Probopyrus floridensis Richardson, 1904 (Isopoda, Bopyridae) parasitizing the freshwater prawn *Macrobrachium potiuna* (Müller, 1880), from São Paulo, Brazil.

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Abstract

This paper reports the ectoparasitism of the palaemonid prawn *Macrobrachium potiuna* (Müller, 1880) by the bopyrid isopod *Probopyrus floridensis* Richardson, 1904, collected at Estação Ecológica Juréia-Itatins and Parque Estadual Ilha do Cardoso, State of São Paulo.It also contains relevant considerations on the prevalence of infestation and provides some comments on the taxonomic controversy regarding *P. floridensis.*

Key words: *Probopyrus floridensis*, ectoparasitism, *Macrobrachium potiuna*

Introduction

Species of the bopyrid isopod *Probopyrus* are branchial ectoparasites of palaemonid prawn (Van Name, 1936; Beck, 1980a; Markham, 1985a; Schuldt and Rodrigues Capítulo, 1985; Markham, 1986). The asymmetrically body shaped bopyrid female always remains adhered to the inner surface of the host branchiostegite, and is usually found accompanied by one smaller, symmetrical male. One of the most conspicuous effect of such parasitism is the obvious unilateral protuberance on the host carapace, where the bopyrid finds shelter and feeds on the host's hemolymph (Van Name, 1936; Carvalho, 1942; Truesdale and Mermilliod, 1977; Beck, 1980a; Schuldt and Rodrigues 1985). Other effects on the host include a reduction on the efficiency of gas exchange by the gills, size reduction of the secondary sex characters and feminization of male prawns, and parasitic castration of female prawns by inhibiting full maturation of the ovaries (Beck, 1980b; Schuldt and Rodrigues Capítulo, 1985; Ordinetz-Collart, 1990).

The pantropical palaemonid prawn *Macrobrachium* sp. is known to harbour many species of *Probopyrus.* Since range distribution of ectoparasites is strongly associated with the geographic distribution of their potential hosts, a fair amount of *Macrobrachium-Probopyrus* association has been reported worldwide (Markham, 1986). Markham (1985a) presents a comprehensive review on this topic for the Americas, whereas Verdi (1991), Alvarez-Leon (1993), Román-Contreras, (1993, 1996) and Masunari et al. (2000) complete the information with more recent data.

According to Brasil-Lima (1998), three species of *Probopyrus, Probopyrus bithynis* Richardson, 1904, *P. floridensis* Richardson, 1904 and *P. palaemoni* Lemos de Castro and Brasil-Lima, 1974 are known to occur in Brazil. Reports on bopyrid isopod infestation in *Macrobrachium* species in Brazil make reference to *P. bithynis* studied by Cordero (1937), Carvalho (1942), Lemos de Castro and Brasil-Lima (1974), Ordinetz-Collart (1990) and, more recently, *P. floridensis* by Masunari *et al.* (2000). The latter bopyrid species is also known to infest other palaemonid prawns belonging to the genus *Palaemonetes* while *Probopyrus palaemoni* is an ectoparasite of *Palaemon pandaliformis* reported by Lemos de Castro and Brasil-Lima (1974) Brasil-Lima (1998).

During field surveys for the BIOTA-FAPESP, a research programme intended to study the biodiversity of the state of São Paulo, Brazil, two specimens of *M. potiuna* were found to be parasitized by *P. floridensis.* Although infestation by *P. floridensis* in *M. potiuna* has recently been reported in the

State of Paraná (Masunari *et al.*, 2000), this is the first report of such parasitism in the State of São Paulo. The host *M. potiuna* is an endemic species in Brazil and its geographical distribution extends from south-eastern to southern regions of that country (Holthuis, 1952; Ramos-Porto and Coelho, 1998; Bond-Buckup and Buckup, 1999). Some comments on prevalence of infestation, number and size of eggs are also presented in this paper.

In recent years, there has been some controversies concerning the taxonomic status of several previously described species of *Probopyrus* from the western Atlantic. Markham (1985a) proposed that *P. floridensis* and *P. bithynis*, as well as most of the known western Atlantic species of *Probopyrus* should be considered as synonymies of *P. pandalicola,* despite the recognition by the author of the high variations in morphology and size among different populations. In the present paper, we express our point of view that *P. floridensis* and *P. pandalicola* should be considered as separate species.

Material and Methods

Specimens of *M. potiuna* were sampled from two different collecting sites (Figure 1). At the Estação Ecológica Juréia – Itatins (24° 22' 05.3"S ; 47° 03' 17.0"W) 29 adults (14 females and 15 males) of *M. potiuna* were sampled from a small, shallow unnamed stream during six different moments (May, August and December 2000; March, May and July 2001). At Ilha do Cardoso (25° 04' 17"S ; 47° 54' 00"W) 15 adults (6 females and 9 males) of *M. potiuna* were collected in August 2001 from a small river known as Poço das Antas. In both localities, prawns were captured with the aid of a hand sieve (mesh: 2.0 mm), which was passed among the partially submerged macrophytes along the banks or used to collect mats of decomposing leaf litter from the bottom. All specimens were immediately fixed in 10% formalin and stored in tagged plastic flasks.

Prawns were identified according to Holthuis (1952) and Gomes-Côrrea (1977), and were sexed according to the presence (male) or absence (female) of the appendix masculina on the second pair of pleopods. Total body length (from the tip of the rostrum to the end of the telson) was

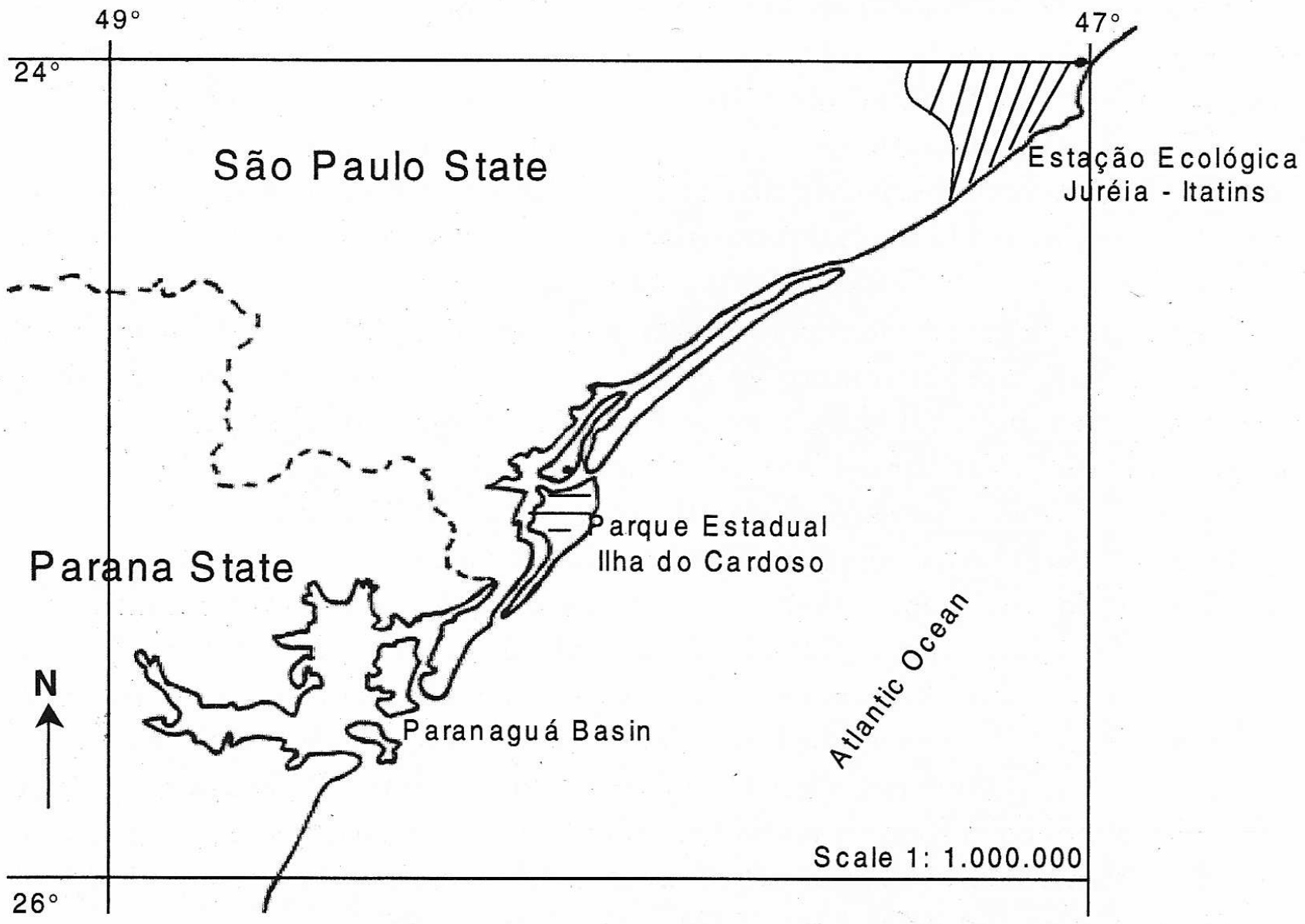

Figure 1: Map of the southern coast of the state of São Paulo showing location of sampling sites: Ilha do Cardoso and Estação Ecológica Juréia-Itatins.

measured with the aid of a Mitutoyo caliper to the nearest 0.1 mm. The bopyrid was identified after Richardson (1904), Lemos de Castro and Brasil-Lima (1974) and Brasil-Lima (1978). Photographs of the ectoparasite and host were taken with a Zeiss Stemi SV6 stereomicroscope.

Bopyrid ectoparasites were carefully detached and removed from the host branchial chamber and placed in a small Petri dish partially filled with freshwater. Number and size of eggs were taken from the egg mass of the sole ovigerous female sampled at Ilha do Cardoso. Eggs of the ovigerous bopyrid female from Juréia-Itatins was not considered because a great number of eggs were lost or damaged during manipulation of the female. Counting of eggs were performed with the aid of a dissecting microscope (Zeiss, model Stemi SV6) and were based on the totality of eggs removed from the marsupium plus some eggs that were recovered from the Petri dish after they had fallen from the marsupium during the removal of female from the branchial chamber. Size of eggs was measured with the aid of a computer imaging processing software (Kontron KS 100, Kontron Elektronik GmbH, Munich) connected to a Zeiss Axioskop light microscope.

Use of the term prevalence followed Margolis *et al.,* (1982) and express the percentage of infested prawns in the sampled population. The bopyrids (male and female) and host from Estação Ecológica Juréia-Itatins were deposited at the Museu de Zoologia – University of São Paulo, under the identification number MZUSP 13521 and MZUSP 13522, respectively.

Results

Only one specimen of *M. potiuna* from each locality was found infested with adult female bopyrid isopod which accounts for the very low prevalence observed at Estação Ecológica Juréia-Itatins (0.03 %, or 1 male in 29 adults; total body length of infested specimen: 29.0 mm) and at Ilha do Cardoso (0.07 %, or 1 female in 15 adults; total body length of infested specimen: 29.4 mm). Infested prawns presented the typical unilateral protuberance on the cephalotorax (Figure 2) so commonly associated with this type of parasitism. All female hosts, either infested or not, sampled in both localities were not ovigerous.

Bopyrid isopods retrieved from infested hosts were identified as *P. floridensis*. Both light brown asymmetric female bopyrids were adults, with fully developed oostegites and ovarian bosses and were ovigerous (Figure 3). Each female bopyrid was accompanied by a whitish-yellow dwarf symmetric male (Figure 4) attached ventrally to the female's pleotelson.

The total number of eggs from the ovigerous bopyrid collected at Ilha do Cardoso was 1545. Eggs were at early embryonic stage and average size (n = 40) was 178.65 ± 7.02 µm.

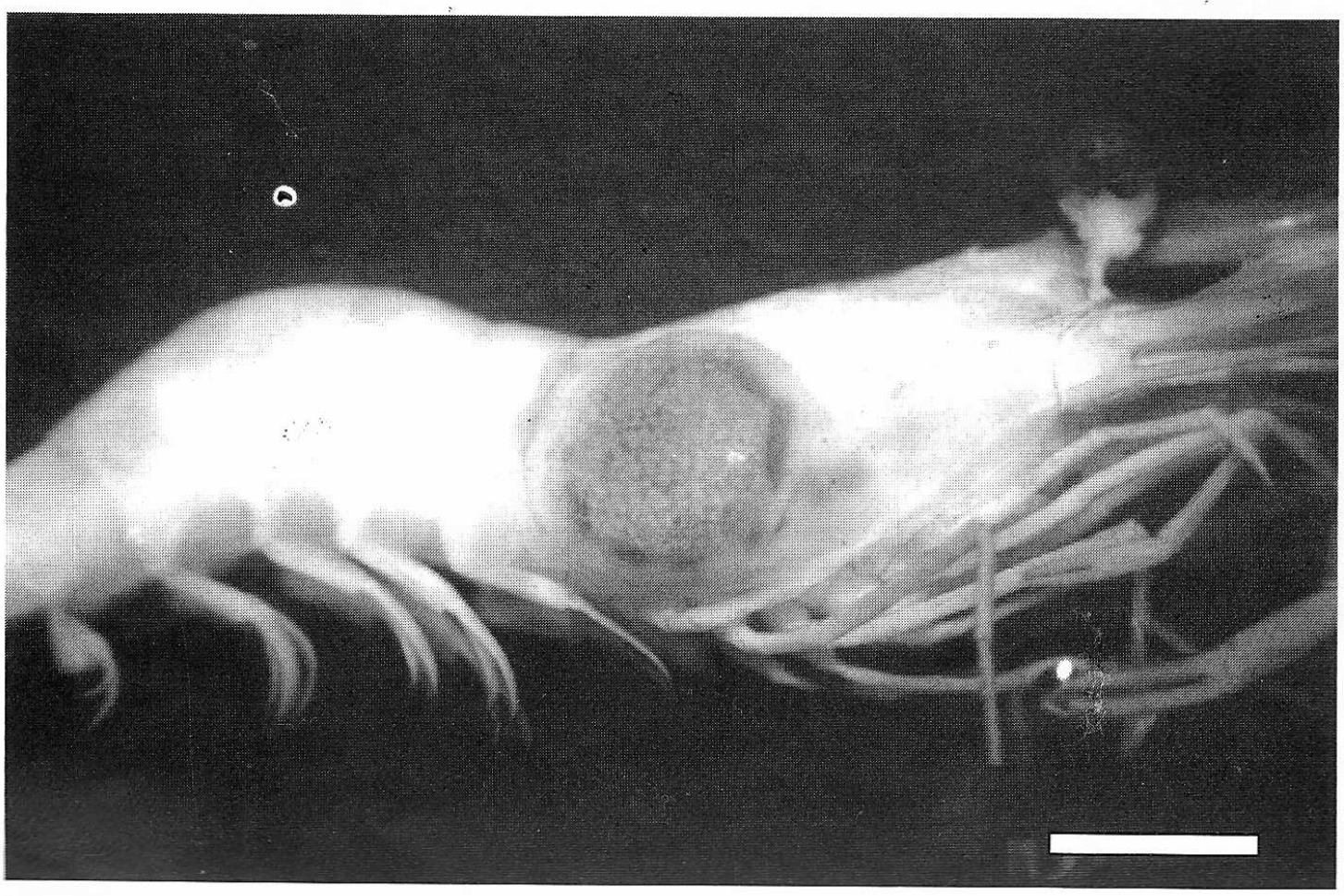

Figure 2: *Macrobrachium potiuna* infested by *Probopyrus floridensis* (lateral view). Bar: 2.5 mm.

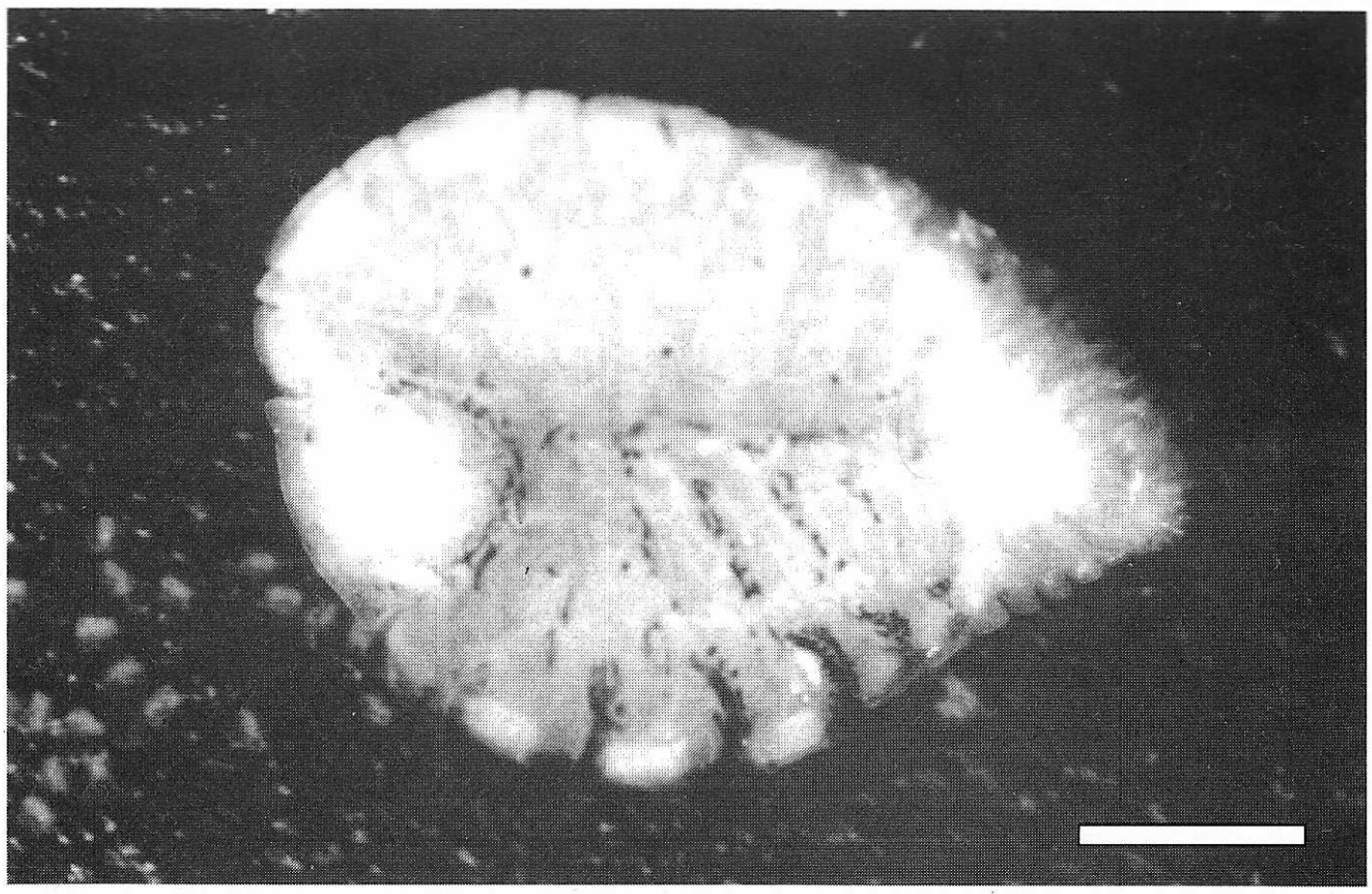

Figure 3: Female of *Probopyrus floridensis* (dorsal view). Bar: 1mm

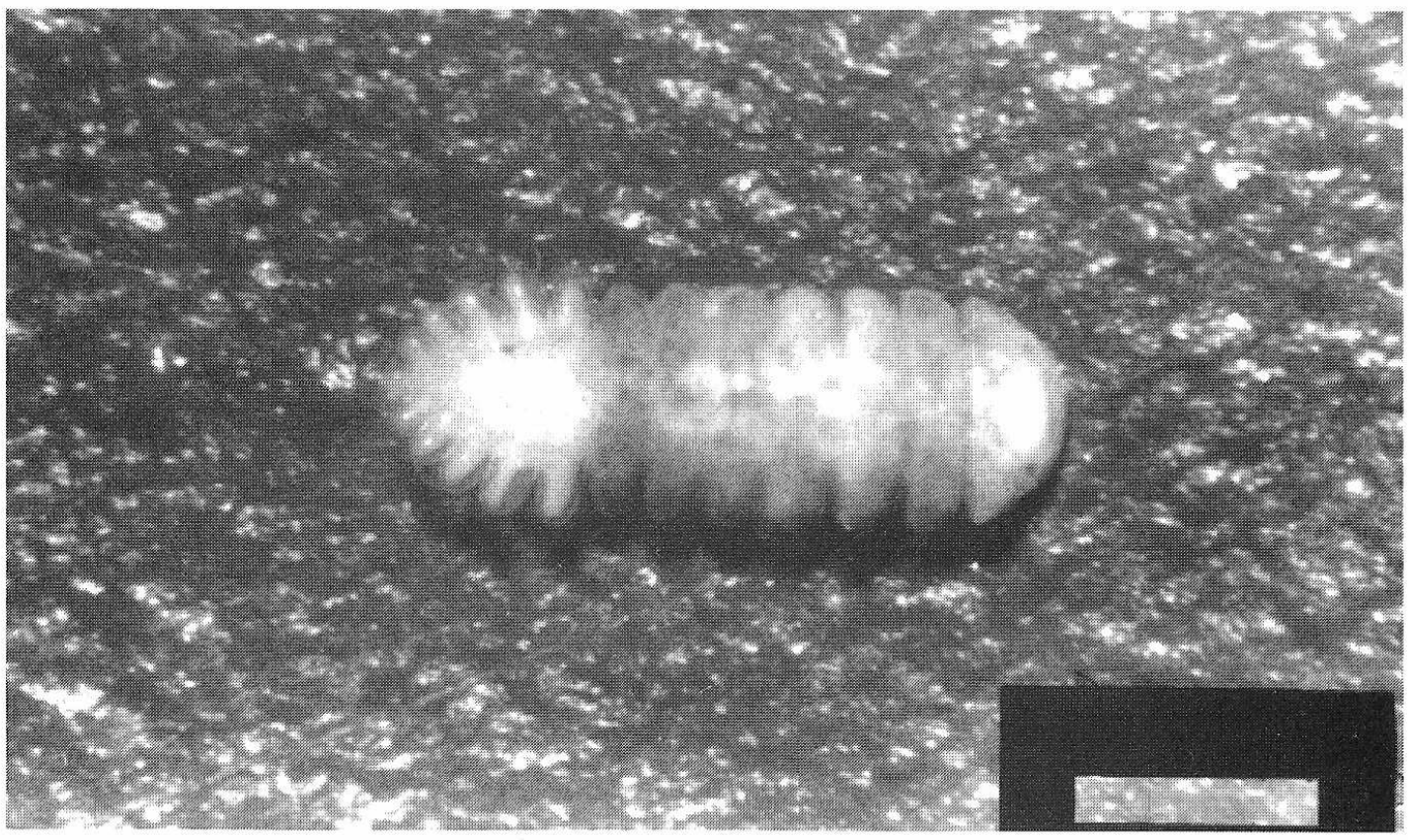

Figure 4: Male of *Probopyrus floridensis* (dorsal view). Bar: 0.4 mm

Discussion

Prevalence of infestation with *P. floridensis* reported in this paper was very low in both localities. It should be emphasised, however, that whilst prevalence data from Estação Ecológica Juréia-Itatins was based on six different sampling occasions between May 2000 and July 2001, that from Ilha do Cardoso was the result of a single sampling in August 2001. According to Masunari *et al.* (2000) prevalence of infestation by *P. floridensis* in *M. potiuna* varies with period of the year. In their one-year study at Paranaguá basin, the authors observed monthly variations in prevalence that ranged from values very close to zero, or even no infestation at all, to as high as 91.7%.

It should be pointed out, however, that Masunari *et al.* (2000) considered all bopyrid life stages that were found associated with the hosts for their calculation of prevalence. In our opinion, only the definitive association between hosts and adult bopyrids, either immature or mature, should be considered for the calculation of prevalence in this type of parasitism. Only after the definitive settlement of one single couple of ectoparasite (with few exception of more than one couple) in the branchial chamber will the typical evolution of the infestation take place such as the outpocketing of the branchiostegite area, interference on reproductive maturation of female host and eventually

culminating with the successful reproduction of the ectoparasite itself. Infestation by the larval forms (cryptoniscus and bopyridium) does not trigger a defence response by the host and no injuries in somatic or germinative tissues of the host have been associated with the presence of these larvae (Schuldt and Damborenea, 1988). Once the definitive settlement of an adult couple in the gill chamber occurs, all remaining larval and immature forms are eliminated or do not develop further in the same host (Masunari *et al.*, 2000).

However, high infestations by larval forms do indicate an intense interface interaction between prawn and bopyrid populations which may lead to high prevalence of a definitive host-parasite association. An excellent evidence of this situation is presented by Masunari *et al* (2000) in which the authors demonstrate that the structure of the parasite population varies with time of the year and that definitive settlement of the couple of parasites occurs within a specific period within the year. These authors observed a gradual shift with time in the structure population of *P. floridensis* infesting *M. potiuna* in Paraná in which a very high infestation composed exclusively by immature forms (June) is gradually replaced by the dominance of mature adults (from August to October).

Masunari *et al.* (2000) pointed out that a population of *P. floridensis* from Paranaguá Basin, State of Paraná, reproduces once a year. This would occur between August and October when the adult stage predominates over immature ones within the ectoparasite population associated with the definitive host. These authors also added that ovigerous female bopyrid (3 in 116 mature females) were observed exclusively in October. In the present study, the observation of one ovigerous female as early as May (from Juréia-Itatins) and August (from Ilha do Cardoso) may indicate that the reproductive period of *P. floridensis* is probably longer than previously thought, especially when the close proximity between these two localities in São Paulo and the Paranaguá Basin in Paraná is considered (Figure 1).

In the present study, female hosts represented 48.3% (14 in 29) and 40.0% (6 in 15) of the collection of prawns from Juréia-Itatins and Ilha do Cardoso, respectively, but none of them was ovigerous. Masunari et al. (2000) also noted a low number of ovigerous females (2 in 111 females) of *M. potiuna* from Paranaguá basin and suggested that this condition could be related to the high prevalence of parasitism by *P. floridensis*. So far, this argument cannot be applied to the present study because prevalence of infestation in each locality was very low and does not explain the absence of ovigerous females of *M. potiuna*. On the other hand, inadequate sampling methods may constitute a reasonable explanation because the original aim of our field work was to conduct an extensive survey on the freshwater decapod diversity in the State of São Paulo and the sampling methods so far employed have been much satisfactory to fully achieve this purpose. If we are to initiate a thorough investigation on prawn and bopyrid association in São Paulo, the sampling methodology should most certainly be re-evaluated.

Richardson's (1904) original description of *P. floridensis* was based on specimens from Florida, U.S.A., found in *Palaemonetes exilipes* Stimpson, 1871. Lemos de Castro and Brasil-Lima (1974) reported this bopyrid from Georgia, U.S.A., parasitizing *Palaemonetes paludosus* (Gibbes, 1850). In Brazil, this bopyrid species is reported as ectoparasite of an unidentified palaemonid prawn collected at São Vicente, near Itararé Beach (Carvalho, 1942); of *P. exilipes* at Guarapari, state of Espírito Santo (Lemos de Castro and Brasil-Lima, 1974) and of *M. potiuna* by Masunari *et al.* (2000).

Contrary to Richardson's (1904) original description, however, eyes are present in the female specimen of *P. floridensis* found associated with *M. potiuna* (Figure 3). This trait, the presence of eyes, has also been noted in other specimens from Georgia (U.S.A.) and Brazil and (Lemos de Castro and Brasil-Lima, 1974; Masunari *et al.*, 2000), which may suggest morphological variations among different populations. Indeed, many authors (see Lemos de Castro and Brasil-Lima, 1974; Markham, 1985a; Román-Contreras, 1996) have pointed out that the great morphological plasticity so frequently observed among different bopyrid populations has contributed to make the precise identification of *Probopyrus* species a difficult task.

As mentioned in the introduction section, Markham (1985a) proposed that *P. floridensis* and *P. bithynis*, as well as most of the known western Atlantic species of *Probopyrus* should be considered as synonymies of *P. pandalicola*. The morphological similarities of these three bopyrid species was already noticed by Van Name (1936).

Besides morphological traits, other important characters and biological information should be considered for diagnosing at the species level. Dale and Anderson (1982) reported that the larval forms from *P. bithynis* and *P. pandalicola* are morphologically very distinct and that epicaridium and cryptoniscus of the latter and *P. floridensis,* although alike at first, presented significant differences in morphometry. Moreover, the cryptonisci of each of the three bopyrid species showed different swimming speed (Dale and Anderson, 1982). According to these authors, *P. pandalicola* and *P. floridensis* should be considered as separate species, an opinion also shared by Román-Contreras (1993).

Another important aspect to be considered is the host-parasite relationship. Although *Probopyrus* species present a large plasticity as far as host specificity is concerned, *P. bithynis* has so far been recognised as ectoectoparasite of several *Macrobrachium* species (Van Name, 1936; Cordero, 1937; Lemos de Castro and Brasil-Lima, 1974; Truesdale and Mermilliod, 1977; Markham, 1985a; Ordinetz-Collart, 1990; Alvarez-Leon, 1993; Verdi, 1991) while *P. floridensis* is reported to infest several species of *Palaemonetes* and *M. potiuna* by Carvalho (1942), Lemos de Castro and Brasil-Lima (1974), Massunari *et al.* (2000). Amongst the *Palaemonetes* species parasitized by *P. pandalicola* studied by Beck (1980b), Anderson and Dale (1981), Markham (1985a), Jiménez and Vargas (1990) the freshwater prawn, *P. paludosus* appears to be the only known common host to this bopyrid species and *P. floridensis* (see Markham, 1985a, for extensive list of hosts).

In terms of habitat, species of *Probopyrus* are found in brackish to freshwater (Markham, 1985b). Both *P. pandalicola* and *P. floridensis* appear to be euryhaline because some of their *Palaemonetes* hosts inhabit either fresh or brackish water habitats. The freshwater prawn, *M. potiuna*, belongs to a group of *Macrobrachium* species that does not depend on brackish water to complete its larval phase. Because larval development is of the abbreviated type (Müller, 1892) and because the potential for dispersal of the newly hatched benthic larvae is very limited, the offsprings of *M. potiuna* tend to be incorporated into their parent population and to remain exclusively in freshwater habitats. The collecting sites, from where the infested prawns was sampled, are rather far away from the sea, indicating that the definitive host-parasite association between this palaemonid prawn and *P. floridensis* is initiated and is carried out in freshwater habitats.

The taxonomical controversy regarding the western Atlantic *Probopyrus* species is far from being settled and this topic is not the subject of the present paper. As pointed out by Markham (1985a), further experimental work are needed to help solving this problem. For this reason, we share the opinion expressed by Dale and Anderson (1982) and Róman-Contreras (1993) that *P. floridensis* and *P. pandalicola* should be treated as separate species until new evidences tell otherwise.

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