**SPECIES OF BRANCHIOBDELLIDAE (ANNELIDA)
ON FRESHWATER CRAYFISH
IN SOUTH TYROL (NORTHERN ITALY).**

**B. OBERKOFER (1), F. QUAGLIO (1), L. FÜREDER (2), M.L. FIORAVANTI (3),
S. GIANNETTO (4), C. MOROLLI (1), G. MINELLI (1)**

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- (1) Centro di Ricerca Interdipartimentale sulle Tecnologie e l'Igiene degli Allevamenti Intensivi delle Piccole Specie, University of Bologna, Via San Giacomo 9, 40126, BOLOGNA, Italy. E-mail : fquaglio@alma.unibo.it
- (2) Institute of Zoology and Limnology, University of Innsbruck, Technikerstrasse 25, 6020, INNSBRUCK, Austria.
- (3) Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, University of Bologna, Via Tolara di Sopra 50, 40064, OZZANO EMILIA (BO), Italy.
- (4) Dipartimento di Patologia, Malattie Infettive e Parassitarie e Ispezione degli Alimenti di Origine Animale, University of Messina, Via S. Cecilia 30, 98123, MESSINA, Italy.

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ABSTRACT

Twenty-eight freshwater crayfish were collected from 8 streams and one small lake in the province of South Tyrol (North Italy) from autumn 1999 to spring 2001, in order to investigate the presence of branchiobdellidans. At 6 of the streams the white clawed crayfish (*Austropotamobius pallipes italicus*) was collected, while the noble crayfish (*Astacus astacus*), the exotic species: signal crayfish (*Pacifastacus leniusculus*) and spiny-cheek crayfish (*Orconectes limosus*) were respectively caught at the other 2 streams and at the lake. Except for *O. limosus*, branchiobdellidans were observed on all crayfish examined. On noble crayfish, specimens of *Branchiobdella italica* and *Branchiobdella hexodonta* were found on the same host. *Branchiobdella italica* was largely recovered from all white clawed crayfish. The specimens of signal crayfish were found to carry only *Xironogiton victoriensis*.

Key-words : Branchiobdellidae, freshwater crayfish, annelids, North Italy.

LES ESPÈCES DE BRANCHIOBDELLIDAE (ANNELIDA) OBSERVÉES SUR LES ÉCREVISSES DANS LE SUD TYROL (ITALIE DU NORD).

RÉSUMÉ

A partir de l'automne 1999 jusqu'au printemps 2001 en Tyrol du Sud, 28 écrevisses ont été prélevées dans 8 ruisseaux et un petit lac dans le but de vérifier la présence des Branchiobdellidae. L'écrevisse à pieds blancs (*Austropotamobius pallipes italicus*) a été trouvée dans 6 ruisseaux, l'écrevisse à pieds rouges (*Astacus astacus*) et l'écrevisse de Californie (*Pacifastacus leniusculus*) dans deux ruisseaux et l'écrevisse américaine (*Orconectes limosus*) dans le lac. A l'exclusion de l'écrevisse américaine, on a trouvé chez toutes les autres espèces des Branchiobdellidae. Chez l'écrevisse à pieds rouges, *Branchiobdella italica* et *Branchiobdella hexodonta* sont présentes. Chez toutes les écrevisses à pieds blancs, on a relevé une grande quantité de *B. italica* alors que chez les écrevisses de Californie on a retrouvé seulement le *Xironogiton victoriensis*.

Mots-clés : Branchiobdellidae, écrevisse, Annelida, Italie du Nord.

INTRODUCTION

In the Italian province of South Tyrol (Northern Italy), four species of freshwater crayfish occur: the autochthonous white clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) and noble crayfish *Astacus astacus* (Linnaeus, 1758) and the two exotic species: the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and spiny-cheek or striped crayfish *Orconectes limosus* (Rafinesque, 1817). At present, *A. pallipes* is scarcely distributed in the water bodies of South Tyrol and *A. astacus* has only been found in a single stream.

The aim of this investigation was to identify the species of branchiobdellidans isolated on the crayfish. So far *Branchiobdella italica*, *Branchiobdella parasita*, *Branchiobdella hexodonta* and *Branchiobdella astaci* were the only branchiobdellidan species reported on *A. pallipes* in North Italy and *B. italica* is the prevalent species (GELDER *et al.*, 1994; NOBILE *et al.*, 1995). Moreover, in this research we investigated the possibility of branchiobdellidan shifting from the autochthonous and European species *A. pallipes* and *A. astacus* to the allochthonous *P. leniusculus* populations (and viceversa) in the Ahr drainage.

MATERIAL AND METHODS

The research was carried out in September 1999 and from February to June 2001. In September 1999, 15 individuals of *P. leniusculus* were collected, while 9 *A. pallipes*, 2 *O. limosus* and a single *A. astacus* were caught from February to June 2001. The crayfish were caught with baited traps and by hand at nine different sites (Figure 1): *A. pallipes* from Moosbachl (Bruneck / St.Georgen), Giessbach (Staben), Hyppolithbach (Naraun), Krebsbach (Kaltern), Krebusbach (Fennberg) and Angelbach (Montiggl); *A. astacus* from Wiesenbach (Gais); *O. limosus* from Vahrn lake (Vahrn); *P. leniusculus* from Auenbachl (Bruneck). The crayfish were transported to laboratory during an approximately 5 hours trip. The specimens were emersed in a 1:1 solution of deionized water and carbonated bottled

water for two minutes. The immobilized branchiobdellidans were removed from the crayfish and placed in stream water to recover, according the Gelder method (GELDER *et al.*, 1994). The worms were examined alive under the microscope, fixed in 70% ethanol and then counted and clarified with lactophenol. The branchiobdellidans from *P. leniusculus* were stained with Borax Carmine and mounted with Glycerine Jelly or Hoyer's fluid. All the branchiobdellidans were examined and measured at the optical microscope with the aid of a « camera lucida ». The identification of the species was obtained on the basis of the jaws, the spermatheca and the spermathecal duct morphology using Gelder's identification key (GELDER and HALL, 1990; GELDER *et al.*, 1994).

Figure 1
Sites of sampling in South Tyrol.

Figure 1
Lieux d'échantillonnage en Tyrol du Sud.

RESULTS AND DISCUSSION

On the exoskeleton of all crayfish caught, from 23 up to 2 107 of branchiobdellidans / host were found, except the spiny-cheek crayfish from Vahrn lake which did not carry any of these organisms.

Branchiobdella italica (Figures 2-5) was the most widespread species, found on each individual of *A. pallipes* and *A. astacus*. The exotic species *Xironogiton victoriensis* (Figures 6-9) was collected only on *P. leniusculus*. *Branchiobdella hexodonta* (Figures 10-11) was observed on *A. astacus*.

Morphological characteristics, number of counted annelids / host, specimens studied and sites sampled are showed in Table I. The dental formulas of the examined annelids are shown in Table II. On the surface of *B. italica* peritricha protozoans of the genus *Epistylis* (Figures 12-13) were often found. The presence of these ciliates has already been reported on crayfish populations as ectoparasites or ectocommensals (BROWN *et al.*, 1993; SCOTT and THUNE, 1986).

Table I

Number of branchiobdellidans / host, number of branchiobdellidans examined, morphometric characteristics and sites sampled in South Tyrol.

Tableau I

Nombre de branchiobdellidae / hôte, nombre de branchiobdellidae examinées, caractéristiques morphologiques et lieux d'échantillonnage en Tyrol du Sud.

crayfish examined	origin	number of branchiobdellidans / host	number of branchiobdellidans examined	species of branchiobdellidan found on crayfish	bodylength	bodyweight
<i>A. pallipes</i> (1)	Angelbach	80	31	<i>B. italica</i>	2.01	0.65
<i>A. pallipes</i> (1)	Giessbach	960	60	<i>B. italica</i>	2.02	0.68
<i>A. pallipes</i> (1)	Krebusbach	23	20	<i>B. italica</i>	2.53	0.75
<i>A. pallipes</i> (1)	Krebusbach	73	34	<i>B. italica</i>	2.72	0.72
<i>A. pallipes</i> (1)	Krebsbach	90	56	<i>B. italica</i>	3.43	0.83
<i>A. pallipes</i> (1)	Hyppolithbach	131	66	<i>B. italica</i>	3.01	0.75
<i>A. pallipes</i> (1)	Hyppolithbach	209	110	<i>B. italica</i>	3.09	0.72
<i>A. pallipes</i> (1)	Moosbachl	262	80	<i>B. italica</i>	2.56	0.67
<i>A. pallipes</i> (1)	Moosbachl	1 150	100	<i>B. italica</i>	2.51	0.63
<i>A. astacus</i> (1)	Wiesenbach	2 107	150/154	<i>B. italica</i>	3.17	0.77
			4/154	<i>B. hexodonta</i>	3.47	0.62
<i>P. leniusculus</i> (15)	Auenbachl	1 500 (average)	350	<i>X. victoriensis</i>	2.16	0.75
<i>O. limosus</i> (2)	Vahrner See	0	0	/	/	/

Table II

Species of branchiobdellidans collected on freshwater crayfish of South Tyrol, their dental formulas and percentage of finding.

Tableau II

Les différentes formules dentaires de Branchiobdellidae du Sud Tyrol et leurs fréquences selon les lieux de capture des écrevisses.

Branchiobdellidan	Dental formulas and percentage of finding						
	6/5	5/5	6/6	7/6	7/5	5/4	4/4
<i>B. italica</i> (from Angelbach)	54.84%	41.93%	3.22%	/	/	/	/
<i>B. italica</i> (from Giessbach)	40.00%	60.00%	/	/	/	/	/
<i>B. italica</i> (from Wiesenbach)	61.69%	20.78%	11.69%	3.89%	1.95%	/	/
<i>B. italica</i> (from Krebusbach)	61.40%	31.58%	3.51%	1.75%	1.75%	/	/
<i>B. italica</i> (from Krebsbach)	47.27%	45.45%	7.27%	/	/	/	/
<i>B. italica</i> (from Hyppolithbach)	53.97%	44.32%	0.57%	/	0.57%	0.57%	/
<i>B. italica</i> (from Moosbachl)	53.33%	45.00%	0.55%	/	/	1.11%	/
<i>B. hexodonta</i> (from Wiesenbach)	100.00%	/	/	/	/	/	/
<i>X. victoriensis</i> (from Auenbachl)	/	20.00%	/	/	/	37.50%	42.50%

Figure 2
A fixed whole borax carmine mount of *Branchiobdella italica*.

Figure 2
Branchiobdella italica fixée avec le colorant borax carminé.

Figure 3
Ventral view of *Branchiobdella italica* observed with the Scanning Electron Microscope (SEM).

Figure 3
Vue ventrale de *Branchiobdella italica* au moyen d'un microscope électronique (SEM).

Figure 4
Branchiobdella italica: jaw with 5/5 teeth (lactophenol).

Figure 4
Branchiobdella italica : mâchoire avec 5/5 dents (lactophenol).

Figure 5
Branchiobdella italica: cephalic portion with oral papillae (SEM). *Epistylis* sp. on the cutaneous surface.

Figure 5
Branchiobdella italica : partie céphalique avec papilles orales (SEM). *Epistylis* sp. sur la surface cutanée.

Figure 6
A fixed whole borax carmine mount of *Xironogiton victoriensis*.

Figure 6
Xironogiton victoriensis fixé avec le colorant borax carminé.

Figure 7
Ventral view of *Xironogiton victoriensis* observed with an Scanning Electron Microscope (SEM).

Figure 7
Vue ventrale de *Xironogiton victoriensis* au moyen d'un microscope électronique (SEM).

Figure 8
Xironogiton victoriensis: jaws with 5 and 4 teeth (Borax carmine).

Figure 9
Xironogiton victoriensis: spermatheca (Borax carmine).

Figure 8
Xironogiton victoriensis : mâchoire avec 5 et 4 dents (Borax carminé).

Figure 9
Xironogiton victoriensis : spermathèque (Borax carminé).

Figure 10
Branchiobdella hexodonta (lactophenol).

Figure 11
Branchiobdella hexodonta: jaw with 6/5 teeth (lactophenol).

Figure 10
Branchiobdella hexodonta (lactophenol).

Figure 11
Branchiobdella hexodonta : mâchoire avec 6/5 dents (lactophenol).

Figure 12
Epistylis sp., peritrichous, ciliated protozoan on the cutaneous surface of *B. italica* (lactophenol).

Figure 13
Epistylis sp., peritrichous, ciliated protozoan on the cutaneous surface of *B. italica* (SEM).

Figure 12
Epistylis sp., protozoaires ciliés, Péritriches sessiles, sur la surface cutanée de *B. italica* (lactophenol).

Figure 13
Epistylis sp., protozoaires ciliés, Péritriches sessiles, sur la surface cutanée de *B. italica* (SEM).

The relationship between branchiobdellidans and their hosts is still poorly known. The worms have been variously described as diatom feeders, blood feeders or as parasites only in the adult stage. It is believed that most species are commensals or perhaps facultative parasites (BISHOP, 1968). GRABDA and WIERZBICKA (1969) provided the only experimental evidence of parasitism in affirming that *B. hexodonta* was living permanently in the branchial cavity of its host. The authors observed tissues of the crayfish in the intestinal tract of *B. hexodonta* by the use of radio-labelled markers. In a recent paper, according to GELDER (1999), branchiobdellidans are defined as ectosymbionts and not parasites. In our investigation, rotifers and chironomids were identified in the intestinal tract of some of *B. italica*. Moreover detritus of unknown nature and origin has been found to occur in different amounts.

YOUNG (1966) has studied the colonization behaviour of these annelids. He found that body contact seems to be essential to their transfer from one host to another. GRABDA and WIERZBICKA (1969) considered this behaviour to be more important in some species than in others. Some signal crayfish samples were collected by GRABER (personal communication) in the Moosbachl, where also a white clawed crayfish population lives. However we did not find on *A. pallipes* any *X. victoriensis*, which was associated with *P. leniusculus*. Either competition between the branchiobdellida species or the incompatibility to colonize of one species might be a possible explanation. GELDER (1999) assumed that a preference in the associations was determined only by branchiobdellidans. It is generally believed that the host is unable to affect or prevent the branchiobdellidans colonization. Although some species of crayfish have been observed scraping their exoskeleton and « combing » their gills with appropriate appendages (GELDER, 1999). In a laboratory tank we have observed a signal crayfish eating *X. victoriensis* from its exuviae. Another possible explanation for the lack of *X. victoriensis* on *A. pallipes* could be the low density of *P. leniusculus* in the Moosbachl. No certain explanation can be given so far. In addition, the capture of the signal crayfish from the Moosbachl is important to understand if it can be an acceptable host for the native branchiobdellidan, *B. italica*.

Quite often, two or more species of branchiobdellidans are found sympatrically on the same hosts. The noble crayfish from Wiesenbach showed to be highly populated by *B. italica* (96.76%) and by some *B. hexodonta* (3.24%). The high number of *B. italica* in comparison with the low presence of *B. hexodonta* allow to consider the possibility of a competition between the two species. Other authors didn't observe this competition. In contrast, during his research carried out in the Bergamo area (Northern Italy), GELDER *et al.* (1994) found more specimens of *B. hexodonta* than of *B. italica* on the same host. In our investigation, *B. italica* from Wiesenbach was bigger compared to *B. italica* found in other streams in South Tyrol. The high number and the size of *B. italica* could be influenced by physical, chemical and biological factors (MORI *et al.*, 2000). The temperature, for example, was 7°C higher than in the other streams. Warmer condition could influence the growth and the colonisation of *B. italica*. Furthermore, high presence of *B. italica* can cause problems of competition and coexistence of other species with similar life styles.

Our investigations provided some interesting and new findings about the occurrence of branchiobdella and their hosts. However more studies are needed to confirm these observations and to obtain more accurate data.

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