft River system and in the Sinnerbach stream (Klotz *et al*., 2013; Jabłońska *et al*., 2018; Weiperth *et al*., 2019; Prati *et al*., 2024b). Therefore, the establishment of *P. reticulata* and *N. davidi* in the thermal park of Juvignac, possibly originating from several aquarium dumping events, is not surprising.

The aforementioned suggests that both species possess certain thermal plasticity, which could thus allow the spread of individuals through the Mosson River during the late months of spring and during summer (when the river reaches the highest temperatures: approximately from 13.5°C up to 22.5°C) (Naiades, 2008). This is particularly plausible for *N. davidi*, a species that has recently shown higher thermal tolerance than *P. reticulata* in the Gillbach, a German stream fed by the cooling water from a coal power plant (Klotz *et al.*, 2013; Lukas *et al.*, 2017). After the input of cooling water was reduced and the temperature of the stream reverted to a more natural thermal regime, *P. reticulata* disappeared while *N. davidi* persisted unperturbed (Prati *et al.*, 2024b). Habitat suitability in Europe for *N. davidi* belonging to the clade B1, which is typically imported from Taiwan, is restricted to the warmer area of the southeastern Mediterranean (Prati *et al.*, 2024b). Nevertheless, it is possible that over time the local *N. davidi* population might adapt to colder water, showing thermal plasticity similar to that from *N. davidi* belonging to clades A and B2 and thus spreading to nearby watercourses in the foreseeable future (Prati *et al.*, 2024b).

The introduction of fish like the Trinidadian guppy into ecosystems already containing fish can lead to competition with native species, declines in native prey species, biodiversity loss, significant modifications of the food web, behavioural changes and alterations in community structure and ecosystem functioning (Strayer, 2010; Havel *et al.*, 2015; Brosse *et al.*, 2021). Similar impacts have been observed after the introduction of shrimp such as *N. davidi* (Weber and Traunspurger, 2016; Schoolmann and Arndt, 2017; Onuki and Fuke, 2022). *Neocaridina davidi* has been documented to replace native shrimp with similar ecological niches, alter meiofaunal assemblages and due to a high feeding rate, strongly influence leaf-litter breakdown in invaded areas (Onuki and Fuke, 2022; Schoolmann and Arndt, 2017; Weber and Traunspurger, 2016). *Neocaridina davidi*, if present in the Mosson River, might outcompete the native shrimp *Atyaephyra desmarestii* Millet, 1831 due to a much higher feeding rate, as shown by Schoolmann and Arnd (2017).

In Europe, several pets, subject to aquarium dumping, such as the topmouth gudgeon *Pseudorasbora parva* Temminck & Schlegel, 1846, the Asian water loaches *Misgurnus* spp. Lacepède, 1803 and the apple snails *Pomacea* spp. Perry, 1811 have become invasive outside thermal waters (Beyer, 2004; Gilioli *et al.*, 2017; Clavero *et al.*, 2023). This trend is likely to increase in the future due to a warming climate (*e.g.*, Dokulil, 2014). A situation that indirectly might pose elevated biosecurity risks, as alien species are often vectors of alien pathogens that can be co-introduced and transmitted to native species (*e.g.*, Simberloff *et al.*, 2013; Roy *et al.*, 2023). Thus, close monitoring is necessary to check for the spread of *P. reticulata* and *N. davidi* and their pathogens, if present, in the nearby Mosson River.

Acknowledgements. – This study is supported by the MNHN, the UAR PatriNat 2006, the UMR BOREA 8067 and the French Agency of Biodiversity (OFB). Our sincere thanks also go to the technicians of the Department of Biology and Ecology at the Faculty of Sciences of Montpellier, particularly Nicolas Moureau, for their assistance in providing equipment, facilities and insightful advice. We also thank Jordi Soliveres for his fieldwork involvement, vital contribution to data collection, and enriching reflections throughout the project. We also express our gratitude to Mélyne Hautecoeur for her assistance with the laboratory work concerning binocular pictures of guppies. Laboratory access and assistance were provided by the "Service de Systématique Moléculaire" of the MNHN (UAR 2AD 2700) with the help of Imane Boumguezid and by the Aquatic Ecology group of the University of Duisburg-Essen. Besides, we express our gratitude to Yaneth Camargo for her contribution to improving the quality of English writing in this article. Lastly, we are thankful for the feedback provided by the reviewers, which enhanced the quality of this article.

REFERENCES

Beyer, K. (2004). Escapees of potentially invasive fishes from an ornamental aquaculture facility: the case of topmouth gudgeon *Pseudorasbora parva*. *J. Fish Biol.,* 65: 326-327. https://doi.org/10.1111/j.0022-1112.2004.00559ac.x

de Bragança, P. H. N., Guimarães, E. C, de Brito, P. S, & Ottoni, F. P. (2020). On the natural occurrence of *Poecilia reticulata* Peters, 1859 (Cyprinodontiformes: Poeciliidae). *Cybium*, 44(4): 309-316. https://doi.org/10.26028/cybium/2020-444-002

Britton, J. R., Lynch, A. J., Bardal, H., Bradbeer, S. J., Coetzee, J. A., Coughlan, N. E., Dalu, T., Tricarico, E., Gallardo, B., Lintermans, M., *et al.* (2023). Preventing and controlling nonnative species invasions to bend the curve of global freshwater biodiversity loss. *Environ. Rev.,* 31: 310-326. https://doi.org/10.1139/er-2022-0103

Brosse, S., Baglan, A., Covain, R., Lalagüe, H., Le Bail, P.-Y., Vigouroux, R., & Quartarollo, G. (2021). Aquarium trade and fish farms as a source of non-native freshwater fish introductions in French Guiana. *Ann. Limnol. – Int. J. Limol.,* 57: 4. https://doi.org/10.1051/limn/2021002

Clavero, M., Suh, J., Franch, N., Aparicio, E., Buchaca, T., Caner, J., Garcia-Rodriguez, S., Llimona, F., Pou-Rovira, Q., Rocaspana, R., *et al.* (2023). Invaders they are a-changing: A recent, unexpected surge of invasive loaches in Catalonia. *Freshw. Biol.,* 68: 621-631. https://doi.org/10.1111/fwb.14051

Cucherousset, J., & Olden, J. D. (2011). Ecological impacts of non-native freshwater fishes. *Fisheries,* 36: 215-230. https://doi.org/10.1080/03632415.2011.574578

Cuinet, A., Denys, G., Besson, S., Marchiset, Y., & Bouchard, J. (2024). First record of the large-scale loach *Misgurnus dabryanus* (Guichenot, 1872) (Teleostei, Cobitidae) in France. *BioInvasions Rec.,* 13: 541-550. https://doi.org/10.3391/bir.2024.13.2.18

Deacon, A. E. (2010). The behavioural ecology of the Trinidadian guppy, *Poecilia reticulata*, as an invasive species. PhD Thesis, Univ. of St. Andrews. http://dx.doi.org/10.1017/CBO9781139939492.016

Deacon, A. E., Ramnarine, I. W., & Magurran, A. E. (2011). How reproductive ecology contributes to the spread of a globally invasive fish. *PLOS ONE,* 6: e24416. https://doi.org/10.1371/journal.pone.0024416

Denys, G., & Manné, S. (2024). First record of an established population of *Misgurnus anguillicaudatus* (Cantor, 1842) (Teleostei, Cobitidae) in France. *BioInvasions Rec.,* 13: 529-540. http://dx.doi.org/10.3391/bir.2024.13.2.17

Denys, G. P. J., Dettaï, A., Persat, H., Hautecoeur, M., & Keith, P. (2014). Morphological and molecular evidence of three species of pikes *Esox* spp. (Actinopterygii, Esocidae) in France, including the description of a new species. *C. R. Biol.,* 337: 521-534. https://doi.org/10.1016/j.crvi.2014.07.002

Dokulil, M. (2014). Impact of climate warming on European inland waters. *Inland Waters,* 4: 27-40. https://doi.org/10.5268/IW-4.1.705

Duggan, I. C. (2010). The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biol. Invasions,* 12: 3757-3770. https://doi.org/10.1007/s10530-010-9768-x

Elvira, B., & Almodóvar, A. (2001). Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. *J. Fish Biol.,* 59: 323-331. https://doi.org/10.1111/j.1095-8649.2001.tb01393.x

Englund, R., & Cai, Y. (1998). The occurrence and description of *Neocaridina denticulata sinensis* (Kemp, 1918) (Crustacea: Decapoda: Atyidae), a new introduction to the Hawaiian Islands. *Bishop Mus. Occas. Pap.,* 58.

FAO (2024). Database on Introductions of Aquatic Species (DIAS) – Fisheries and Aquaculture. Available from: https://www.fao.org/fishery/en/knowledgebase/79

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biol.,* 22: 151-163. https://doi.org/10.1111/gcb.13004

Ghedotti, M. J. (2000). Phylogenetic analysis and taxonomy of the poecilioid fishes (Teleostei: Cyprinodontiformes). *Zool. J. Linn. Soc.,* 130: 1-53. https://doi.org/10.1006/zjls.1999.0213

Gilioli, G., Schrader, G., Carlsson, N., Van Donk, E., Van Leeuwen, C. H. A, Martín, P. R, Pasquali, S., Vilà, M., & Vos, S. (2017). Environmental risk assessment for invasive alien species: A case study of apple snails affecting ecosystem services in Europe. *Environ. Impact Assess. Rev.,* 65:1-11. https://doi.org/10.1016/j.eiar.2017.03.008

Gippet, J. M. W., & Bertelsmeier, C. (2021). Invasiveness is linked to greater commercial success in the global pet trade. *Proc. Natl. Acad. Sci. USA,* 118: e2016337118. https://doi.org/10.1073/pnas.2016337118

Godin, J.-G. J., & McDonough, H. E. (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.,* 14: 194-200. https://doi.org/10.1093/beheco/14.2.194

Havel, J. E., Kovalenko, K. E., Thomaz, S. M., Amalfitano, S., Kats, L.B. (2015). Aquatic invasive species: challenges for the future. *Hydrobiologia,* 750: 147-170. https://doi.org/10.1007/s10750-014-2166-0

Hebert, P. D. N., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B*, 270(Suppl. 1). https://doi.org/10.1098/rsbl.2003.0025

Holitzki, T. M., MacKenzie, R. A., Wiegner, T. N., & McDermid, K. J. (2013). Differences in ecological structure, function, and native species abundance between native and invaded Hawaiian streams. *Ecol. Appl.,* 23: 1367-1383. https://doi.org/10.1890/12-0529.1

Jabłońska, A., Mamos, T., Gruszka, P., Szlauer-Łukaszewska, A., & Grabowski, M. (2018). First record and DNA barcodes of the aquarium shrimp, *Neocaridina davidi*, in Central Europe from thermally polluted River Oder canal, Poland. *Knowl. Manage. Aquat. Ecosyst.*, 14. https://doi.org/10.1051/kmae/2018004

Juvignac Municipality (2023). Le parc des Thermes. Available from: https://www.juvignac.fr/le-parc-des-thermes/

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.,* 20: 1160-1166. https://doi.org/10.1093/bib/bbx108

Keith, P., Vigneux, E., & Marquet, G. (2002). Atlas des Poissons et des Crustacés d'Eau douce de Polynésie française. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Keith, P., Marquet, G., Valade, P., Bosc, P., & Vigneux, E. (2006). Atlas des Poissons et des Crustacés d'Eau douce des Comores, Mascareignes et Seychelles. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Keith, P., Poulet, N., Denys, G., Changeux, T., Feuteun, E., & Persat, H. (2020). Les Poissons d'eau douce de France. 2e édit. Mèze & Paris, Biotope éditions & Muséum national d'histoire naturelle.

van Kleef, H., van der Velde, G., Leuven, R. S. E. W., & Esselink, H. (2008). Pumpkinseed sunfish (*Lepomis gibbosus*) invasions facilitated by introductions and nature management strongly reduce macroinvertebrate abundance in isolated water bodies. *Biol Invasions,* 10: 1481-1490. https://doi.org/10.1007/s10530-008-9220-7

Klotz, W., Miesen, F., Hüllen, S., & Herder, F. (2013). Two Asian fresh water shrimp species found in a thermally polluted stream system in North Rhine-Westphalia, Germany. *Aquat. Invasions,* 8: 333-339. https://doi.org/10.3391/ai.2013.8.3.09

Lambea-Camblor, A., Morcillo, F., Muñoz, J., & Perdices, A. (2023). Genetic and ecological approaches to introduced populations of pumpkinseed sunfish (*Lepomis gibbosus*) in Southwestern Europe. *Diversity,* 15: 1059. https://doi.org/10.3390/d15101059

Lim, P., Meunier, J., Keith, P., Noël, P. Y. (2002). Atlas des Poissons et des Crustacés d'Eau douce de la Martinique. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Lockwood, J. L., Welbourne, D. J., Romagosa, C. M., Cassey, P., Mandrak, N. E., Strecker, A., Leung, B., Stringham, O. C., Udell, B., Episcopio-Sturgeon, D. J., *et al.* (2019). When pets become pests: the role of the exotic pet trade in producing invasive vertebrate animals. *Frontiers Ecol. Environ.,* 17: 323-330. https://doi.org/10.1002/fee.2059

Lukas, J., Kalinkat, G., Kempkes, M., Rose, U., & Bierbach, D. (2017). Feral guppies in Germany – a critical evaluation of a citizen science approach as biomonitoring tool. *Bull. Fish Biol.*, 17(1/2): 13-27.

Maceda-Veiga, A., Escribano-Alacid, J., De Sostoa, A., & García-Berthou, E. (2013). The aquarium trade as a potential source of fish introductions in southwestern Europe. *Biol. Invasions,* 15: 2707-2716. https://doi.org/10.1007/s10530-013-0485-0

Maciaszek, R., Kamaszewski, M., Łapa, P., & Strużyński, W. (2018). Epibionts of ornamental freshwater shrimps bred in Taiwan. *Ann. Warsaw Univ. Life Sci. – SGGW – Anim. Sci.,* 57: 133-142. https://doi.org/10.22630/AAS.2018.57.2.13

Magalhães, A. L. B. de, & Jacobi, C. M. (2013). Invasion risks posed by ornamental freshwater fish trade to southeastern Brazilian rivers. *Neotrop. Ichthyol.,* 11: 433-441. https://doi.org/10.1590/S1679-62252013005000003

Marquet, G., Keith, P., & Vigneux E. (2003). Atlas des Poissons et des Crustacés d'Eau douce de Nouvelle-Calédonie. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Millar, N. P., Reznick, D. N., Kinnison, M. T., & Hendry, A. P. (2006). Disentangling the selective factors that act on male colour in wild guppies. *Oikos,* 113: 1-12. https://doi.org/10.1111/j.0030-1299.2006.14038.x

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.,* 37: 1530-1534. https://doi.org/10.1093/molbev/msaa015

Monti, D., Keith, P., & Vigneux, E. (2010). Atlas des Poissons et des Crustacés d'Eau douce de la Guadeloupe. Publications scientifiques du Muséum national d'Histoire naturelle, Paris.

Naiades (2008). Relevé de températures de la Mosson, Montpellier. Naïades. Available from: https://naiades.eaufrance.fr/acces-donnees#/physicochimie/resultats?debut=01-01-2007&fin=29-03-2024&wkt=POLYGON((3.8170865178108206%2043.615044233107454,3.8175103068351737%2043.61511802505507,3.8175317645072933%2043.61551028599317,3.817006051540374%2043.61538600557682,3.8170865178108206%2043.615044233107454))

Nekrasova, O., Tytar, V., Pupins, M., Čeirāns, A., Marushchak, O., & Skute, A. (2021a). Distribution of viviparous american fish species in Eastern Europe on the example of *Gambusia holbrooki* Girarg, 1859 and *Poecilia reticulata* Peters, 1859 in the context of global climate change. *In*: The 1st International Electronic Conference on Biological Diversity, Ecology and Evolution. MDPI: p. 9. https://doi.org/10.3390/BDEE2021-09398

Nekrasova, O., Tytar, V., Pupins, M., Čeirāns, A., Marushchak, O., & Skute, A. (2021b). A GIS modeling study of the distribution of viviparous invasive alien fish species in Eastern Europe in terms of global climate change, as exemplified by *Poecilia reticulata* Peters, 1859 and *Gambusia holbrooki* Girarg, 1859. *Diversity,* 13: 385. https://doi.org/10.3390/d13080385

Nekrasova, O. D., Marushchak, O., Marushchak, M., Bolotova, K. M., Čeirāns, A., & Skute, A. (2023). Phenotypic study of population and distribution of the *Poecilia reticulata* (Cyprinodontiformes, Poeciliidae) from Kyiv Sewage System (Ukraine). *Zoodiversity,* 57(4). https://doi.org/10.15407/zoo2023.04.301

Nowak, M., Szczerbik, P., & Tatoj, K. W. P. (2008). Non-native freshwater fishes in Poland: an overview. *AACL Bioflux,* 1: 173-191.

Onuki, K., & Fuke, Y. (2022). Rediscovery of a native freshwater shrimp, Neocaridina denticulata, and expansion of an invasive species in and around Lake Biwa, Japan: Genetic and morphological approach. *Conserv. Genet.,* 23: 967-980. https://doi.org/10.1007/s10592-022-01467-1

Patoka, J., Magalhães, A. L. B., Kouba, A., Faulkes, Z., Jerikho, R., & Vitule, J. R. S. (2018). Invasive aquatic pets: failed policies increase risks of harmful invasions. *Biodivers. Conserv.,* 27: 3037-3046. https://doi.org/10.1007/s10531-018-1581-3

Poeser, F., Kempkes, M., & Isbrücker, I. J. H. (2005). Description of *Poecilia (Acanthophacelus) wingei* n. sp. from París Peninsula, Venezuela, including notes on *Acanthophacelus* Eigenmann, 1907 and other subgenera of *Poecilia* Bloch and Schneider, 1801 (Teleostei, Cyprinodontiformes, Poeciliidae). *Contrib. Zool.* 74(1/2): 97-115. https://doi.org/10.1163/18759866-0740102007

Prati, S., Enß, J., Grabner, D. S., Huesken, A., Feld, C. K., Doliwa, A., & Sures, B. (2023). Possible seasonal and diurnal modulation of *Gammarus pulex* (Crustacea, Amphipoda) drift by microsporidian parasites. *Sci. Rep.,* 13: 9474. https://doi.org/10.1038/s41598-023-36630-2

Prati, S., Faivre, L., Mazancourt, V. de, Sures, B., & Valade, P. (2024a). Paradise under threat: the successful invasion of the freshwater shrimp *Neocaridina davidi* and its parasites on La Réunion Island. BioRxiv. https://doi.org/10.1101/2024.08.23.609356

Prati, S., Grabner, D. S., Hupało, K., Weiperth, A., Maciaszek, R., Lipták, B., Bojko, J., Bérces, F., & Sures, B. (2024b). Invisible invaders: range expansion of feral *Neocaridina davidi* offers new opportunities for generalist intracellular parasites. *Biol. Invasions*, 26: 2499-2523. https://doi.org/10.1007/s10530-024-03324-3

Rodd, F. H., Reznick, D. N., & Sokolowski, M. B. (1997). Phenotypic plasticity in the life history traits of guppies: Responses to social environment. *Ecology,* 78: 419-433. https://doi.org/10.1890/0012-9658(1997)078%5b0419:PPITLH%5d2.0.CO;2

Roy, H. E., Tricarico, E., Hassall, R., Johns, C. A., Roy, K. A., Scalera, R., Smith, K. G., & Purse, B. V. (2023). The role of invasive alien species in the emergence and spread of zoonoses. *Biol. Invasions,* 25: 1249-1264. https://doi.org/10.1007/s10530-022-02978-1

Schneider, R., Prati, S., Grabner, D., & Sures, B. (2022). First report of microsporidians in the non-native shrimp Neocaridina davidi from a temperate European stream. *Dis. Aquat. Org.,* 150: 125-130. https://doi.org/10.3354/dao03681

Schoolmann, G., & Arndt, H. (2017). Leaf-litter preferences of the introduced freshwater shrimps *Atyaephyra desmarestii* and *Neocaridina davidi*. *Crustaceana,* 90: 1715-1730. https://doi.org/10.1163/15685403-00003736

Shih, H.-T., Cai, Y., Niwa, N., Yoshigou, H., & Nakahara, Y. (2024). Integrative taxonomy reveals freshwater shrimp diversity (Decapoda: Atyidae: Neocaridina) from Kyushu and Southern Honshu of Japan, with a discussion on introduced species. *Zool. Stud.*, 63: 18. https://doi.org/10.6620/ZS.2024.63-18

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., *et al*. (2013). Impacts of Biological Invasions: What's What and the Way Forward. *Trends Ecol. Evol.,* 28: 58-66. https://doi.org/10.1016/j.tree.2012.07.013

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshw. Biol.,* 55: 152-174. https://doi.org/10.1111/j.1365-2427.2009.02380.x

Wallace, C. K., Bright, L. A., Marx, J. O., Andersen, R. P., Mullins, M. C., & Carty, A. J. (2018). Effectiveness of rapid cooling as a method of euthanasia for young zebrafish (*Danio rerio*). *J. Am. Ass. Lab. Anim. Sci.,* 57: 58-63.

Weber, S., & Traunspurger, W. (2016). Influence of the ornamental red cherry shrimp *Neocaridina davidi* (Bouvier, 1904) on freshwater meiofaunal assemblages. *Limnologica,* 59: 155-161. https://doi.org/10.1016/j.limno.2016.06.001

Weiperth, A., Gábris, V., Danyik, T., Farkas, A., Kuříková, P., Kouba, A., & Patoka, J. (2019). Occurrence of non-native red cherry shrimp in European temperate waterbodies: A case study from Hungary. *Knowl. Manage. Aquat. Ecosyst.*, 420: 9. https://doi.org/10.1051/kmae/2019002

Annex 1

Species	GenBank Accession number	Locality	Source
Poecilia reticulata	PQ483117	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483118	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483119	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483120	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483121	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483122	France (thermal park, Juvignac)	Current study
Poecilia reticulata	PQ483123	France (thermal park, Juvignac)	Current study
Poecilia reticulata	KJ554388	Germany (Rhine)	Geiger *et al*., 2014
Poecilia reticulata	KP700521	Trinidad and Tobago	Bagley *et al*., 2015
Poecilia reticulata	KU692775	Indonesia (Kabupaten Sukabumi)	Dahruddin *et al*., 2017
Poecilia reticulata	KX832885	China	Without link to any publication
Poecilia reticulata	LC154814	Japan (Aichi Higashiyama Zoo)	Without link to any publication
Poecilia reticulata	MF591705	India	Prasannan & Pillai, 2017
Poecilia reticulata	MK355287	Colombia	Papamija *et al*., 2019
Poecilia reticulata	MK464042	Brazil	Bagley *et al*., 2019
Poecilia reticulata	MT523240	Uganda	Decru *et al*., 2022
Poecilia reticulata	MT523700	Uganda	Decru *et al*., 2022
Poecilia reticulata	MT523703	Uganda	Decru *et al*., 2022
Poecilia reticulata	MW591135	Malaysia	Jamaluddin *et al*., 2022
Poecilia reticulata	MW591138	Malaysia	Jamaluddin *et al*., 2022
Poecilia chica	LC153110	Japan (Aichi Higashiyama Zoo)	Without link to any publication
Poecilia latipinna	KP700518	Mexico	Bagley *et al*., 2015
Poecilia latipunctata	HQ955837	Mexico (El Mante)	Without link to any publication
Poecilia maylandi	LC153119	Japan (Aichi Higashiyama Zoo)	Without link to any publication
Poecilia mexicana	KP700471	Nicaragua (Rio La Conquista)	Bagley *et al*., 2015
Poecilia parae	LC154810	Japan (Aichi Higashiyama Zoo)	Without link to any publication
Poecilia petenensis	HQ564568	Mexico (Campeche, Hampolol)	Without link to any publication
Poecilia picta	KP700520	Trinidad and Tobago	Bagley *et al*., 2015
Poecilia sphenops	KP700463	Nicaragua (tributary to Rio Grande at La Trinidad)	Bagley *et al*., 2015
Poecilia sulphuraria	KC992996	Mexico	Pfenninger *et al*., 2014
Poecilia velifera	GU807009	Mexico (Tulum)	Without link to any publication
Poecilia vivipara	GU701911	Brazil	Pereira *et al*., 2013
Poecilia wingei	LC153761	Japan (Aichi Higashiyama Zoo)	Without link to any publication
Poecilia wingei	LC153771	Japan (Aichi Higashiyama Zoo)	Without link to any publication

REFERENCES OF THE ANNEXES

Bagley, J.C., Alda, F., Breitman, M.F., Bermingham, E., van den Berghe, E.P. & Johnson, J.B. (2015). Assessing species boundaries using multilocus species delimitation in a morphologically conserved group of Neotropical freshwater fishes, the Poecilia sphenops species complex (Poeciliidae). *PLoS ONE*, 10(4): e0121139. https://doi.org/10.1371/journal.pone.0121139

Bagley, J. C., de Aquino, P. D. P. U., Breitman, M. F., Langeani, F., & Colli, G. R. (2019). DNA barcode and minibarcode identification of freshwater fishes from Cerrado headwater streams in Central Brazil. *J. Fish Biol*., 95(4): 1046-1060. https://doi.org/10.1111/jfb.14098

Chen, Q. H., Chen, W. J., Zheng, X. Z., & Guo, Z. L. (2020). Two freshwater shrimp species of the genus *Caridina* (Decapoda, Caridea, Atyidae) from Dawanshan Island, Guangdong, China, with the description of a new species. *ZooKeys*, 923: 15-32. https://doi.org/10.3897/zookeys.923.48593

Dahruddin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R. & Hubert, N. (2017). Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: Taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Mol. Ecol. Resour*., 17(2): 288-299. https://doi.org/10.1111/1755-0998.12528

Decru, E., Vranken, N., Maetens, H., Mertens de Vry, A., Kayenbergh, A., Snoeks, J. & Van Steenberge, M. (2022). DNA barcoding the Lake Edward basin: High taxonomic coverage of a tropical freshwater ichthyofauna. *Hydrobiologia*, 849(8): 1743-1762. https://doi.org/10.1007/s10750-022-04812-0

Geiger, M. F., Herder, F., Monaghan, M. T., Almada, V., Barbieri, R., Bariche, M., Berrebi, P., Bohlen, J., Casal-Lopez, M., Delmastro, G. B., Denys, G. P. J., Dettai, A., Doadrio, I., Kalogianni, E., Kärst, H., Kottelat, M., Kovačić, M., Laporte, M., Loren-

Annex 2

Species	Genbank Accession number	Locality	Source
Neocaridina davidi	PP593877	France (thermal park, Juvignac)	Current study
Neocaridina davidi	PP593878	France (thermal park, Juvignac)	Current study
Neocaridina davidi	AB300185	USA (Oahu, Hawaii)	Shih and Cai, 2007
Neocaridina davidi	MG734263	Taiwan (Dajia)	Han *et al*., 2019
Neocaridina davidi	MG734283	Taiwan (Gangkong)	Han *et al*., 2019
Neocaridina davidi	MG734285	Taiwan (Lanyang)	Han *et al*., 2019
Neocaridina davidi	OR610860	Germany	Prati *et al*., 2024
Neocaridina davidi	OR610861	Germany	Prati *et al*., 2024
Neocaridina davidi	OR610862	Slovakia	Prati *et al*., 2024
Neocaridina davidi	OR610863	Germany, Hungary, Poland and Slovakia	Prati *et al*., 2024
Neocaridina davidi	OR610865	Hungary	Prati *et al*., 2024
Neocaridina davidi	AB563155	Taiwan (Penghu)	Without link to a publication
Neocaridina davidi	MN336443	Israel (Yarden)	Levitt-Barmats *et al*., 2019
Neocaridina davidi	MN336453	Israel (Yarqon)	Levitt-Barmats *et al*., 2019
Neocaridina davidi	MN336477	Israel (Yarden)	Levitt-Barmats *et al*., 2019
Neocaridina davidi	MW069612	China (Henan)	Zhou *et al*., 2021
Neocaridina davidi	MW069661	China (Henan)	Zhou *et al*., 2021
Neocaridina davidi	NC_023823	China	Yu *et al*., 2014
Neocaridina davidi	LC699738	Japan (Seri River)	Onuki and Fuke, 2022
Neocaridina davidi	LC699745	Japan (Shiratori River)	Onuki and Fuke, 2022
Neocaridina davidi	LC659919	Japan (Kakinohana spring)	Nagai and Imai, 2021
Neocaridina palmata	MN701612	China (Qingyuan)	Chen *et al*., 2020
Neocaridina denticulata	LC699750	Japan (Miya River)	Onuki and Fuke, 2022
Neocaridina denticulata	LC699682	Japan (Chinai River)	Onuki and Fuke, 2022
Neocaridina iriomotensis	LC659943	Japan (Iriomotejima Island)	Nagai and Imai, 2021

zoni, M., Marčić, Z., Özuluğ, M., Perdices, A., Perea, S., Persat, H., Porcelotti, S., Puzzi, C., Robalo, J., Šanda, R., Schneider, M., Šlechtová, V., Stoumboudi, M., Walter, S., & Freyhof, J. (2014). Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. *Mol. Ecol. Resour.*, 14(6): 1210-1221. https://doi.org/10.1111/1755-0998.12257

Han, C. C., Hsu, K. C., Fang, L. S., Cheng, I. M., & Lin, H. D. (2019). Geographical and temporal origins of *Neocaridina* species (Decapoda: Caridea: Atyidae) in Taiwan. *BMC Genet.*, 20: 86. https://doi.org/10.1186/s12863-019-0788-y

Jamaluddin, J., Lavoué, S., Alshari, N., Ghazali, S., Ahmad, A., Tan, A., Leng, C. & Mohd Nor, S. (2022). Reassessing fish diversity of Penang Island's freshwaters (northwest Peninsular Malaysia) through a molecular approach raises questions on its conservation status. *Biodivers. Conserv.*, 31(5-6): 1551-1576. https://doi.org/10.1007/s10531-022-02409-6

Levitt-Barmats, Y. A., Yanai, Z., Cohen, T. M., & Shenkar, N. (2019). Life-history traits and ecological characteristics of the ornamental shrimp *Neocaridina denticulata* (De Haan, 1844), recently introduced into the freshwater systems of Israel. *Aquat. Invasions*, 14: 684-702. https://doi.org/10.3391/ai.2019.14.4.08

Nagai, H. & Imai, H. (2021). First record of the invasive alien species *Neocaridina* sp. from Okinawajima Island, central Ryukyus, Japan. *Bull. Biogeogr. Soc. Jpn*, 76: 51-57.

Onuki, K., & Fuke, Y. (2022). Rediscovery of a native freshwater shrimp, *Neocaridina denticulata*, and expansion of an invasive species in and around Lake Biwa, Japan: genetic and morphological approach. *Conserv. Genet.*, 23: 967-980. https://doi.org/10.1007/s10592-022-01467-1

Papamija, C., Buitrago-Bitar, M. A., Londono, J. M., & Munoz, J. E. (2019). Molecular characterization of fish fauna in two natural reserves from Colombia. *Poecilia reticulata* isolate cytochrome oxidase subunit 1 (CO1) gen – Nucleotide – NCBI (nih.gov).

Pereira, L., Hanner, R., Foresti, F. & Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genet.*, 14: 20. https://doi.org/10.1186/1471-2156-14-20

Pfenninger, M., Lerp, H., Tobler, M., Passow, C., Kelley, J., Funke, E., Greshake, B., Erkoc, U., Berberich, T. & Plath, M. (2014). Parallel evolution of cox genes in H2S-tolerant fish as key adaptation to a toxic environment. *Nature Comm.*, 5: 3874. https://doi.org/10.1038/ncomms4873

Prasannan, K., & Pillai, P. M. (2017). Barcoding of selected hill-stream fishes of southern Western Ghats, Kerala, India. *Poecilia reticulata* isolate 39Q cytochrome oxidase subunit 1 (CO1) gen – Nucleotide – NCBI (nih.gov).

Prati, S., Grabner, D. S., Hupało, K., Weiperth, A., Maciaszek, R., Lipták, B., Bojko, J., Bérces, F., & Sures, B. (2024). Invisible invaders: range expansion of feral *Neocaridina davidi* offers new opportunities for generalist intracellular parasites. *Biol. Invasions*, 26: 2499-2523. https://doi.org/10.1007/s10530-024-03324-3

Shih, H. T., & Cai, Y. (2007). Two new species of the land-locked freshwater shrimps genus, *Neocaridina* Kubo, 1938 (Decapoda: Caridea: Atyidae), from Taiwan, with notes on speciation on the island. *Zool. Stud.*, 46: 680-694.

Yu, Y. Q., Yang, W. J., & Yang, J. S. (2014). The complete mitogenome of the Chinese swamp shrimp *Neocaridina denticulata sinensis* Kemp 1918 (Crustacea: Decapoda: Atyidae). *Mitochondrial DNA*, 25: 204–205. https://doi.org/10.3109/19401736.2013.796465

Zhou, C. J., Feng, M. X., Tang, Y. T., Yang, C. X., Meng, X. L., & Nie, G. X. (2021). Species diversity of freshwater shrimp in Henan Province, China, based on morphological characters and COI mitochondrial gene. *Ecol. Evol.*, 11: 10502-10514. https://doi.org/10.1002/ece3.7855