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Another hitchhiker exposed: *Diceratocephala boschmai* (Platyhelminthes: Temnocephalida) found associated with ornamental crayfish *Cherax* spp.

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Abstract – There are known plenty tiny invertebrate species associated with decapod crustaceans. Our contribution documents the first record of New Guinean ornamental *Cherax* crayfish epibiont, *Diceratocephala boschmai* (Platyhelminthes: Temnocephalida) found in pet trade in the Czech Republic. Correct species assignment was confirmed by genetic barcoding. The pathway for introductions of organisms unintentionally moved in association with ornamental crayfish via the international trade has been highlighted. Although *Cherax* species survive in thermal waters in Europe, even potential introduction to these habitats posed threat to native crayfish ectosymbionts (branchiobdellids) thought similar ecological niche.

Keywords: Ectosymbiont / alien species / ornamental species / pet trade / Europe / New Guinea

Résumé – Un autre hitchhiker dévoilé: *Diceratocephala boschmai* (Platyhelminthes: Temnocephalida) trouvé associé aux écrevisses ornementales *Cherax* spp. On connaît de nombreuses espèces de petits invertébrés associés à des crustacés décapodes. Notre contribution documente le premier enregistrement de l'épibionte de l'écrevisse *Cherax* ornementale de Nouvelle-Guinée, *Diceratocephala boschmai* (Platyhelminthes : Temnocephalida) trouvé dans le commerce des animaux de compagnie en République tchèque. L'attribution correcte des espèces a été confirmée par un code-barres génétique. La voie d'introduction d'organismes déplacés involontairement en association avec des écrevisses ornementales via le commerce international a été mise en évidence. Alors que les espèces de *Cherax* survivent dans les eaux thermales en Europe, même une introduction potentielle dans ces habitats constitue une menace pour les ectosymbiontes d'écrevisses indigènes (branchiobdellidés) dont la niche écologique est similaire.

Mots-clés : Ectosymbionte / espèces exotiques / espèces ornementales / commerce des animaux de compagnie / Europe / Nouvelle-Guinée

Anthropogenic translocation of species together with subsequent biological invasions are considered among major threats causing biodiversity alteration in the aquatic ecosystems worldwide (Rodríguez *et al.*, 2005). One of the main pathways for non-native species introduction is international pet trade (Patoka *et al.*, 2018; Marková *et al.*, 2020). Although decapod crustaceans are relatively new to the pet trade (Chucholl, 2013; Faulkes, 2015; Patoka *et al.*, 2015), especially colourful species of *Cherax* quickly became very popular (Patoka, 2020). With increasing number of imports from Indonesia, likelihood to import of so-called hitchhikers increase as well (Patoka *et al.*, 2015). Alien epibionts are usually overlooked (Dörr *et al.*, 2011; Patoka *et al.*, 2016a,b, 2020; Duggan and Pullan, 2017; Duggan *et al.*, 2018) unless they represent real threat to ecosystem where their hosts were introduced (Ohtaka *et al.*, 2005). Up to now, mostly North American representatives of brachniobdellids (Annelida: Branchiobdellida) were introduced and found in European waters (James *et al.*, 2015; Parpet and Gelder, 2020) following their North American crayfish hosts (mostly *Procambarus clarkii* and *Pacifastacus leniusculus*). Natural epibionts of the North Hemisphere crayfish are branchiobdellids (Annelida), while Southern hemisphere crayfish are infested

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by temnocephalids (Platyhelminthes) (Gelder, 1999). Populations of Australian *Cherax* crayfish are already established in some European localities (Scalici *et al.*, 2009; Jaklič and Vrezec, 2011; Mazza *et al.*, 2018; Weiperth *et al.*, 2019; Weiperth *et al.*, 2020; Arias Rodríguez and Torralba Burrial, 2021), however up to now only *Temnosewellia minor* was reported from Italy associated with *C. destructor* (Chiesa *et al.*, 2015; Vecchioni *et al.*, 2021), and with North American *P. clarkii* (Mazza *et al.*, 2018). Just recently, *Scutariella japonica* has been recorded on freshwater shrimps in thermally polluted waters in Poland (Maciaszek *et al.*, 2021). Nevertheless, occurrence of temnocephalids out of their natural range was reported on crayfish in Uruguay (Volonterio, 2009), South Africa (Mitchell and Kock, 1988; Du Preez and Smit, 2013; Tavakol *et al.*, 2016) and Thailand (Ngamniyom, 2020). Therefore, the aim of this study was to point out on unintentional translocation of hitchhikers through international pet trade of their hosts, and to suggest preventive measures against introduction out of their natural range.

In total, 40 adult individuals of various *Cherax* species (*C. alyciae*, *C. gherardii*, *C. peknyi*, and several individuals of *Cherax* sp. "Black Scorpion" and *C.* cf. *boesemani*) were superficially inspected for potential epibionts after their arrival in one batch import from Indonesia on 13 October 2020, when no obvious epibionts were found. After two months in aquaria within closed recirculation system, one adult temnocephalidan was found on the bottom part of carapace in one *Cherax* individual. Together with adult individual, many eggs were recorded in surroundings (Fig. 1). Later more adult individuals were found on this specific crayfish individual, attached under rostrum (2 ind.), on the bottom of carapace (2 ind.) and at the base of abdomen (2 ind.).

Two temnocephalidans were removed from crayfish and placed directly into lysis buffer. Total genomic DNA was extracted using E.Z.N.A.® Tissue DNA Mini Kits (Peqlab, Erlangen, Germany). Fragments of mitochondrial genes Cytochrome c oxidase subunit I and nuclear 28S rDNA were amplified using PCR primers 450F/1200R and Ltem180/Ltem1000R, respectively following protocol of Hoyal Cuthill *et al.* (2016). PCR products were purified with NucleoSpin® (Macherey-Nagel, Düren, Germany) and sequenced on an ABI automatic capillary sequencer (series 373) (Macrogen, Seoul, Korea), using amplification primers.

Morphology as well as genetic barcoding showed clear assignment to the species of *Diceratocephala boschmai* Baer, 1953. Blast analysis reveals 99% identity with COI sequence MK421403 and 404, and 28S rRNA sequence KM588103 of *D. boschmai* available at GenBank database. Sequences originated in this study are deposited in GenBank under accession numbers MZ128776 for COI and MZ087752 for 28S gene.

Despite our first observations of two adult individuals of *D. boschmai* surrounded by their eggs on one crayfish host, during following 60 days the host was highly infested. Moreover, temnocephalids subsequently infested other crayfish individual which was kept separately but in the same recirculation system. This fact is highlighting potential of high spreading ability under suitable conditions (water temperature was 25 ± 2 °C). The temnocephalid associated with their *Cherax* crayfish hosts can be probably found in many species collected in wild in New Guinea. This assumption was confirmed by temnocephalid eggs recorded on adult

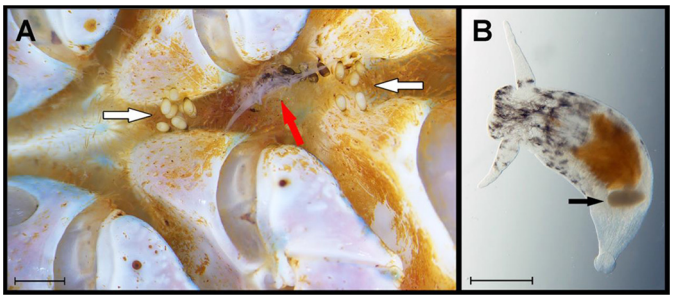

Fig. 1. Position of *Diceratocephala boschmai* (red arrow) and its eggs (white arrows) attached on the bottom part of carapace (A), and detail of epibiont with egg ready to be laid (black arrow) as well as gut content (B). The scale bar is equal to one millimeter.

C. monticola (Fig. 2) offered for sale in Wibama market in Wamena, Papua Province, Indonesian part of New Guinea in 2017 within astacological expedition to Yumugima cave system (Patoka *et al.*, 2017) by two authors of this publication (Patoka and Bláha, 2017, unpublished data).

Presence of alien epibionts in Europe following the aquatic invasions of decapods is actual topic (Chiesa *et al.*, 2015; James *et al.*, 2015; Mazza *et al.*, 2018; Parpet and Gelder, 2020; Maciaszek *et al.*, 2021; Vecchioni *et al.*, 2021). Global pet trade with increasing attractivity of ornamental decapods together with their current commercial availability is considered as a relevant risk of alien hitchhikers future introductions (Chucholl and Wendler, 2017; Yonvitner *et al.*, 2020). Additionally, the high invasive potential of *C. quadricarinatus*, the species widely used in aquaculture and introduced to many countries out of the original area of distribution has recently been discussed (Akmal *et al.*, 2021; Haubrock *et al.*, 2021) and thus representing high potential risk of native epibionts introductions. Even if policymakers generally focused on regulation of spread and introductions of potentially invasive species in European Union (Regulation (EU) No 1143/2014), the current legislative framework is ineffective in many cases (Patoka *et al.*, 2018). Our finding of *D. boschmai* on imported crayfish showed insufficient preventive measures on wild-caught crayfish in the place of export. However even including such measures do not ensure epibionts on transported animals or plants (Patoka *et al.*, 2016b; Duggan *et al.*, 2018). It is worth mentioning that certain epibionts, including temnocephalids would potentially spread in Europe similarly to North American branchiobdellids (Parpet and Gelder, 2020; Vecchioni *et al.*, 2021) as already shown on *Scuteriella japonica*, temnocephalid epibiont of freshwater shrimps (Maciaszek *et al.*, 2021). The only difference that their distribution would be related to warmer south parts, thermal or thermally polluted streams in Europe.

Together with previously reported host opportunism of ectosymbionts (James *et al.*, 2017; Mazza *et al.*, 2018) they could serve as vectors of pathogens to their non-indigenous hosts (Ngamniyom, 2020). We presume potential direct risk for native European epibionts based on our observation. Digestive tract of individuals of *D. boschmai* contained periphyton, cyclopoid copepods and chironomid larvae suggesting omnivorous feeding strategy. In case of common occurrence, smaller European branchiobdellids such as

Fig. 2. Temnocephalid eggs located on thorax carapace (white arrow) of adult *Cherax monticola*.

B. pentadonta or *B. hexadonta* could become a prey for bigger *D. boschmai*.

To reduce the spreading probability of the epibionts, the attention should be therefore focused on strict multiplied three-phases preventive measures according to manipulation with wild-caught animals and their exporting: (i) disinfection bath of wild-caught individuals; (ii) quarantine before adding to stock tanks; and (ii) regular periodical sanitation of stock tanks. Similar procedure is suggested for pet shops in areas of imported animals as well as to final customer who should follow the preventive procedure for newly purchased animals.

Future experiments focused on behaviour of *D. boschmai* in interactions with native branchiobdellids to ascertain the potential risk are recommended.

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