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Epibionts of ornamental freshwater shrimps bred in Taiwan

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Abstract: *Epibionts of ornamental freshwater shrimps bred in Taiwan.* One of the major problems in breeding *Neocaridina davidi* in Taiwanese aquaculture ponds are epibionts found on the body of ornamental shrimp. These organisms affect shrimp wellbeing by causing distress which leads directly to shrimp weakness, loss of colour and even casualties. They can also be observed in imported shrimps which put in danger individuals bred in Europe, mostly characterised by high level of inbreeding and sensitivity to pathogens. Microscopic analyses indicated presence of six freshwater shrimp epibionts. Some of them showing parasitic lifestyle (*Cladogonium ogishimae*, *Saprolegnia* sp., *Scutariella japonica*), others (phyla Ciliophora and Rotifera) may indicate level of organic matter in water. To allow an effective treatment and control of the spread of parasites, all of their preferred locations on shrimp body observer in this study should be checked and become a vital part of diagnostic methods. Researches on ornamental freshwater shrimps' epibionts are important to achieve success in shrimp breeding as well as to effectively monitor epibiont populations globally, especially that in some regions they may become potentially invasive organisms to the native crustaceans.

Key words: epibionts, parasites, aquarium, shrimps, aquaculture

INTRODUCTION

Freshwater shrimps belong to the most common crustaceans kept in aquarium. Their small sizes, intensive colouration and large diversity of possible patterns make them valuable to breeders and became reason of rising quantity of colour varieties available in global aquarium trade (Hung et al. 1993, Jayachandran and Raji 2005, Heerbrandt and Lin 2006, Barbier 2010).

Colour intensity, the most important feature of these pet animals, is affected by multiple factors including wellbeing that depends on shrimp health and environment conditions. Shrimp quality is verified by professional judges during valued international contests, where colour intensity and wellbeing of the pets are especially high-priced (Maciaszek 2016).

Growing market of aquarium shrimp causes establishment of new aquaculture farms adapted to producing mass quantities of low-cost crustaceans. Shallow, concrete-based ponds filled with rainwater are also much cheaper in keeping than aquarium farms. In case of Taiwanese breeders, aquaculture ponds usually do not have any additional filtration as they are exposed to wind blows which results in high rates of organic matter dispersed in water. Each year one pond may produce thousands of crustaceans which then are exported mainly to Europe to find their final destination in European aquariums (Maciaszek 2016).

Unfortunately, aquaculture ponds create suitable conditions for development of other, potentially undesirable organisms called epibionts. These organisms may affect shrimp wellbeing by causing distress which leads directly to shrimp weakness, loss of colour and even casualties. They can be also observed in imported shrimp which can put in danger individuals bred in Europe, characterised by high level of inbreeding and sensitivity to pathogens. Lack of effective treatment due to the relatively small knowledge of parasites found in freshwater shrimp farms may result in escalating of the problem. Except observations made by Patoka et. al (2015) current available literature on shrimps' parasites is almost completely restricted to marine species (Johnson 1989, Lightner and Redman 1998, Chakraborti and Bandyapadhyay 2011). Therefore, the aim of this study was to estimate seasonal changes in population of common freshwater shrimp epibionts as well as to identify species and their preferred locations on shrimp body.

MATERIAL AND METHODS

Present study was conducted in two seasons: spring (May) and autumn (October) during the period of 2012–2015. Live *Neocaridina davidi* (Bouvier 1904) adults bred in aquaculture ponds (Crimson Taiwan, New Taipei City, Taiwan) were collected in trials of 600 shrimps per season and transported in groups of 200 individuals to minimize possible effect on water parameters changes and casualties in result. Shrimp were transported inside styro boxes in 10-litre plastic aquarium fish bags half-filled with pond water and half with oxygen under pressure. Day-long (24 h) airplane imports in constant temperature of 20°C were performed once per season. Imported shrimp were taken under observation in Kumak Shrimp – aquarium shrimp farm (Konstancin-Jeziorna, Poland).

After initial acclimatization in 10-litre quarantine tanks equipped with air pump sponge filtration only (each), shrimps were checked for epibionts presence in four determined locations preferred by epibionts:

- location A – rostrum, antennas and antennules;
- location B – gills;
- location C – chelipeds and pereiopods;
- location D – pleopods, uropods and telson.

Studies were conducted using camera Canon 600D (Canon Inc., Japan) to allow an accurate examination a plastic Petri dish was used to keep shrimp inside the aquarium (trapping shrimp inside the dish and pressing against side wall until it calmed down). Additional observations and species identification were made with binocular Nikon SMZ1000 (Nikon Corporation, Japan) in the Department of Ichthyobiology, Fisheries and Biotechnology in Aquaculture of Warsaw University of Life Sciences – SGGW (Warsaw, Poland). Epibiont identification was made using available literature and identification keys (Matjašič 1980, Shiel 1995, Foissner and Berger 1996, Niwa and Ohtaka 2006, Diéguez-Uribeondo et al. 2007).

Results were statistically summarized with PQStat ver. 1.6.4.121. Epibionts quantity in different locations was analyzed with χ^2 test of independence. Comparisons between epibionts were examined with multiple χ^2 test with use of Benferroni correction. Equivalence of epibionts' distribution in different locations was analyzed with χ^2 test for compatibility. Differences between quantities of epibionts and seasons were examined using two-way analysis of variance (ANOVA) for repeated measures. Dependent factor (repeated measure) was season (spring and summer) and independent factor (grouping factor) was epibiont. Post-hoc analyses in epibionts were examined with Tukey's test while differences between seasons were analyzed using contrast method. Value of P level used as statistically significant was $P < 0.05$ when statistically highly significant value was $P < 0.01$.

RESULTS AND DISCUSSION

Microscopic analyses indicated presence of six freshwater shrimps' epibionts: *Saprolegnia* sp. (Nees von Esenbeck 1823), *Scutariella japonica* (Matjašič 1980), *Vorticella* sp. (van Leeuvenhoek 1702), *Stentor* sp. (Ehrenberg 1831), representatives of phylum Rotifera (Cuvier 1817) as well as *Cladogonium ogishimae* (Hirose and Akiyama 1971, Matsuyama-Serisawa et al. 2014, Imai et al. 2017) that was not reported outside of Japan before. Location preferences of each epibiont species in representative group of 1,200 shrimps (600 per season) imported in 2013 were presented in Table 1. The biggest diversity of identified epibionts was

TABLE 1. Selected epibionts observed in examined parts of shrimp's body

Location		*Cladogonium ogishimae*	Rotifera	*Saprolegnia* sp.	*Scutariella japonica*	*Stentor* sp.	*Vorticella* sp.
A	*n*	18	799	19	877	708	319
	%	2.4	46.4	6.8	39.4	51.4	14.9
B	*n*	7	398	24	988	4	12
	%	0.9	23.1	8.6	44.4	0.3	0.6
C	*n*	358	310	106	176	303	905
	%	47.9	18.0	37.9	7.9	22.0	42.2
D	*n*	364	216	131	183	362	907
	%	48.7	12.5	46.8	8.2	26.3	42.3
χ^2 test of independence		$\chi^2 = 40.25$, $df = 15$, $P < 0.0001$					
Cladogonium ogishimae			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rotifera		< 0.0001		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Saprolegnia sp.		< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001
Scutariella japonica		< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001
Stentor sp.		< 0.0001	< 0.0001	< 0.0001	< 0.0001		< 0.0001
Vorticella sp.		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
χ^2 test for compatibility		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

n – quantity of shrimps settled by 1 epibiont observed in group of 1,200 individuals; % – percentage quantity of 1 epibiont observed in examined parts of shrimp's body.

observed in locations C and D (Table 1). These locations were occupied mostly by: *Cladogonium ogishimae* (47.9 and 48.7%, respectively), *Scutariella japonica* (37.9 and 46.8%, respectively) and *Vorticella* sp. (42.2 and 42.3%, respectively). Location A was taken in vast majority by rotifers (46.4%). Comparing to other examined epibionts, location B was particularly preferred by *Scutariella japonica* (44.4%) and rotifers (23.1%). Observations affirmed possible parasitic lifestyle of *Saprolegnia* sp. (Fig. 1) and *Cladogonium ogishimae* (Matsuyama-Serisawa et al. 2014) that were observed especially on the pleopods responsible for incubating host's eggs. Epibionts belonging to phylum Ciliophora were recorded in all examined locations except gills. This confirms ciliates usage of shrimp movable body parts only as a way of getting plankton which is their source of food (Psenner 1995). The ciliates also used additional surface created by *Cladogonium ogishimae* structures (Fig. 2). Similar preferences were observed for rotifers (Fig. 3) and scutariellids (Fig. 4) which used mainly location A as an opportunity for catching microorganisms. Parasitic behaviour of *Scutariella japonica* was clearly visible especially on gills where it layed eggs causing destruction to the host body structures (Fah and Christianus 2013, Klotz et al. 2013).

Statistically highly significant ($\chi^2 = 40.25$, $df = 15$, $P < 0.0001$) difference was examined in distribution depended on epibiont. Multiple comparisons indicated highly significant differences ($P < 0.0001$) between each pair of examined epibionts. The same result ($P < 0.0001$) was obtained using

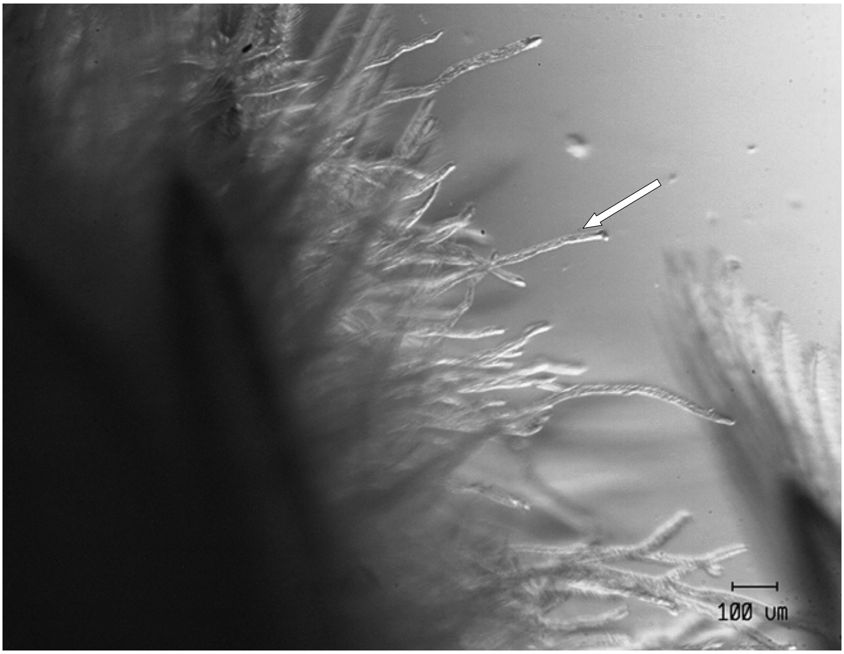

FIGURE 1. *Saprolegnia* sp. (white arrow) detected on pleopods of *Neocaridina davidi*

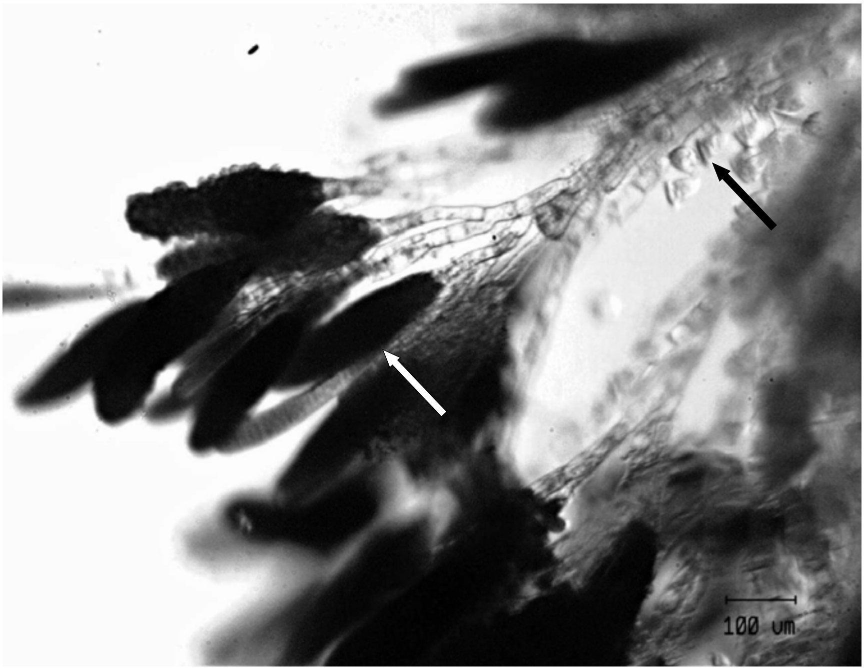

FIGURE 2. *Cladogonium ogishimae* observed on *Neocaridina davidi* pleopods. Structures of this species may be used by other organisms such us *Vorticella* sp. (black arrow)

FIGURE 3. Rotifera representatives (black arrow) observed in rostrum region

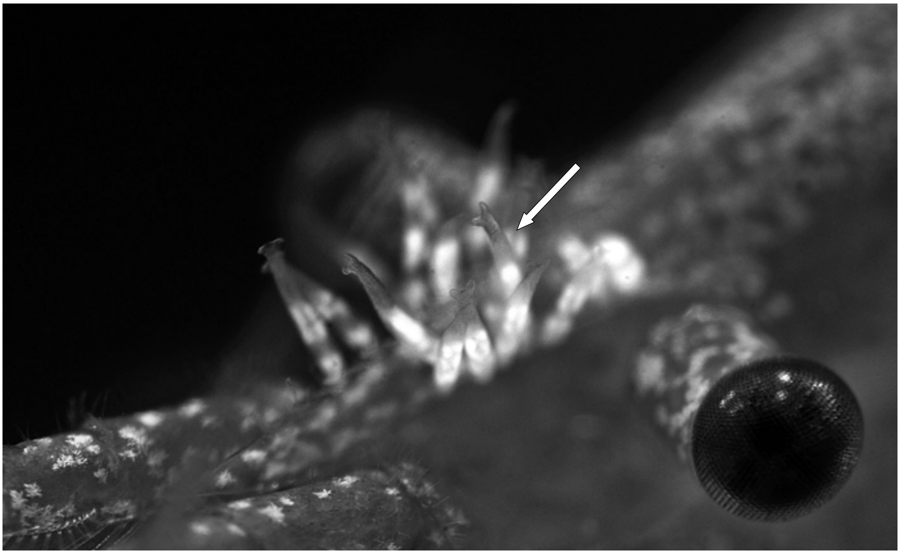

FIGURE 4. *Scutariella japonica* adults (white arrow) saddle in *Neocaridina davidi* rostrum

Bonferroni correction, what explains specific distribution of preferred location for each examined epibiont. Hypothesis involving equivalence of epibiont distribution was verified and in each case statistically highly significant ($P < 0.0001$) incompatibility with normal distribution was obtained. Epibiont location was not random, all examined epibionts preferred some locations.

Results obtained in four-year period indicates decreasing presence of most shrimps' epibionts (*Cladogonium ogishimae*, Rotifera, *Saprolegnia* sp., *Stentor* sp., *Vorticella* sp.) bred in aquaculture ponds what is clearly visible in changes of trial average (Table 2). It could be the effect of increasing, but still insufficient knowledge of shrimp parasites as well as possible improvement of ponds filtration quality. Although records achieved for individuals infected by *Scutariella japonica* (min. 69.5%) (Table 2) compared to Niwa and Ohtaka (2006) results (58%) indicates increasing quantities of this species in the aquarium trade. Observation shows *Scutariella japonica* can use shrimp as its own mobile base (for example by attaching itself to the rostrum) to obtain organic material from the water column as well as living its parasitic lifestyle using shrimp as its host (for example attaching to the gills). Thanks to this ability to adapt *Scutariella japonica* can be dangerous to the aquarium shrimp keeping as it can easily infect not only weakened individuals but also a healthy ones.

Statistically significant differences [$F(5;18) = 3.85$, $P = 0.0151$] between examined epibionts were observed. Post-hoc Tukey's test figured out that significant differences occurred between *Cladogonium ogishimae* and *Scutariella japonica*. Statistically highly significant [$F(1;\ 15) = 17.03$, $P = 0.0006$] were found between seasons. Epibionts quantity found on shrimps was bigger

TABLE 2. Selected epibionts observed in examined parts of shrimp’s body

Years of observations	Scientific name												Trial average
	Cladogonium ogishimae		Rotifera		*Saprolegnia* sp.		*Scutariella japonica*		*Stentor* sp.		*Vorticella* sp.		
	spring	autumn	spring	autumn	spring	autumn	spring	autumn	spring	autumn	spring	autumn	
2012	125.3	57.0	193.0	114.0	189.0	112.3	143.0	139.0	143.0	138.7	200.0	192.3	145.55
2013	79.3	42.0	160.7	105.7	35.7	8.0	175.0	154.3	175.7	196.7	60.3	105.7	108.26
2014	25.0	11.7	159.0	119.0	17.3	9.0	185.3	155.0	19.0	11.7	145.0	132.0	82.42
2015	10.0	2.0	155.3	132.0	8.0	4.7	200.0	182.3	14.3	3.3	157.0	109.7	81.55
$\bar{x}$	59.90	28.18	167.00	117.68	62.50	33.50	175.83	157.65	88.00	87.60	140.58	134.93	
SD	52.79	25.68	17.48	11.01	85.12	52.57	24.17	18.02	83.48	95.54	58.49	39.96	
Median	52.15	26.85	159.85	116.50	26.50	8.50	180.15	154.65	81.00	75.20	151.00	120.85	
ANOVA	scientific name $F(5;18) = 3.85$, $P = 0.0151$, seasons $F(1; 15) = 17.03$, $P = 0.0006$, interaction $F(5; 18) = 1.86$, $P = 0.1524$												
Contrast	$P = 0.0281$		$P = 0.0016$		$P = 0.0425$		$P = 0.1880$		$P = 0.9763$		$P = 0.6756$		

in spring than autumn. Higher temperatures during springtime cause less water oxygenation and speeds up shrimps metabolism which is the main reason for increasing rates of toxic nitrogenous compounds which are responsible for shrimps weakening (Jiang et al. 2000, Figueroa-Lucero et al. 2012). Interaction between epibionts and season was not statistically significant [$F(5; 18) = 1.86$, $P = 0.1524$]. Differences between seasons are comparable to all examined epibionts. Contrast analysis indicated significant difference between seasons in *Cladogonium ogishimae* ($P = 0.0281$) and *Saprolegnia* sp. ($P = 0.0425$) as well as highly significant in Rotifera ($P = 0.0016$). No significant differences were found in *Scutariella japonica* ($P = 0.1880$), *Stentor* sp. ($P = 0.9763$) and *Vorticella* sp. ($P = 0.6756$).

CONCLUSIONS

Aquaculture ponds create favorable conditions for *Neocaridina davidi* shrimps' epibionts development, especially ectoparasites. Decreasing quantity of most epibionts in the four-year period is most probably a result of breeders increasing awareness of other organisms present in ponds and related to them possible shrimp casualties. Some of those organisms have parasitic lifestyle (*Cladogonium ogishimae*, *Saprolegnia* sp., *Scutariella japonica*), others (phyla Ciliophora and Rotifera) may be useful as indicators of level of organic matter in water. To allow an effective treatment and control of the spread of parasite, all of their preferred locations on shrimp body observed in this study should be checked and become a vital part of diagnostic methods and should be assisted by assuring of right transport conditions, post import acclimatization, optimal filtration and water parameters.

This work contains initial studies on shrimps' epibionts. Further researches on ornamental freshwater shrimps' epibionts are important to achieve success in shrimp breeding as well as to effectively monitor epibiont populations globally, especially that in some regions they may become potentially invasive organisms to the native crustaceans.

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Streszczenie: *Epibionty krewetek akwariowych hodowanych na Tajwanie*. Epibionty są jednymi z głównych problemów w hodowli krewetek *Neocaridina davidi* w stawach hodowlanych na Tajwanie. Ich obecność wpływa negatywnie na dobrostan krewetek poprzez wywoływanie stresu, osłabienia, upadków oraz utraty ubarwienia.

Epbionty stwierdza się również na krewetkach pochodzących z importu, co jest szczególnie niebezpieczne dla osobników hodowanych w Europie charakteryzujących się wysokim poziomem chowu wsobnego oraz słabą odpornością na patogeny. Analiza mikroskopowa wykazała obecność sześciu gatunków symbiontów krewetek słodkowodnych. Niektóre z nich prowadzą pasożytniczy tryb życia (*Cladogonium ogishimae*, *Saprolegnia* sp., *Scutariella* sp.), inne (typ Ciliophora oraz typ Rotifera) mogą być wykorzystane jako wskaźniki ilości materii organicznej w wodzie. Wykazane w obserwacjach miejsca ciała krewetek preferowane przez pasożyty powinny stanowić nieodłączną część metod ich diagnostyki pozwalającej na efektywne leczenie. Badania na epibiontach krewetek akwariowych są szczególnie istotne dla sukcesywnej hodowli tych skorupiaków, a także dla prowadzenia efektywnego monitoringu populacji epibiontów, które w niektórych regionach świata mogą stać się gatunkami potencjalnie inwazyjnymi dla naturalnie występujących skorupiaków.

Słowa kluczowe: epibionty, pasożyty, akwarium, krewetki, akwakultura

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