

CRAYFISH EPIBIONTS *BRANCHIOBDELLA* SP. AND *HYSTRICOSOMA CHAPPUISI* (ANNELIDA: CLITELLATA) IN GREECE

M. SUBCHEV (1), E. KOUTRAKIS (2) and C. PERDIKARIS (3)

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- (1) Institute of Zoology, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria
E-mail: subchev@yahoo.com
 - (2) Fisheries Research Institute, National Agricultural Research Foundation, GR-64007 Nea Peramos, Kavala, Greece
E-mail: manosk@inale.gr
 - (3) Department of Aquaculture & Fisheries, Technological Educational Institute of Epirus, Irinis & Eilias 1, GR-46100 Igoumenitsa, Greece
E-mail: kperdik@otenet.gr

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ABSTRACT

Specimens of the three known Greek crayfish species, *Astacus astacus* (Linnaeus, 1758), *Astacus leptodactylus* Eschscholtz, 1823 and *Austropotamobius torrentium* (Schrank, 1803), were collected from seven localities in Greece and were examined for the epibionts of *Branchiobdella* sp. (Annelida: Clitellata) and the aelosomatid *Hystriocosoma chappuisi* Michaelsen, 1926 and the copepod *Nitocrella divaricata* (Chappuis, 1923). As a result, three branchiobdellid species, *Branchiobdella parasita* (Braun, 1805), *Branchiobdella pentodonta* Whitman, 1882 and *Branchiobdella hexodonta* Grüber, 1883 were found. This was also the first report of *B. pentodonta* from Greece as well as *H. chappuisi*, the latter being found on crayfish gills in one of the localities investigated. Moreover, the current work represents the first documentation of the presence of *B. parasita* and *B. hexodonta* in several localities in Greece.

Key words: crayfish, crayfish epibionts, *Branchiobdella* sp., *Hystriocosoma chappuisi*, Greece

EPIBIONTES D'ECREVISSSES, *BRANCHIOBDELLA* SP. ET *HYSTRICOSOMA CHAPPUISI* (ANNELIDA: CLITELLATA) EN GRÈCE

RÉSUMÉ

Des spécimens des trois espèces d'écrevisses connues pour être présentes en Grèce *Astacus astacus* (Linnaeus, 1758), *Astacus leptodactylus* Eschscholtz, 1823 et *Austropotamobius torrentium* (Schrank, 1803) ont été prélevés dans sept localités grecques et ont été étudiés en vue d'une éventuelle présence d'épibiontes du genre *Branchiobdella* Annelides (Annelida : Clitellata), de aelosomatid *Hystriocosoma chappuisi* Michaelsen, 1926 et le copépode *Nitocrella divaricata* (Chappuis, 1923). En conséquence, trois espèces de branchiobdellidés, *Branchiobdella parasita* (Braun, 1805), *Branchiobdella pentodonta* Whitman, 1882 et *Branchiobdella hexodonta* Grüber, 1883 ont été trouvées. En particulier, *B. pentodonta* est signalée pour la première fois pour la faune grecque, ainsi

que *H. chappuisi* trouvé sur des branchies d'écrevisses dans une des localités étudiées. En outre, les travaux en cours représentent la première documentation de la présence de *B. parasita* et *B. hexodonta* dans plusieurs localités en Grèce.

Mots-clés : Écrevisses, épibiontes d'écrevisses, *Branchiobdella* sp., *Hystriocosoma chappuisi*, Grèce.

INTRODUCTION

Freshwater crayfish have an important ecological role in freshwater ecosystems and particularly in the European biodiversity. They have been significant in the social, cultural and economic life of Europeans, mainly as a source of fine food since the Middle Ages. The degradation of freshwater habitats, mainly due to pollution and other anthropogenic impacts, has increased the scientific interest on their ecology and conservation (SOUTY-GROSSET *et al.*, 2006).

Recent studies on Greek crayfish fauna indicated the presence of three indigenous species, *Astacus astacus* (Linnaeus 1758), *Astacus leptodactylus* Eschscholtz, 1823 and *Austropotamobius torrentium* (Schrank, 1803) (MACHINO and HOLDICH, 2006; SOUTY-GROSSET *et al.*, 2006). In particular, details on distribution and some biological and ecological peculiarities of these three species were reported by KOUTRAKIS *et al.* (2005), KOUTRAKIS *et al.* (2007) and PERDIKARIS *et al.* (2007). However, no information exists for the indigenous European crayfish epibionts in Greece, especially for the annelids of the European genus *Branchiobdella* and other gill inhabitants, such as the aelosomatid *Hystriocosoma chappuisi* Michaelsen, 1926 and the copepod *Nitocrella divaricata* (Chappuis, 1923). Investigations on these epibionts not only add to the current knowledge on Greek fauna, but also verify whether European gill inhabiting *Branchiobdella* species are also present. This is important because there is evidence that *Branchiobdella astaci* Odier, 1823 and *Branchiobdella hexodonta* Grüber, 1883 are harmful to crayfish since they dwell and injure crayfish gills, by feeding on them (KOZAROV *et al.*, 1972; WIERZBICKA and ŚMIETANA, 1999).

The aim of this work was to report the presence of crayfish epibionts in Greece, referring particularly to *Branchiobdella* species and *H. chappuisi*.

MATERIALS AND METHODS

Crayfish samples belonging to the species *A. astacus*, *A. leptodactylus* and *A. torrentium* from seven localities in Greece (Table I and Figure 1) were collected, according to the modified protocol proposed by MACHINO and FÜREDER (2005), and examined for branchiobdellidan species and gill epibionts *H. chappuisi* and *N. divaricata*. Specimens coming from Evros River, Aggitis Cave and River, Kalamas River and Bougazi Creek were collected and fixed from 2002 to 2005, while the collection of crayfish specimens from Vathitopos River and Pageo Mountain were performed during summer 2006.

In most cases the crayfish were fixed in 80 % alcohol immediately after their collection. Crayfish from Kalamas River, however, were frozen before their examination for branchiobdellidans. Some of the branchiobdellidan specimens found on crayfish from this locality were separated from the host and fixed in 80 % alcohol for further species identification.

Determination of the branchiobdellid species was performed based on body and jaw morphological characteristics as originally described by ODIER, 1823, WHITMAN, 1882, GRUBER, 1883, CANEGALLO, 1928, MOSZYNSKI, 1937, 1938 and SUBCHEV, 1978). However, in the case of *B. parasita* (Braun, 1805), the more accurate description of HENLE (1835) was used. The species descriptions presented in KASPRZAK (1979) were used for *H. chappuisi* identification.

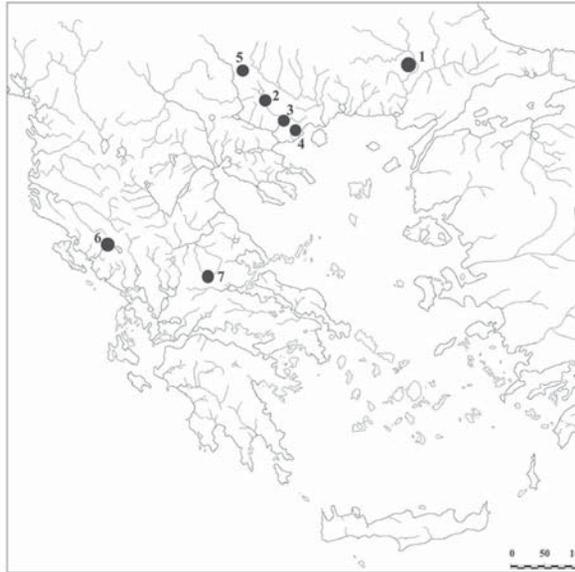


Figure 1

Map of Greece indicating the localities where the crayfish specimens were collected (background modified from ECONOMIDIS, 1995). 1 = Evros River, near Didimotihio, 2 = Aggitis Cave, 3 = Aggitis River, 4 = Tributary of Marmara River in Pageo, 5 = Vathitopos River, Vathitopos, 6 = Kalamas River, close to Vrodismeni, 7 = Bougazi Creek, close to dried Xyniada Lake.

Figure 1

Carte de la Grèce indiquant les localités où les spécimens d'écrevisses ont été collectés (fond modifié d'ECONOMIDIS, 1995). 1 = Fleuve Evros, près de Didimotihio, 2 = Aggitis Cave, 3 = Rivière Aggitis, 4 = Affluent de Marmara en Pageo, 5 = Rivière Vathitopos, Vathitopos, 6 = Rivière Kalamas, près de Vrodismeni, 7 = Creek Bougazi, à proximité de l'ancien lac Xyniada.

Table I

Branchiobdella sp. presence in different crayfish species investigated in seven localities around Greece.

Tableau I

Observations de *Branchiobdella* sp. sur différentes espèces d'écrevisses, selon une enquête réalisée dans sept localités en Grèce.

Locality	Host (number of crayfish in the sample)	<i>Branchiobdella</i> sp. found		
		<i>B.</i> <i>parasita</i>	<i>B.</i> <i>pentodonta</i>	<i>B.</i> <i>hexodonta</i>
1. Evros River (Maritza) near Didimotihio	<i>A. leptodactylus</i> (1)	-	+	+
2. Aggitis Cave	<i>A. torrentium</i> (1)	-	-	+
3. Aggitis River	<i>A. torrentium</i> (5)	+	-	+
4. Tributary of Marmara River in Pageo	<i>A. torrentium</i> (2)	-	+	+
5. Vathitopos River in Valthitopos	<i>A. torrentium</i> (3)*	+	+	+
6. Kalamas River near Vrodismeni	<i>A. astacus</i> (9)	+	-	-
7. Bougazi Creek, close to the dried Xyniada Lake	<i>A. astacus</i> (3)	+	-	+

*three exuviae were fixed in the same sample

RESULTS

Branchiobdellidans were found on crayfish from all seven localities investigated (see Table I) and *H. chappuisi* only from one locality. The copepod *N. divaricata* was not found during the current investigation. The epibionts found in association with the crayfish specimens in each locality are described in the following sections in a geographic order, starting from northern Greece and progressing south.

Evros (Maritza) River

Thirteen adult and nine juvenile specimens of *Branchiobdella pentodonta* Whitman, 1882 were found in the debris of a jar containing one male narrow-clawed crayfish *A. leptodactylus*, described in PERDIKARIS *et al.* (2007) and found in 2005 in the Evros River, near the city of Didimotihio. The specimen found confirmed the presence of *A. leptodactylus* in the Evros River and introduced the possibility of *A. leptodactylus* being indigenous to Greece. Adult worms presented the typical appearance for this species with a flattened and broadened body form and typical jaw formation, with large lateral and median teeth, as well as one or two additional teeth between the median and lateral ones on each side.

In addition, three adult and six juvenile specimens of *B. hexodonta* were collected from the gills of the same crayfish. Typical jaw formation and dentation for this species – two big lateral and four and three smaller median teeth equal in size, for the dorsal and ventral jaw respectively, was observed in two dissected branchiobdellidan specimens. The other typical feature of the species – a furrow dividing the head in two parts – was also clearly visible in all branchiobdellidans.

Aggitis Cave

Twenty-nine adults and forty seven juveniles of *B. hexodonta* were found in the debris of a jar with one *A. torrentium* crayfish, described in KOUTRAKIS *et al.* (2005). The crayfish was collected in the river that runs throughout the Aggitis Cave (7,100 m from the cave entrance) in 2002. In addition, twenty adults and five juveniles of the same species were found in the gills of the examined crayfish.

Aggitis River

Five *A. torrentium* crayfish were collected from the Aggitis River. The river originates from the Aggitis Cave and becomes a tributary of the Strymon (Struma) River that flows into the Aegean Sea. Five adult and sixteen juvenile specimens of *Branchiodella parasita*, as well as two adult *B. hexodonta* specimens were found in the debris. *B. parasita* specimens were identified by the relatively big body size and large, equally sized, triangular teeth, which are typical for this species. A total of ten adults and nine juveniles of *B. hexodonta*, were found on the gills of the crayfish.

Tributary of Marmara River in Pageo Mountain

A tributary of the Marmara River close to the Mesoropi village, originates from the Pageo Mountain, in a difficult area to approach. Most of the crayfish observed in this river were heavily infested by branchiobdellidans and their cocoons that covered almost the entire crayfish body. One hundred and thirty nine adults and one hundred and twenty six juveniles of *B. pentodonta* were found in the debris of the jar, where two *A. torrentium* crayfish were fixed. In addition to this, twenty-four adults and two juveniles of *B. hexodonta* were found on the crayfish gills.

Vathitopos River

Three small crayfish and three crayfish exuviae were collected from the Vathitopos River, close to the Vathitopos village. Nine *B. parasita* adults, one hundred and twelve *B.*

pentodonta adults and twenty-three *B. hexodonta* adults were found in the debris of the jar where the crayfish and exuviae were fixed. Additionally, some more juveniles of these three epibiont species were also found in the same sample. Sixteen adults and three juveniles of *B. hexodonta* were found on the gills. More than thirty *H. chappuisi* were also collected both as single zooids and two-zooid chains from the gills of the crayfish from this locality. The external morphology of the *H. chappuisi* matched closely to the drawings of fixed specimens given in KASPRZAK (1976).

Kalamas River

Only specimens of *B. parasita* were isolated and identified from nine frozen *A. astacus* trapped in the Kalamas River, near the Vrodismeni village. The branchiobdellidans were in a very bad condition and specimen identification was based mainly on jaw morphology. In addition, a few branchiobdellidan specimens collected from living crayfish and fixed in alcohol also matched all of the characteristic features for this species.

Bougazi Creek

Representatives of two *Branchiobdella* species were found in the debris of a jar containing three poorly preserved *A. astacus* specimens, coming from the Bougazi Creek, close to the dried Xyniada Lake. Both *B. parasita* and *B. hexodonta* were identified mainly by their jaw morphology. Some specimens in a very bad condition, presumably *B. parasita* juveniles and *B. hexodonta* juveniles and adults, were found also on the gills.

DISCUSSION

Only four branchiobdellid species were known in Europe until the beginning of the 20th century: *B. astaci*, *B. parasita*, *B. pentodonta* and *B. hexodonta*. Later, forty-three new branchiobdellid species were described for the Old World (PIERANTONI, 1912; CANEGALLO, 1928; MOSZYNSKI, 1937; 1938 and GEORGEVITCH, 1957). POP (1965) revised the European branchiobdellidans and accepted as valid species only the previously mentioned four species. He gave CANEGALLO's (1928) species, *Branchiobdella italica* Canegallo, 1928, a subspecies status - *B. pentodonta italica* and described a new subspecies *B. pentodonta orientalis* Pop, 1965. KARAMAN (1967, 1970) and KOZAROV *et al.* (1972) showed, independently of each other, that Pop's *B. pentodonta orientalis* matched all the features of *B. balcanica* Moszynski, 1937 and *B. insolita* Moszynski, 1937 and thus they were junior synonyms. KARAMAN (1967, 1970) accepted *B. balcanica* because *B. insolita* was its junior synonym, while KOZAROV *et al.* (1972) preferred *B. insolita* because the description of the reproductive system of this species in MOSZYNSKI (1937) was more detailed compared to that of *B. balcanica* and supplemented with a picture showing clearly the details of the spermatheca and male reproductive system. KARAMAN (1967, 1970) restored also the species status of *B. italica* and thus together with *B. balcanica* and *B. kozarovi* Subchev, 1978, the number of the endemic recognised European branchiobdellid species was raised to seven. Recently, NESEMANN and HUTTER (2002) described one more European species from Austria – *Branchiobdella papillosa* Nesemann & Hutter, 2002. The authors however did not present details of the reproductive system and therefore the species needs further examination for its status confirmation.

NESEMANN and NEUBERT (1999) cited unpublished data on the occurrence of *B. parasita* and *B. hexodonta* in Greece, giving no details on their hosts and localities. To the best of our knowledge, no other data about Greek branchiobdellid fauna are available in the literature. Thus, our work presents the first accurately documented information on *B. parasita* and *B. hexodonta* in Greece and the first report for *B. pentodonta* for this country.

Branchiobdella species found in Greece in the current investigation were the three most widely distributed species in Europe, particularly in the Balkans. All three species were also found in Bulgaria (KOZAROV *et al.*, 1972; SUBCHEV and STANIMIROVA, 1998); Serbia (MOSZYNSKI, 1937; KARAMAN, 1967, 1970); Former Yugoslav Republic of Macedonia (F.Y.R.O.M.), Bosnia and Herzegovina, Slovenia (KARAMAN, 1967, 1970) and Croatia (KLOBUČAR *et al.*, 2006). However, in Montenegro only *Branchiobdella hexodonta* and *B. pentodonta* were found (GELDER, 1999). There are no available data on branchiobdellidans in Albania and the European part of Turkey, which have a common border with Greece.

For the rest of the European branchiobdellidans, *B. astaci* is known from the following Balkan countries: Bulgaria (KOZAROV *et al.*, 1972; SUBCHEV and STANIMIROVA, 1998); Serbia (MOSZYNSKI, 1937) and Croatia (KLOBUČAR *et al.*, 2006). *B. italica* is known only from *A. pallipes*, which is not present in Greece, and for this reason it is not expected to be found in this country. *Branchiobdella kozarovi* is known so far from Bulgaria - Danube tributaries and along the Black Sea (SUBCHEV and STANIMIROVA, 1998), and Ukraine (BOSHKO, 1983a), and recently from Poland (WIERZBICKA and ŚMIETANA, 1999).

According to BOSHKO (1983b), the distribution of *H. chappuisi* extends across the Ukraine, Poland, former Czechoslovakia, Romania, former East Germany and F.Y.R.O.M. SUBCHEV and STANIMIROVA (1986; 1998) found that this crayfish gill dwelling species was quite common in Bulgaria. This species was found also in the south-west and south-east parts of this country near to the border with Greece. Thus, the presence of *H. chappuisi*, reported here for the first time, was not unexpected for Greece. Until now, Greece represents the most southern distribution limits of this species.

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