Knowl. Manag. Aquat. Ecosyst. 2021, 422, 19
© R. Maciaszek *et al.*, Published by EDP Sciences 2021
https://doi.org/10.1051/kmae/2021018

www.kmae-journal.org

Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Office français de la biodiversité

RESEARCH PAPER OPEN ACCESS

First report of *Scutariella japonica* (Matjašič, 1990), a temnocephalid epibiont from South-East Asia, found on introduced ornamental freshwater shrimp in European waters

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Received: 22 February 2021 / Accepted: 22 April 2021

Abstract – This contribution presents the first record of ornamental shrimp epibiont, *Scutariella japonica* (Platyhelminthes: Temnocephalida) in European waters. The species lives on freshwater Atyidae shrimp from temperate, subtropical and tropical zones of South-East Asia. In total, 120 individuals of *Neocaridina davidi* shrimp were caught in thermally polluted canal of Oder river, near the city of Gryfino, in the northwest part of Poland. In that group, 5.83% were infected with scutariellids. Among shrimp, females were mostly infected (85.71%). Since ornamental shrimp released into thermally polluted water bodies have been also noticed in surrounding waters of natural temperature regime in Europe, *S. japonica* may spread further following potential expansion of the *Neocaridina* shrimp in Oder river. It is possible that other crustaceans, both native and alien which are present at this location, may also become vectors of this epibiont.

Keywords: Alien species / ornamental pet trade / Scutariellidae / Vorticellidae / *Neocaridina davidi* / thermally polluted waters

Résumé – Premier signalement de *Scutariella japonica* (Matjašič, 1990), un épibionte temnocéphalide d'Asie du Sud-Est, trouvé sur des crevettes d'eau douce ornementales introduites dans les eaux européennes. Cette contribution présente le premier signalement de l'épibionte des crevettes d'ornement, *Scutariella japonica* (Platyhelminthes: Temnocephalida) dans les eaux européennes. L'espèce vit sur des crevettes Atyidae d'eau douce provenant des zones tempérées, subtropicales et tropicales de l'Asie du Sud-Est. Au total, 120 individus de crevettes *Neocaridina davidi* ont été capturés dans un canal thermiquement pollué de la rivière Oder, près de la ville de Gryfino, dans le nord-ouest de la Pologne. Dans ce groupe, 5,83% étaient infectés par des scutariellidés. Parmi les crevettes, les femelles étaient principalement infectées (85,71%). Comme les crevettes d'ornement relâchées dans des plans d'eau thermiquement pollués ont également été remarquées dans les eaux environnantes au régime de température naturel en Europe, *S. japonica* pourrait se propager davantage suite à l'expansion potentielle des crevettes *Neocaridina* dans le fleuve Oder. Il est possible que d'autres crustacés, à la fois indigènes et exotiques, présents à cet endroit, deviennent également des vecteurs de cet épibionte.

Mots clés : Espèces exotiques / commerce des animaux d'ornement / Scutariellidae / Vorticellidae / *Neocaridina davidi* / eaux thermiquement polluées

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1 Introduction

Releases of non-indigenous crustaceans into natural water ecosystems are known to have major negative effects on the native species and food chains (Twardochleb *et al.*, 2013; Šidagytė *et al.*, 2017; Zhang *et al.*, 2019). However, it can get even worse if these species are accompanied by other organisms, such as epibionts, often accidentally introduced to European waters. Some of them, like *Temnosewellia minor* (Haswell, 1887) or *Aphanomyces astaci* (Schikora, 1906) can be transmitted to other crustaceans what may have an impact on natural biodiversity (Diéguez-Uribeondo and Söderhäll, 1993; Chiesa *et al.*, 2015)).

Ornamental freshwater shrimp *Neocaridina davidi* (Bouvier, 1904) of the family Atyidae, belongs to the most common crustaceans in global aquarium pet market. The shrimp is characterised by widespread variety of colour and quickly became an interest of aquarists. This has led to mass production of these crustaceans in aquaculture ponds mainly in Taiwan (Maciaszek *et al.*, 2018). As a result of intentional or unintentional releases from pet traders, *N. davidi* representatives are present also in waters outside its native range. Originally occurred in South-East Asia (Klotz *et al.*, 2013), *N. davidi* representatives have colonised new locations in temperate, subtropical and tropical zones around the world as an alien species (Englund and Cai, 1999; Mitsugi *et al.*, 2018; Levitt-Barmats *et al.*, 2019; Onuki, 2021). Effects of their uncontrolled releases have been described in European natural and commercially induced thermal waterbodies in Germany, Poland and Hungary (Klotz *et al.*, 2013; Jabłońska *et al.*, 2018a; Weiperth *et al.*, 2019). In Hungary, shrimp were even reported to be found in colder waters, more specific to water temperature regime of Central Europe (Weiperth *et al.*, 2019).

Unfortunately, *N. davidi* is known to be a host of at least two specific epibionts, including temnocephalid, *Scutariella japonica* (syn. *Cardinicola japonica*) (Matjašič, 1990) and branchiobdellidan, *Holtodrilus truncatus* (Liang, 1963). Both species were observed coexisting in host gill chambers in native populations of the shrimp in Taiwan (Ohtaka *et al.*, 2015). They were accidentally introduced in Japan, with wild-caught shrimp imported for fishing purposes (Niwa and Ohtaka, 2006). It is likely that they are also widespread in aquaculture ponds or aquarium hobby, as they can be found with imported ornamental shrimp and at least one of them, *S. japonica* has been already confirmed in both types of shrimp farming (Klotz *et al.*, 2013; Maciaszek *et al.*, 2018; Maciaszek *et al.*, 2020). Aquaculture ponds create favourable conditions for the development of shrimp epibionts including representatives of Vorticellidae, mainly because of the high levels of organic matter in water and plankton, which could be recognizable as an additional source of their food. It is the main reason, *S. japonica* adults are often observed hunting for food at rostral area of shrimps. The species can be also found attached to pereiopods or pleopods. These locations belong to suitable for *S. japonica*, thanks to water moves regularly delivering organic matter (Maciaszek *et al.*, 2018). Similar location preferences can be observed in vorticellids, however, their colonies are found also in other body parts (Patoka *et al.*, 2016a; Liao *et al.*, 2018; Maciaszek *et al.*, 2018). *Scutariella japonica* is known to spend most of its life in host gills where is lays eggs (Matjašič, 1990). Presence of this epibiont on shrimp in most cases does not impact host health as long as it occurs in relatively small numbers. Mass quantities of *S. japonica* specimens (as well as vorticellids) can be dangerous especially for moulting crustaceans. Shrimp may not survive this process due to stress caused by the activity of the epibiont as well as host weakness can be used as a potential gate for infections of other organisms (Liao *et al.*, 2018; Maciaszek *et al.*, 2018).

In Europe, several genera of Scutariellidae are known to be epibionts of freshwater crustaceans. Among them, only representatives of genus *Scutariella* have been reported from shrimp of the family Atyidae (Matjašič, 1990). Most species can be found in caves, where they often live on endemic shrimp. However, one species, *Scutariella didactyla* (Mrázek, 1907) is the only European representative of the genus found in branchial cavity of *Atyaephyra vladoi* (Jabłońska *et al.*, 2018b) occurring in Skadarsko lake and its tributaries (Matjašič, 1990; Jabłońska *et al.*, 2018b).

If introduced, *S. japonica* as a new alien epibiont species may become a possible threat to European native species, potential hosts as well as their epibionts, including other epibiotic platyhelminths like dalyellid *Varsoviella kozminskii* (Gieysztor and Wiszniewski, 1947), living on gammarids. As scutariellids are known not only from shrimp, it is likely that in some cases, they could be transmitted also by other crustaceans, including crayfish (Matjašič, 1990; Ohtaka *et al.*, 2012). The spread can be intensified by thermal pollution of waters or climate change, since warmer waters create more favourable conditions for *S. japonica* (Maciaszek *et al.*, 2018).

In this study, we provide first report on the occurrence of epibiotic *S. japonica* in European thermally polluted waterbodies.

2 Material and methods

2.1 Locality

Sampling site was located at thermally polluted canal (53.2101 °N; 14.4638 °E) situated south of Gryfino in north-west Poland, ca. 7 km from the border with Germany (Fig. 1), which is a part of Dolna Odra Power Plant cooling system and therefore contains heated waters. According to measurements provided by Fisheries Research Station of West Pomeranian University of Technology, average year water temperature in this location is 20.9 °C while the minimum and maximum are 7.5 °C and 34.2 °C. The canal is known for the presence of both native and alien species. The latter are represented mainly by fishes such as *Cyprinus carpio* (Linnaeus, 1758), *Lepomis gibbosus* (Linnaeus, 1758), *Neogobius melanostomus* (Pallas, 1811) and invertebrates like crayfish *Faxonius limosus* (Rafinesque, 1817) or bivalve *Sinanodonta woodiana* (Lea, 1834). Aquarium-related species have been also noticed. Estabilished populations of another bivalve *Corbicula fluminea* (Müller, 1774), shrimp *N. davidi* and fish *Piaractus brachypomus* (Cuvier, 1818) have been found being present in this location for at least 10 years. Preying on shrimp in this site was observed in most fish species, mainly *L. gibbosus* and *N. melanostomus*.

2.2 Animals collection, epibiont detection and observation

In total, 120 adult individuals of *N. davidi* (min. 1.5 cm length) including 85 females and 35 males, were collected from the Ciepły Canal of Dolna Odra Power Plant. Shrimp were caught using long-handled dip net and flashlight at the waterside controlled by Fisheries Research Station of West Pomeranian University of Technology. They were collected from mostly rocky or sand substrata and depth not exceeding 0.5 m. Survey carried out on March 10, 2018 was made during nighttime and lasted ca. 120 minutes. Collected shrimp were observed for selected epibionts (Annellida, Ciliata, Platyhelminthes) presence under digital microscope Keyence VHX-7000 at 500–1500x magnification. Among epibionts, presence of vorticellids was used only as an indicator of organic matter quantity in the water, which can impact presence of scutariellids (Maciaszek *et al.*, 2018; Kondrateva *et al.*, 2019). Isolated epibionts were examined for morphological features using available literature (Matjašič, 1990; Ahn and Min, 2016).

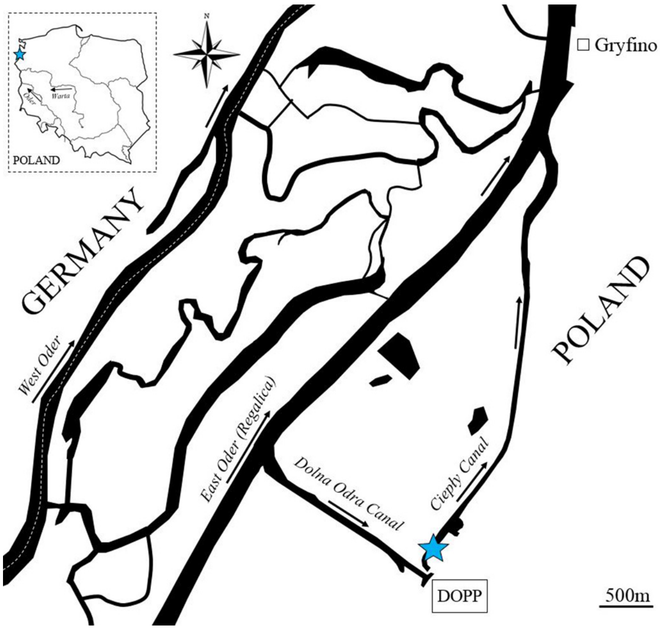

Fig. 1. Location of sampling site (blue asterix) on the map of Poland and Ciepły Canal of Dolna Odra Power Plant (DOPP).

3 Results

Total of six adult representatives and 15 eggs of scutariellids were found in gills area of seven infected shrimp (5.83%) among examined group of 120 *N. davidi* shrimp (Tab. 1). Despite difference in sex ratio in infected shrimp, females were more preferred (85.71%) than males (14.29%). Vorticellids were found in 99 shrimp (82.5%), out of which 69.07% were females and 30.93% were males. They were mostly attached to shrimp pleopods and pereiopods. In contrast to males, females were hosts for more vorticellid specimens. Individuals free of examined epibionts (both vorticellids and scutariellids) constituted 17.5% of the group. In 10 shrimp (8.33%) melanized damages in gills were observed. Detailed data on selected epibionts presence was presented in Table 1.

All scutariellids were identified as *S. japonica* (Matjašič, 1990) (Fig. 2). Collected individuals were characterised by following features: body transparent white, reaching 2.1 mm when not extended (similar size was noticed after preservation in 96% ethyl alcohol); two tentacles, each with cylindrical base and independent sucker; two eyes present, well developed; pharyngeal glands absent or not well developed; two testes and separated vitelline glands located on each side of the body; adhesive attachment organ, single, horseshoe-shaped (Fig. 3).

Table 1. Occurrence of examined epibionts (*S. japonica*, Vorticellidae) in *N. davidi* shrimp collected from the thermally polluted canal of Oder River.

N. davidi		Scutariellidae			Vorticellidae
No.	Sex	Adults quantity	Eggs quantity	Confirmed activity (damages in gills)	Confirmed presence (live individuals)*
1	F	2	8	+	++
2	F	1	3	+	++
3	F	1	2	+	++
4	M	1			
5	F	1			
6	F		1	+	++
7	F		1	+	+
8–13	F (3)			+	++
	M (2)			+	+
14–120	F (32)				++
	F (25)				+
	M (27)				+
	F (16)				
	M (5)				

*Higher quantity (hundreds) of specimens marked with "++".

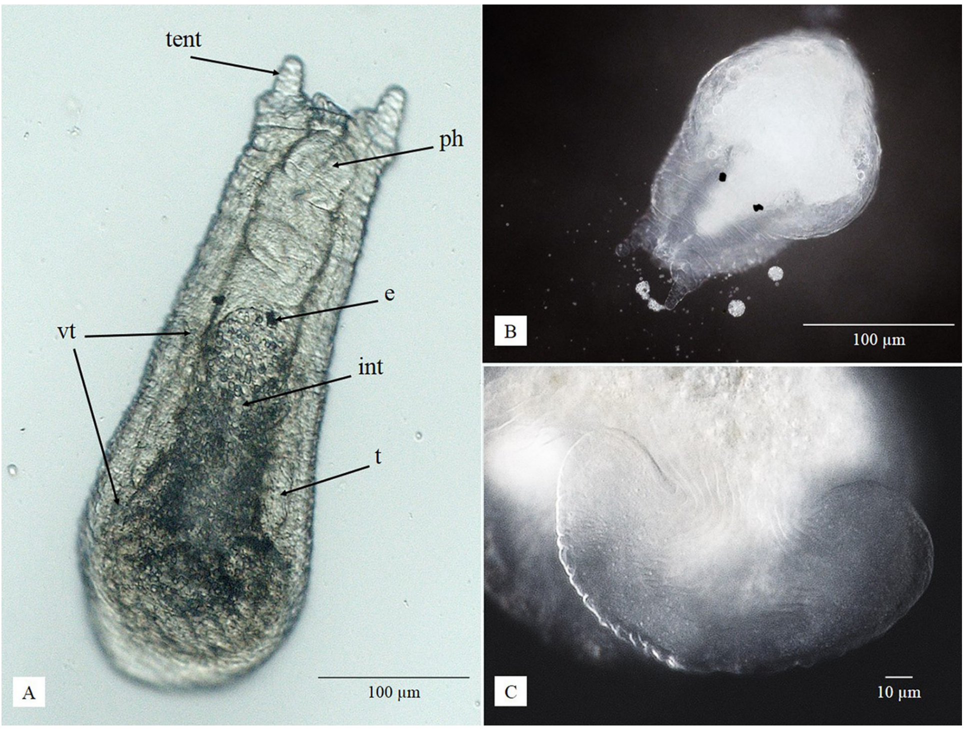

Fig. 2. Adult representative of *Scutariella japonica* collected from Oder River: live specimen (A, B); horseshoe-shaped adhesive attachment organ (C); e – eye, int – intestine filled up with the shrimp's hemolymph, ph – pharynx, t – testis, tent – tentacle, vt – vitelline glands.

4 Discussion

We provide first documented data on the occurrence of *S. japonica* in European waters, more precisely, thermally polluted canal of Dolna Odra Power Plant in Poland. The species can be easily distinguished from most European members of the genus by presence of the eyes and simultaneously occurrence of characteristic, horseshoe-shaped adhesive attachment organ which is heart-shaped in *S. didactyla* (Matjašič, 1990). Among other known representatives with developed eyes, *S. japonica* is distinct from *S. indica* (Annendale, 1912) by presence of two, instead of four testes (Matjašič, 1990), and unlike *S. sinensis* (Wang and Chen, 2018) it has not well developed pharyngeal glands (Chen *et al.*, 2018).

Among examined *N. davidi* shrimp, occurrence of *S. japonica* in gill chambers and vorticellids in external body parts reflects most suitable body parts for these epibionts (Matjašič, 1990; Ohtaka *et al.*, 2012; Maciaszek *et al.*, 2018). In comparison to most studies which had been carried out on Asian populations (Niwa and Ohtaka, 2006; Ohtaka *et al.*, 2012; Ohtaka *et al.*, 2015), including those from aquaculture ponds (Maciaszek *et al.*, 2018), much smaller prevalence of *S. japonica* on *N. davidi* was found in our study. It may suggest that the habitat conditions of Ciepły Canal are not proper for the species, although vorticellids quantity implies optimal levels of organic matter in the water (Maciaszek *et al.*, 2018). On the other hand, it could be an effect of increased predation on shrimp infected by this epibiont due to possible host stress (Liao *et al.*, 2018; Maciaszek *et al.*, 2018). In most cases *S. japonica* adults or eggs were observed at once with melanized damages in host gills, except two hosts in which only adults were found, possibly recently invaded. Simultaneous occurrence of scutariellids and the damages implies that activity of the epibionts somehow leads to partial melanization of gills, however it has not been earlier reported (Ohtaka *et al.*, 2012). Potential reinvasion on the same, previously moulted shrimp should not be excluded, especially that in both cases, hosts were also free of vorticellids or scutariellid eggs, which can be removed with shrimp moult (Klotz *et al.*, 2013). Among infected shrimp, females more likely presented damages in the cuticle, suggesting that the activity of *S. japonica* might be higher on them than on males, what is supported also by the presence of eggs only on females. This is most likely a result of more surface available to attach on females which grow bigger than males (Vazquez *et al.*, 2017), what can also be observed in quantity of vorticellids, which was also lower in males. On the other hand, females are often characterised with body covered by more intense pigmentation (Tomas *et al.*, 2019), which probably creates favourable shelter conditions for scutariellids hiding in host gill chambers.

Occurrence of *S. japonica* in Ciepły Canal of Oder river is most likely a result of accidental introduction with ornamental shrimp, *N. davidi*, that have been observed in the site since 2003 (Jabłońska *et al.*, 2018a). However, based on the quantity of alien species in the area, possible origin like separate releases or numerous introductions of ornamental shrimp should not be excluded. Therefore, release of and first observation of *N. davidi* on this location may not be connected. As Jabłońska *et al.* (2018a) proposed, introduction of shrimp, including infected ones, was apparently an action of irresponsible owner and originated from aquarium culture. *Neocaridina davidi* from Ciepły Canal presents similar features with specimens occurring in aquaria as well as in

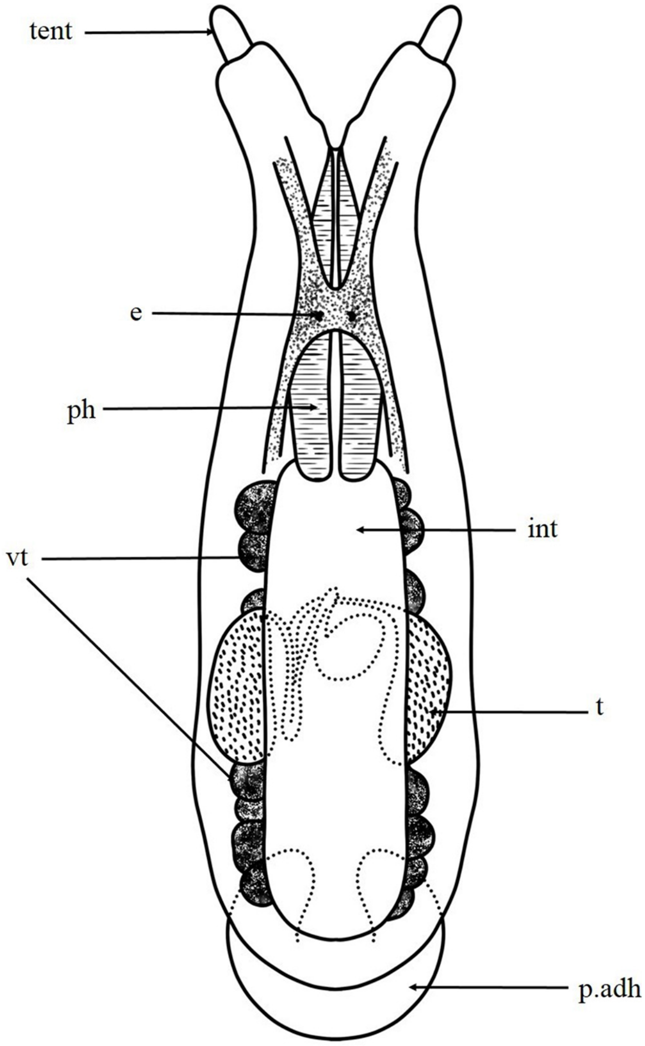

Fig. 3. Dorsal view of collected *Scutariella japonica* based on Matjašič (1990): e – eye, int – intestine, p.adh – posterior adhesive organ, ph – pharynx, t – testis, tent – tentacle, vt – vitelline glands.

the wild in Taiwan. This may confirm origin of examined *S. japonica*, which is probably the same species as observed in European aquaria, Taiwanese seminatural farms and natural waterbodies (Ohtaka *et al.*, 2015; Maciaszek *et al.*, 2018). If so, it is expected that vorticellids found in *N. davidi*, might also be introduced aliens (Patoka *et al.*, 2016a). However, this would not be a surprise, since until now there are no control methods introduced on large scale. Unwanted hitchhikers are still present in imported shrimp from Asia as well as in the European pet trade (Patoka *et al.*, 2016a; Patoka *et al.*, 2016b; Maciaszek *et al.*, 2018).

Further spread of *S. japonica* in European waters and its possible transmission to other crustaceans is potentially dangerous to native species. Presence of *S. japonica* may affect growth and reproduction of host, by causing stress and problems with moulting and health as well (Matjašič, 1990; Maciaszek *et al.*, 2018). As scutariellids are known to be epibionts of freshwater shrimp, also can be transmitted to other crustaceans such as crayfish, these can be their potential vectors. Unfortunately, all these crustaceans can be found in Ciepły Canal. Although *N. davidi* has not yet been reported as an escaper from the location, it is possible that the shrimp has already colonised surrounding areas, following other aliens (Łabęcka and Domagała, 2018; Weiperth *et al.*, 2019) supporting spread of *S. japonica*. Moreover, Oder river is located in west part of geographic range of *Atyaephyra desmarestii* (Millet, 1831), the most likely European host for *S. japonica*. In contrast to *Atyaephyra vladoi* (Jabłońska *et al.*, 2018b) from the ancient Skadar Lake system, *A. desmarestii* is not known for the presence of *S. didactyla*, the only known European scutariellid found in shrimp living in surface waters (Matjašič, 1990; Pešić *et al.*, 2018). If infected, the shrimp could become a potential vector of *S. japonica* and transmit this epibiont to south-west parts of the continent, finally reaching sites of other endemic shrimp and in some cases, their native scutariellids. However, invasion of this epibiont would not impact shrimp populations living in brackish water, which is deadly for scutariellids (Gottstein and Kerovec, 2002; Niwa and Ohtaka, 2006). Luckily, possible transmission to crayfish may be limited by other epibionts, including European branchiobdellidans, which in contrast to *H. truncatus*, are not harmoniously coexisting with scutariellids, as they prey on them (Gelder, 1999; Ohtaka *et al.*, 2012). In Polish waters, *S. japonica* may eventually compete with exemplary *V. kozminskii* in gills of gammarids. Although possible cohabitation of two epibiotic platyhelminths (scutariellids) is known from Ceylon (Matjašič, 1990), impact of new invader on European species both native as well as above mentioned aliens remains unknown.

To sum up, the occurrence of *S. japonica* in Ciepły Canal of Odra river creates opportunities for its further spread in Europe. This may be supported by transmission to native as well as nonindigenous crustaceans and may have negative impact on native species.

We, therefore, urge for more control over this epibiont, using preventive methods that need to be yet developed, such as proper quarantine for ornamental shrimp (at least two-three weeks in aquarium allowing observation of all specimens and removing moults) and baths in saline solutions (30 seconds bath in 40g sodium chloride per 1l of aquarium water) (Maciaszek, 2016), which are still rarely used in aquarium hobby. Population of *N. davidi* should be covered by monitoring and if possible, wiped out from the site. We recommend catching shrimp and their epibionts for experimental purposes or moving them to educational aquariums, for promotion of responsible aquarium hobby.

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Cite this article as: Maciaszek R, Świderek W, Kaliszewicz A, Karaban K, Szpakowski B. 2021. First report of *Scutariella japonica* (Matjašič, 1990), a temnocephalid epibiont from South-East Asia, found on introduced ornamental freshwater shrimp in European waters. *Knowl. Manag. Aquat. Ecosyst.*, 422, 19.