ECOLOGY, BEHAVIOR AND BIONOMICS

An Aquatic Microhymenopterous Egg-Parasitoid of *Argia insipida* Hagen in Selys (Odonata: Coenagrionidae) and Biological Observations in the Central Amazon, Brazil

Ranyse B Querino[1], Neusa Hamada[2]

[1]*Embrapa Roraima, BR 174, km 8, Distrito Industrial, 69301-970, Boa Vista, RR; ranyse@cpafrr.embrapa.br*
[2]*INPA, Coordenação de Pesquisas em Entomologia, C. postal 478, Av. André Araújo, 2936, Petrópolis, 69011-970, Manaus, AM; nhamada@inpa.gov.br*

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Um Microimenóptero Aquático Parasitóide de Ovos de *Argia insipida* Hagen in Selys (Odonata: Coenagrionidae) e Observações Biológicas na Amazônia Central, Brazil

RESUMO - Foi estudada a interação entre *Argia insipida* Hagen in Selys, o parasitóide de ovos *Pseudoligosita longifrangiata* (Viggiani) (Hymenoptera: Trichogrammatidae) e a planta *Tonina fluviatilis* (Eriocaulacea), utilizada como substrato de oviposição de *A. insipida*. O local de estudo foi um curso de água com intensa correnteza, contendo macrófitas como *T. fluviatilis*. As informações sobre parasitóide de ovos aquáticos são escarsas. Este é o primeiro registro do parasitismo de ovos de *A. insipida* por *P. longifrangiata* no Brazil e o primeiro registro da ocorrência de *P. longifrangiata* no país. Ovos de *A. insipida* parasitados e não-parasitados foram observados somente 0-5 cm abaixo da superfície da água. O período de maior incidência de casais de *A. insipida* realizando posturas foi entre 13:00h e 14:00h. As folhas de *T. fluviatilis* tornam-se amareladas e secam quando um grande número de ovos é inserido nas folhas.

PALAVRAS-CHAVE: Inseto aquático, Zygoptera, *Pseudoligosita*, Trichogrammatidae

ABSTRACT - The tritrophic interaction *Argia insipida* Hagen in Selys, the Trichogrammatidae egg parasitoid *Pseudoligosita longifrangiata* (Viggiani) and the host plant *Tonina fluviatilis* (Eriocaulacea), which is a substrate for egg deposition of *A. insipid*, was investigated. The study locality was a stream with rapids where macrophytes such as *T. fluviatilis* grow. Information on aquatic egg parasitoids is scarce. This is the first record of egg parasitism of *A. insipida* by *P. longifrangiata* in Brazil, and the first record of occurrence of *P. longifrangiata* in the country. Parasitized and unparasitized eggs of *A. insipida* were observed only on leaves 0-5 cm below the water surface. The maximum number of pairs of *A. insipida* laying eggs in the study area was observed between 13:00h and 14:00h. Leaves of *T. fluviatilis* become yellowish and dry out when large numbers of eggs of *A. insipida* are laid on them.

KEY WORDS: Aquatic insect, damselfly, *Pseudoligosita*, Trichogrammatidae

Despite the large number of studies that have been done on neotropical Odonata, many aspects of their biology and ecology remain unknown, although a number of behavioral and community studies conducted in Brazil are available (e.g. De Marco 1998, De Marco & Santos-De Marco 1998, De Marco & Cardoso 2004). *Argia insipida* Hagen in Selys is widely distributed in South America (Colombia, Venezuela, Trinidad and Tobago, Guiana, Surinam, French Guyana and Brazil) (Lencioni 2006) but very little is known about its biology.

Biotic interactions are among the factors that influence the utilization of any given habitat. Many Odonata species perform endophytic egg laying on aquatic plants, and their eggs are potentially vulnerable to attack by predators and parasitoids. According to Corbet (1962), predation of Odonata eggs by other animals is not known; however, eggs of endophytic species have occasionally been observed in the stomachs of insectivorous fishes. Westfall & Tennessen (1996) reported that immature stages of an aquatic mite (*Hydracarina* spp.) might prey on eggs of Odonata. Trichoptera larvae (Odontoceridae: *Marilia* sp.) were observed feeding on endophytic eggs of Odonata in Manaus municipality (pers. obs.). Existing information on parasitoids and predators of dragonfly eggs is largely qualitative (Corbet 1999). The only detailed information of egg mortality in Odonata is associated with parasitism by small Hymenoptera (Hagen 1996, Corbet 1999). Literature on Odonata egg parasitism is scarce: Hagen (1996) compiled records for North America, and Fursov (1985) elaborated a review of European Chalcidoidea (Hymenoptera) parasitizing eggs of aquatic insects.

The definition of aquatic Hymenoptera adopted here is that of Hagen (1996), which includes all Hymenoptera species that parasitize any life stage of aquatic insects. Most of these species belong to the families Eulophidae, Mymaridae, Trichogrammatidae and Scelionidae. In Brazil, the only Hymenoptera egg parasitoid of Odonata reported is *Hydrophylita lestesi* (Costa Lima 1960) (Trichogrammatidae), which was observed parasitizing eggs of *Lestes* sp. (Lestidae) on leaves of *Hedychium coronarium* (Costa Lima 1960). Additional information found in the literature indicates *Lathromeroidea* sp. (Trichogramatidae) as a parasitoid of gerrid eggs (Henriquez & Spence 1993) and *Trichogramma* species parasitizing eggs of Sialidae (Megaloptera) (Azan & Anderson 1969, Barnard 1977).

Trichogrammatidae includes species that are parasitoids of eggs of several insects orders. It is one of the smallest families of Chalcidoidea, containing only about 55 genera in the New World (Pinto 2006). Except for the genus *Trichogramma*, which is used in biological control programs worldwide, this family is not well known in the Neotropical region. Information on the biology and ecology of these species is scarce because the majority of species are collected with traps; the host insects and their associated plants are therefore unknown.

Pseudoligosita is a cosmopolitan genus, and like other Oligositinae genera in South America it occurs in Argentina, Bolivia, Brazil, Colombia, Uruguay and Venezuela (Pinto 2006). Species in this genus have been cited as parasitoids of eggs of Hemiptera (Auchenorrhyncha), Orthoptera (Tettigoniidae) and Coleoptera (Chrysomelidae: Hispinae) (Pinto & Viggiani 2004). *Pseudoligosita longifrangiata* (Viggiani) had only been collected previously in Argentina and Uruguay with no information on its host (Viggiani 1981). Information on the biology of aquatic microhymenopterous parasitoids is scarce, especially in the Neotropics.

The objectives of this study were to provide information on the egg parasitoids of *A. insipida,* on the interaction between this damselfly, its egg parasitoid and the host plant for its eggs, and on the bionomics of *A. insipida*.

Material and Methods

Collections in the Onça stream, located at km 20 on the AM-240 Highway (02º00'52''S/60º01'43''W) in Presidente Figueiredo municipality, state of Amazonas, Central Amazon region, were performed in June and October 2003 and in July 2004. The stream had a rocky bottom with rapids and sand banks where macrophytes such as *Tonina fluviatilis* (Eriocaulacea) were present. Under the Köppen classification, the predominant climate in this area is included in group A (Rainy Tropical Climate), which has annual precipitation above 2000 mm. The relative humidity is high (85% to 95%) and the average annual temperatures range from 24ºC to 26ºC (RADAMBRASIL 1978).

In order to study egg parasitism on *A. insipida* we collected branches of the aquatic plant *T. fluviatilis* at the edges of the stream, in places with turbulent water. Leaves and branches with eggs of *A. insipida* were transferred to glass containers in the laboratory and kept at room temperature until either the parasitoid emerged or the larvae of *A. insipida* hatched. Both parasitoids and Odonata were fixed in 70% alcohol. Parasitoid identification was based on Doutt & Viggiani (1968), Viggiani (1981), Pinto (1997) and Pinto & Viggiani (2004). For determination of the parasitism percentage, 50 leaves of *T. fluviatilis* were collected and the eggs of *A. insipida* present on the leaves were counted and transferred to plastic recipients. Hatched Odonata larvae and emerged adult parasitoids were counted.

Oviposition activity of *A. insipida* on *T. fluviatilis* was observed for the first time in June 2003. At this time, adults of both sexes of *A. insipida* were collected and preserved in entomological envelopes for confirmation of the specific identification. In October 2003 and June 2004, oviposition activity observations were performed in a five-meter stretch of the stream where one edge was completely occupied by *T. fluviatilis*. Observations were done during two days at 30-minute intervals from 6:00h to 18:00h on the first day and from 6 a.m. to 4 p.m. on the second day. Due to the narrow width of the stretch analyzed, it was possible to directly count the individuals of *A. insipida* from a distance 1-2 meters.

Although *A. insipida* was the only Odonata observed ovipositing at the study site we placed one couple of this species in a small cage together with pieces of the host plant without eggs, in order to obtain freshly oviposited eggs for comparison with the eggs collected in the field. The cage was made of a two-liter transparent plastic bottle with several circular holes in the lower part of the bottle to allow water circulation. This cage was placed in the stream in an area with low water velocity and the bottom of the bottle was fixed to a rock. The male and female placed in this cage were captured during oviposition activity at the study site; they remained in the cage from 10:00h to 18:00h. After this period, the plant was removed from the cage, placed in a plastic recipient and transported to the laboratory in an icebox. In the laboratory, the eggs were photographed for morphological characterization.

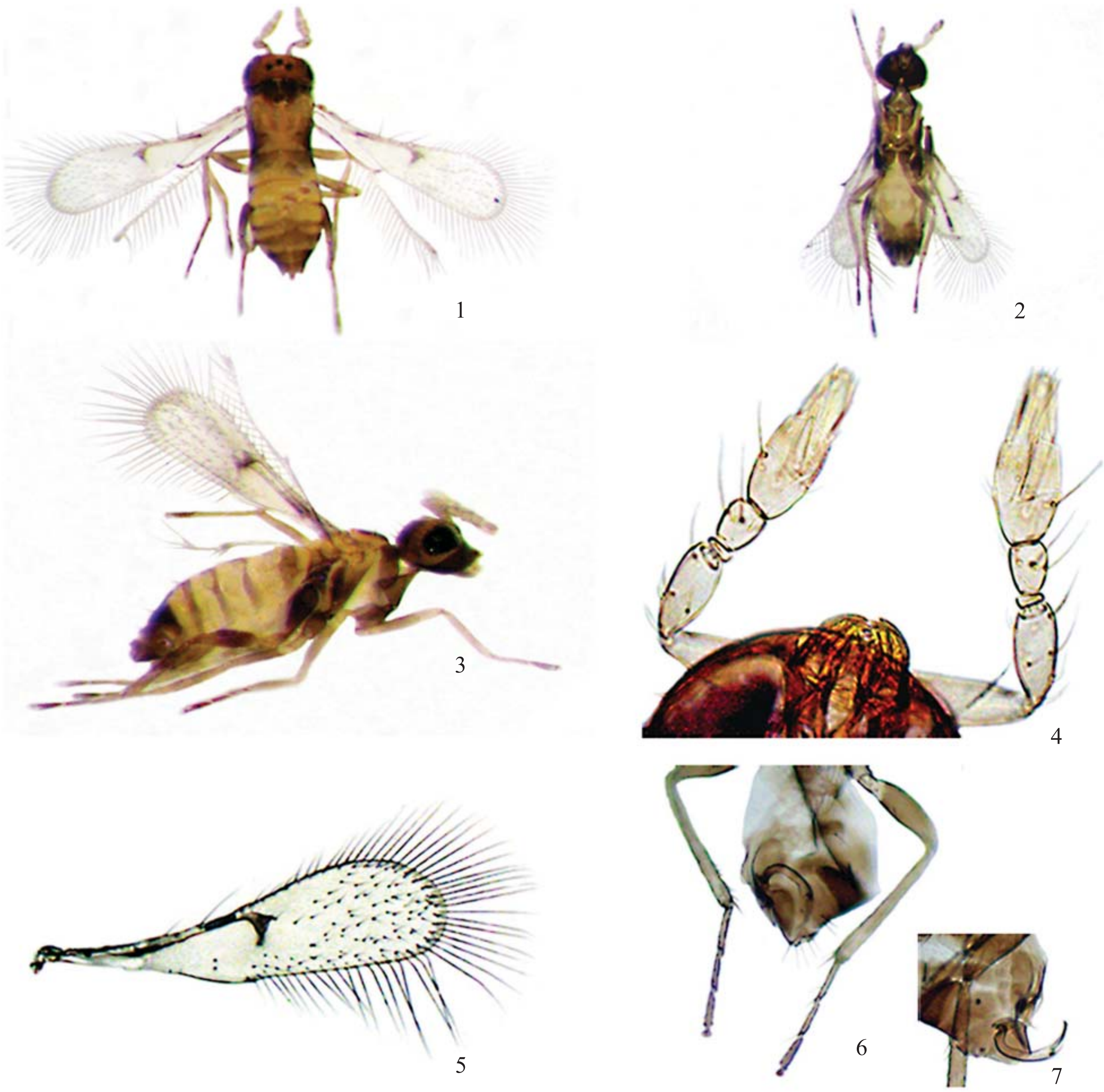
Since the 5-m stretch studied had areas that differed in terms of water turbulence, we divided the stretch into three sections: the first section had a small riffle and therefore had higher water velocity; the middle section had lower water velocity, and the third section had another small riffle. In order to determine if there were any differences between these sections as oviposition sites, 20 branches of the host plant were collected in from each section of the stream. In each section branches were also collected separately from two levels: 5 cm below and 5 cm above water surface. Mean number of eggs per leaf was determined by counting the eggs on 50 leaves from each of the sections and levels sampled (n = 300). During the sampling period, the stream was also searched to check for oviposition activity of *A. insipida* on other aquatic plants.

Results and Discussion

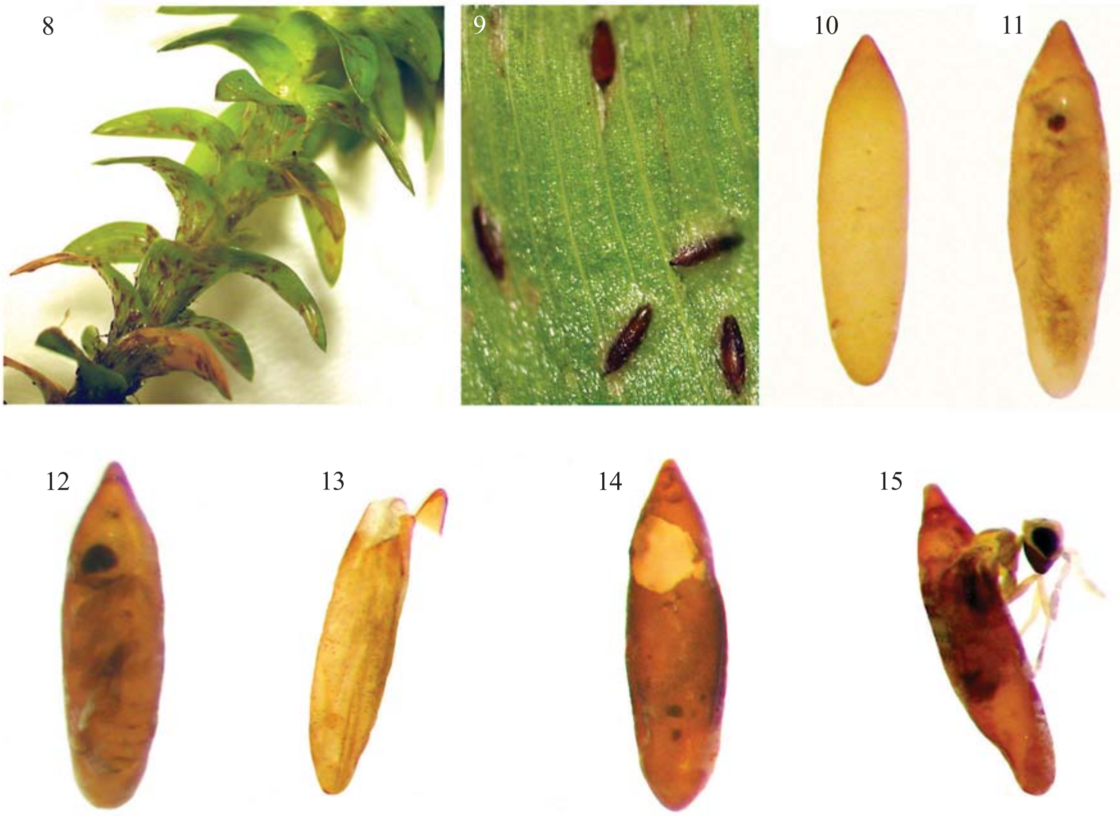
Adults of *A. insipida* are sexually dimorphic in color pattern: males are blue with black patches, while females are grayish with black spots on thorax.

This is the first record of *P. longifrangiata* in Brazil and the first report of *A. insipida* egg parasitism by *P. longifrangiata* associated with *T. fluviatilis*. Other species of Odonata were seen flying and perching on the plants, but only *A. insipida* was observed in tandem and engaged in oviposition activity at the study site.

Pseudoligosita longifrangiata (Figs 1-7) was the only species collected on eggs of *A. insipida* laid on leaves and branches of *T. fluviatilis*. Eggs of *A. insipida* are individually and endophytically laid both on leaves and on branches with no ordering pattern (Figs 8-9). They are elongated, cylindrical and yellow, with relatively sharp ends (Figs 10-15), as is characteristic of species with endophytic egg laying (Corbet 1962). A translucent egg chorium allows detection of clear differences in the Odonata or parasitoid development. Where Odonata embryos are present, the eggs have light coloration and the embryo body shape can be visualized, including the eyes, which are at the lateral sides at late stages of development (Fig 11). In the presence of the parasitoid, the egg has a darker coloration and its pupa can be clearly visualized through the chorium (Fig 12). Larval hatching of *A. insipida* occurs through the anterior end of the egg (Fig 13), while parasitoid emergence takes place through a lateral



Figs 1-7 *Pseudoligosita longifrangiata*, adult male. 1. Dorsal view. 2. Ventral view. 3. Lateral view. 4. Antenna. 5. Forewing. 6. Genitalia, ventral view. 7. Genitalia, lateral view.



Figs 8-15 *Argia insipida* eggs. 8-9. Placed endophytically on leaves and branches of *Tonina fluviatilis*. 10. Recently laid. 11. Lateral view of the embryo. 12. Lateral view of the parasitoid pupae. 13. Unparasitized spent egg after hatching of damselfly larva. 14. Parasitized spent egg after emergence of wasp. 15. Parasitoid emerging from egg.

circular hole (Fig 14). These differences allow determination of whether or not spent eggs were parasitized.

The observed percentage of eggs of *A. insipida* parasitized was 10.6%; no information of this nature is available for other species in the Neotropics. However, Corbet (1999) reported that the percentage of Odonata eggs parasitized in several parts of the world varied from 12% to 90%, depending on factors such as the part of the plant used for oviposition, host species, and parasitoid species, among others.

Diagnostic features by which *P. longifrangiata* (Figs 1-7) can be recognized are: Color – yellow with dark-brown stains on the mesonotum, mesopleura, thigh and femur. Head – lower region has a distinctly dark color. Antenna – club with three segments, the distal segment with no terminal sensilla, with projection; globoid funicular segment. Forewing – long marginal vein, with three long setae; glabrous area next to the stigmal vein; fringe with long setae, approximately as long as the wing. Ovipositor almost as long as the posterior tibia. Male genitalia – copulative organ simple, tubular and strongly curved ventrally. References to the taxonomic literature can be found in Pinto & Viggiani (2004) and Pinto (2006).

In the studied site *A. insipida* laid eggs only on *T. fluviatilis*, which is a small-size plant with dense ramification. Leaves become yellowish and dry when large numbers of eggs of *A. insipida* are laid. In areas with high densities of Odonata performing endophytic egg laying, plants are known to be at risk of dying or of being damaged due to excessive oviposition (Corbet 1962).

Oviposition was only performed on leaves 0-5 cm below the water surface; no eggs were observed on the upper part of the plant or above the water surface. In the studied stretches, higher numbers of eggs were collected in the first and third sections of the study site; both are places where the water velocity and turbulence were more intense. Also, in these two sections, a higher number of couples engaged in oviposition activity was observed, suggesting an aggregation behavior during this activity; this explains the higher number of eggs collected at these sites and hence the greater damage to the plant tissue. Females laid eggs while in tandem with males. Males either remained still in the air in a vertical position while holding the female's prothorax or more commonly, holding the plant with his legs. Females remained submerged for several minutes during oviposition, while males remained out of the water. The mean number of eggs per leaf was 13.5 below the water surface and zero above the surface.

Males were the first to be observed in the studied stretch, between 8:00h and 8:30h (Fig 16). The maximum number of adults was observed between 13:00h and 14:00h. Solitary males flew over and remained perched on plants near the oviposition sites. When couples in tandem appeared, solitary males approached them and tried to copulate with females. Solitary males remained at the site until 16:30h. Couples arrived at the observation site already in tandem between 21:00h and 21:30h; they perched briefly on the plants and

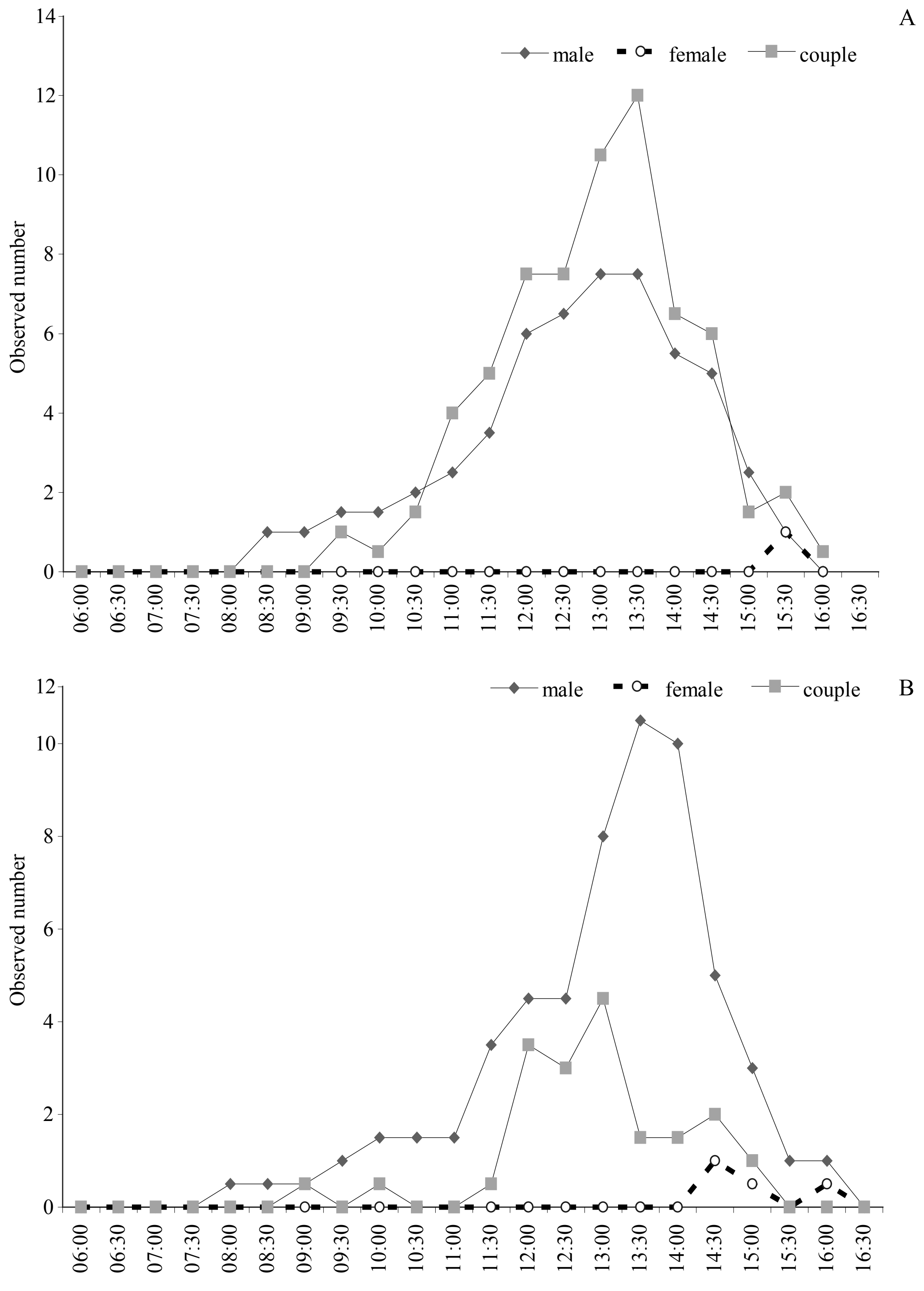


Fig 16 Oviposition activity of *Argia insipida* on leaves and branches of *Tonina fluviatilis* at a site in Presidente Figueiredo municipality, Amazonas state. A. In October 2003. B. In July 2004.

then females started their oviposition activity. Maximum number of couples in oviposition activity was observed between 13:00h and 13:30h. No single females were observed at the site and pair bonding occurs elsewhere.

Three newly hatched *A. insipida* adults were observed over the plants in October 2003; the first adult and its exuviae were seen at 7:50h; the second adult was seen at 11:20h near its exuviae and remained stretching its wings until 12:10h when a sudden noise induced it to fly to the shrubby vegetation at the stream's edge. The third adult was removed from the water at 14:14h.

Natural enemies of Odonata, such as spiders and a Vespidae, were also observed at the study site. An adult of *A. insipida* was stung by a wasp and fell down paralyzed.

Many aspects of the biology and ecology of neotropical Odonata species remain unknown. Parasitism under water is a highly specialized type of biotic interaction. It is unknown how these tiny and fragile parasitoid wasps are able to overcome water current forces in order to find their Odonata hosts. The natural history of many aquatic insects, including microhymenopterans, remains unknown, and systematic work on this group deserves special attention as a prerequisite for the natural-history studies that would be needed to obtain this knowledge.

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