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# Phoretic associations between *Nanocladius asiaticus* (Diptera, Chironomidae) and its hosts *Gestroiella* (Heteroptera, Naucoridae) and *Euphaea masoni* (Odonata, Euphaeidae) in streams in Western Thailand

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**Abstract** – Phoretic associations between larvae of *Nanocladius* (*Plecopteracoluthus*) *asiaticus* (Diptera, Chironomidae) and two species in the genus *Gestroiella* (Heteroptera, Naucoridae) were studied in Kanchanaburi and Ratchaburi Provinces (Western Thailand). *Gestroiella siamensis* was used by chironomid larvae as a host more frequently than was *Gestroiella limnocoroides*. Moreover, *N. asiaticus* larvae were associated symphoretically with nymphs of the damselfly *Euphaea masoni* (Odonata, Euphaeidae). This is the first report of a symphoretic association involving *E. masoni*. Approximately 44% of the population of naucorids harboured symphoretic chironomids, whereas only 13% of damselfly nymphs were hosts of the chironomid larvae. Most of the brachypterous male (59%) naucorids hosted chironomid larvae. The attachment site of the chironomids was frequently along the mesofemur and mesosternum of the naucorid hosts. The chironomids were attached to the ventral sides of abdominal segments of *E. masoni* nymphs. There was a positive correlation in body length between chironomids and naucorids ($r = 0.389$, $P < 0.01$). There are many benefits of symphoresis in the Chironomidae, such as improved feeding opportunities, increased mobility, suitable pupation sites and decreased predation risks.

**Key words:** *Nanocladius* (*Plecopteracoluthus*) *asiaticus* / *Gestroiella* / *Euphaea masoni* / phoretic associations / Thailand

## Introduction

Phoretic association is a relationship in which one organism lives on the body of another organism. This association occurs among various organisms in aquatic environments (Tokeshi, 1993). The family Chironomidae (Diptera) have been found to associate themselves phoretically with other aquatic insects, most notably Plecoptera (Dosdall and Mason, 1981; Giberson *et al.*, 1996; Dorvillé *et al.*, 2000), Odonata (Dosdall and Parker, 1998; Vescovi Rosa *et al.*, 2009), Ephemeroptera (Epler, 1986; Callisto and Goulart, 2000), Trichoptera (White *et al.*, 1980; Callisto and Goulart, 2000), Megaloptera (Tracy and Hazelwood, 1983; De La Rosa, 1992; Hayashi, 1998) and Hemiptera (Roback, 1977).

Phoretic relationships between larvae of the genus *Nanocladius* and other aquatic insect species have been reported; *Nanocladius* (*Plecopteracoluthus*) sp. and stonefly nymph *Pteronarcys biloba* Newman (Giberson *et al.*, 1996) and *Kempnyia tijucana* (Dorvillé & Froehlich) (Dorvillé *et al.*, 2000), *N. bubrachiatus* Saether and many leptophlebiid mayfly nymphs (*Traverella* sp. and *Thraulodes* sp.) (Epler, 1986; Callisto and Goulart, 2000). Larvae of the *Nanocladius branchicolus* Saether attached to the stonefly *Acroneuria lycorias* (Newman) (Plecoptera, Perlidae) (Dosdall and Mason, 1981) and the damselfly *Argia moesta* (Hagen) (Odonata, Coenagrionidae) (Dosdall and Parker, 1998). In addition, *Nanocladius downesi* (Steffan) attached to *Corydalus cornutus* Linnaeus (Megaloptera, Corydalidae) (Tracy and Hazelwood, 1983).

Phoresy by chironomid larvae and pupae of *Nanocladius* (*Plecopteracoluthus*) *asiaticus* Hayashi on corydalid larvae is known in several species, such as *Protohermes grandis* (Thunberg), *P. costalis* (Walker), *Neochauliodes sinensis* (Walker), *Neurhermes maculipennis*

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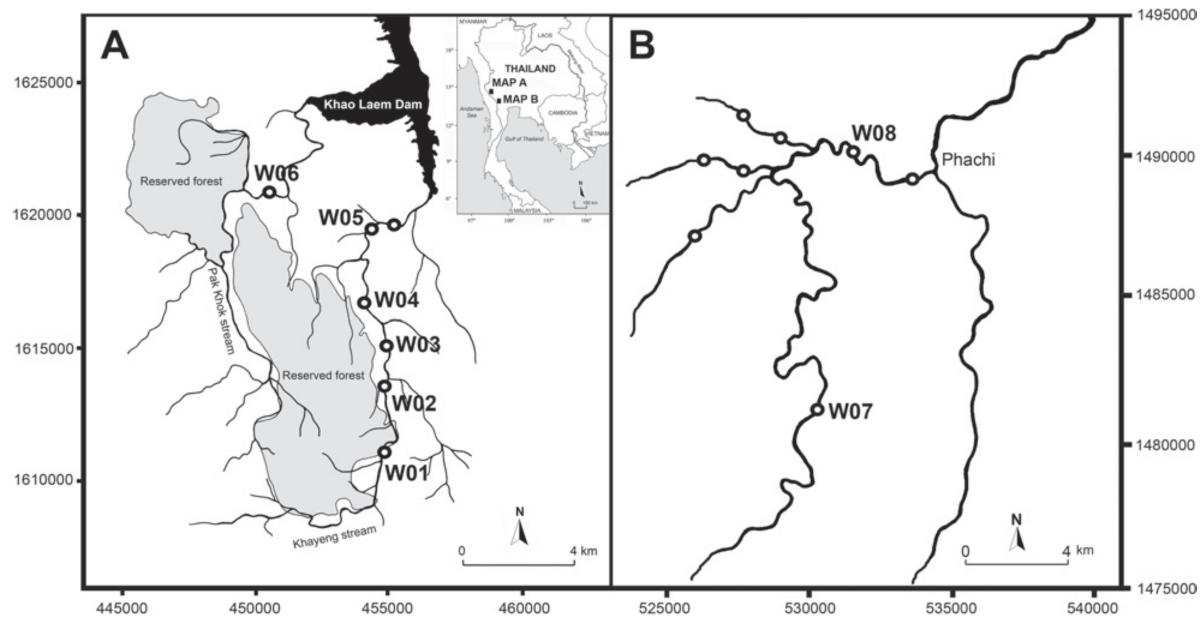


**Fig. 1.** Map illustrating the study sites in Kanchanaburi (A) and Ratchaburi (B) Provinces (Western Thailand).

(G. Gray in Cuvier) and *Parachauliodes continentalis* (van der Weele) (Hayashi, 1998). In addition, Polhemus *et al.* (2008) reported an association between *N. asiaticus* and two species of naucorids, *Gestroiella siamensis* Polhemus, Polhemus & Sites and *Gestroiella limnocoroides* Montandon. However, the detail of the phoretic mode has not been established. The aim of the present study was to describe the phoretic relationships of both larvae and pupae between *N. asiaticus*, its host *E. masoni* Selys and two species in the genus *Gestroiella* in streams in Kanchanaburi and Ratchaburi Provinces (Western Thailand).

## Materials and methods

### Study area

The study was conducted in third- and fourth-order tributaries of the Maeklong River (Fig. 1(A)) and the Phachi River (Fig. 1(B)). The first study area lies within the Maeklong tributary, located in Western Thailand. Sampling sites were chosen at the upstream part of the Khoa Lam reservoir in Huai Khayeng Settlement, Thong Pha Phum District, Kanchanaburi Province. Six sampling sites (W01–W06) were selected from the Huai Khayeng and Huai Pak Kok streams. All sampling sites were surrounded by different types of land (forested, agricultural and residential). The site selected was located approximately 20 km up from the Khao Laem reservoir. The second study area is situated in the Phachi stream, Ratchaburi Province, Western Thailand. Two sampling sites (W07–W08) were chosen from the stream. Physical characteristics of each site are summarized in Table 1. The stream was characterized by an alternation of riffles and pools. The stream bed was mostly composed of boulders (10–30%), cobbles (30–60%), pebbles (15–40%), gravels (10–20%) and sand (5–20%).

### Materials collection, processing and identification

Samples were collected using kick nets from at eight sampling sites (Fig. 1). Materials used in this study were collected during the field trips in December 2008, April 2009 and 2013, December 2013 and April 2014. Specimens were preserved in 90% ethanol and identified as species based on Hayashi (1998) for chironomids, Polhemus *et al.* (2008) for naucorids and Asahina (1985) for damselflies. Numbers of individuals, position and body length of attached chironomids on naucorid hosts were recorded. The body length (the distance between the anterior part of the head and the posterior part of the last abdominal segment) of chironomids and their hosts was measured using an ocular micrometer. The relationships of body length between naucorids and chironomids were analysed using Pearson's correlation coefficient. The correlation coefficient between chironomids and odonates was not calculated because of the small number of samples. Only five chironomids (2.5–4.0 mm in body length) were collected from the sternal portion of the Odonata specimens (6.5–12.0 mm in body length). Photographs of specimens were taken using a NIKON SMZ800 stereoscopic microscope. All material was deposited in the collection of ZMKU (Zoological Museum, Kasetsart University, Bangkok, Thailand).

During the field sampling, environmental factors such as conductivity, pH, dissolved oxygen concentration and water temperature were measured in the field using a multi-probe YSI Incorporated® model 556 MPS. Current

**Table 1.** Physical characteristics of eight sampling sites.

| Site code | Latitude | Longitude | Elevation (m) | Stream order | Location | Substrate composition |
|---|---|---|---|---|---|---|
| W01 | 14°34′58″N | 98°34′52″E | 256 | 3 | Ban Pra Chum Mai | Cobble 60% pebble 15% gravel 15% sand 10% |
| W02 | 14°36′20″N | 98°34′38″E | 206 | 4 | Ban Huai Khayeng | Cobble 50% pebble 25% gravel 15% sand 10% |
| W03 | 14°37′35″N | 98°34′22″E | 195 | 4 | Phuiraya | Cobble 40% pebble 35% gravel 15% sand 10% |
| W04 | 14°37′59″N | 98°34′06″E | 182 | 4 | Ban Pak Lum Pilock | Boulder 50% cobble 10% gravel 20% sand 20% |
| W05 | 14°38′58″N | 98°34′56″E | 166 | 4 | Ban Tao Tan | Cobble 30% pebble 50% gravel 15% sand 5% |
| W06 | 14°39′34″N | 98°32′02″E | 176 | 3 | Huai Pak Kok | Boulder 10% cobble 40% pebble 30% gravel 10% sand 10% |
| W07 | 13°24′28″N | 99°16′52″E | 200 | 3 | Kang Som Maew | Boulder 20% cobble 40% pebble 40% gravel 10% sand 10% |
| W08 | 13°30′56″N | 99°20′42″E | 110 | 4 | Pavothai resort | Cobble 30% pebble 50% gravel 15% sand 5% |

**Table 2.** Physico-chemical characteristics and occurrence of phoretic association (%) of eight sampling sites during field sampling.

| | Parameters | | | | | | | | | Occurrence of phoretic association (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Site code | Water temperature (°C) | pH | Conductivity (μS.cm$^{-1}$) | Dissolved oxygen (mg.L$^{-1}$) | Total dissolved solids (mg.L$^{-1}$) | Current velocity (m.s$^{-1}$) | Depth (cm) | N-$NO_3^-$ (mg.L$^{-1}$) | P-$PO_4^{3-}$ (mg.L$^{-1}$) | |
| W01Apr | 28.30 | 7.00 | 360 | 5.40 | 230 | 0.60 | 18 | 0.17 | 0.02 | 3.2 |
| W02Apr | 25.00 | 7.90 | 371 | 5.80 | 241 | 0.70 | 16 | 0.30 | 0.05 | 27.9 |
| W03Apr | 26.50 | 7.60 | 373 | 5.20 | 235 | 0.20 | 35 | 0.50 | 0.04 | 5.1 |
| W04Apr | 27.42 | 7.46 | 299 | 7.31 | 185 | 0.40 | 18 | 0.30 | 0.02 | 3.8 |
| W05Apr | 28.00 | 8.50 | 402 | 5.50 | 249 | 0.30 | 18 | 0.30 | 0.05 | 9.5 |
| W06Apr | 29.90 | 7.50 | 165 | 6.20 | 98 | 0.20 | 15 | 0.50 | 0.07 | 13.9 |
| W07Apr | 28.55 | 8.10 | 297 | 5.90 | 181 | 0.50 | 28 | 0.20 | 0.06 | 5.7 |
| W08Apr | 32.27 | 8.00 | 314 | 6.69 | 179 | 0.50 | 15 | 0.30 | 0.05 | 1.9 |
| W02Dec | 23.67 | 8.09 | 493 | 8.57 | 135 | 0.20 | 33 | 0.23 | 0.27 | 9.5 |
| W04Dec | 23.44 | 8.23 | 493 | 8.52 | 135 | 0.20 | 23 | 0.70 | 0.05 | 3.0 |
| W05Dec | 23.02 | 8.28 | 395 | 8.74 | 170 | 0.25 | 20 | 0.43 | 0.05 | 5.1 |
| W06Dec | 21.35 | 8.10 | 118 | 9.68 | 59 | 0.04 | 40 | 0.33 | 0.04 | 11.4 |

velocity was measured using a Global Water® flow-meter model FP 111. Concentrations of orthophosphate and nitrate were determined within 24 h after sample collection, using a standard method provided by Hach Company (1992).

## Results

Physico-chemical parameters varied over time (Table 2), in correlation with progressive water withdrawal, implying an increase of temperature, nitrate, orthophosphate, conductivity and total dissolved solids, and a decrease of oxygen concentration, current velocity, depth and submerged area. pH values also slightly varied across time, but without showing any particular pattern. Pearson's correlation analysis revealed that there was no statistically significant correlation between percentage of the occurrence of phoretic and environmental variables. There was no significant correlation between current and the occurrence of phoretic association ($r = 0.228$, $P > 0.05$) (Fig. 2). However, the phoretic association was observed in riffle areas, possibly because the microhabitat of hosts (two species of *Gestroiella*).

Of 361 collected naucorids, 158 (44%) specimens harboured *N. asiaticus*. Among them, *G. siamensis* (90%) were used as hosts more frequently than *G. limnocoroides*. *Nanocladius asiaticus* larvae were attached to various body parts (mesofemur, mesosternum, meso- and metacoxae and scutellum) of their hosts by a silken net (Fig. 3). They attached mostly along the mesofemur (54%) and mesosternum (34%) of the naucorid hosts (Fig. 4). The chironomids were mostly located along the mesofemur (80%) and mesosternum (10%) of *G. limnocoroides*, whereas they occurred on various body parts of *G. siamensis* (Fig. 4). Meanwhile, chironomids were mostly observed in the brachypterous form of both species of naucorids, *G. limnocoroides* (86% in females) and *G. siamensis* (32 and 64% in females and males, respectively). A small proportion of chironomids (3.5%) were observed in the macropterous females. Of the naucorids associated with chironomids, 91% hosted single specimens; rarely were two or three chironomids found on one host. Pupation (6%) occurred on the mesofemur of naucorids. An analysis of the body size of two species of naucorids showed that *G. limnocoroides* (ranging from 5.2 to 15.5 mm, $n = 143$) is the majority of host of chironomids, while *G. siamensis* ranged from 15.0 to 16.5 mm

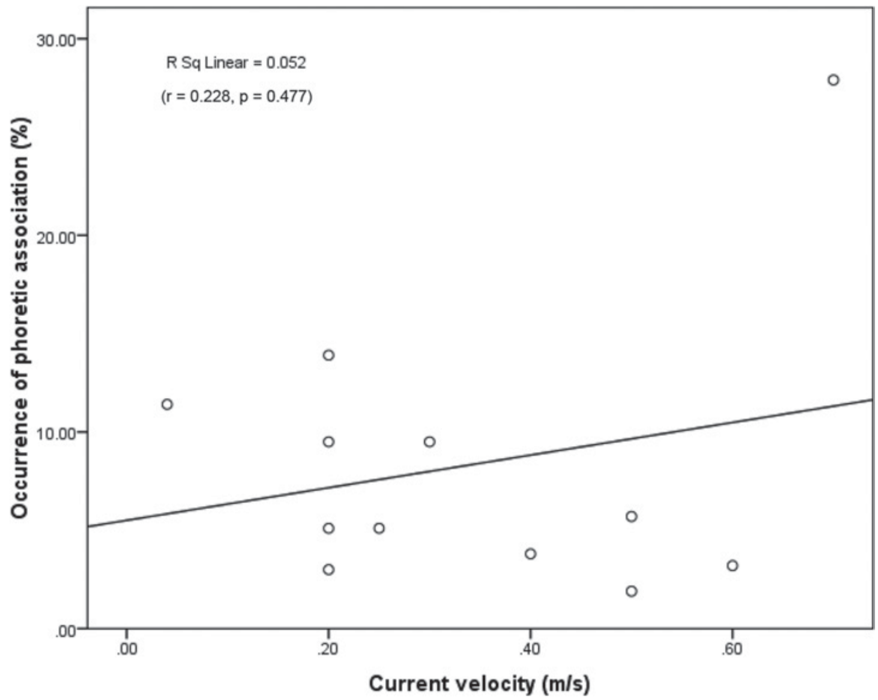


**Fig. 2.** Scatter plot showing the relationship between occurrences of phoretic association (%) of *N. asiaticus* and two species of *Gestroiella* and current velocity.

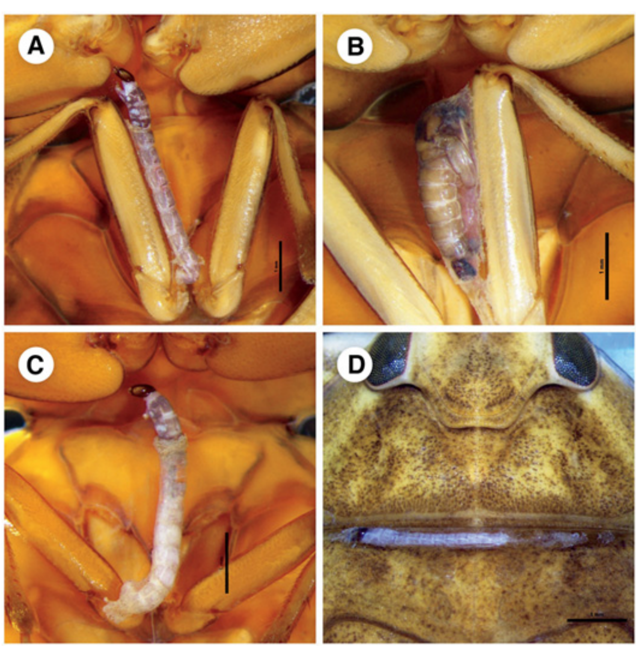


**Fig. 3.** *Nanocladius asiaticus* symphoresy on mesofemur (A, B), mesosternum (C), and scutellum (D) of *G. siamensis*.

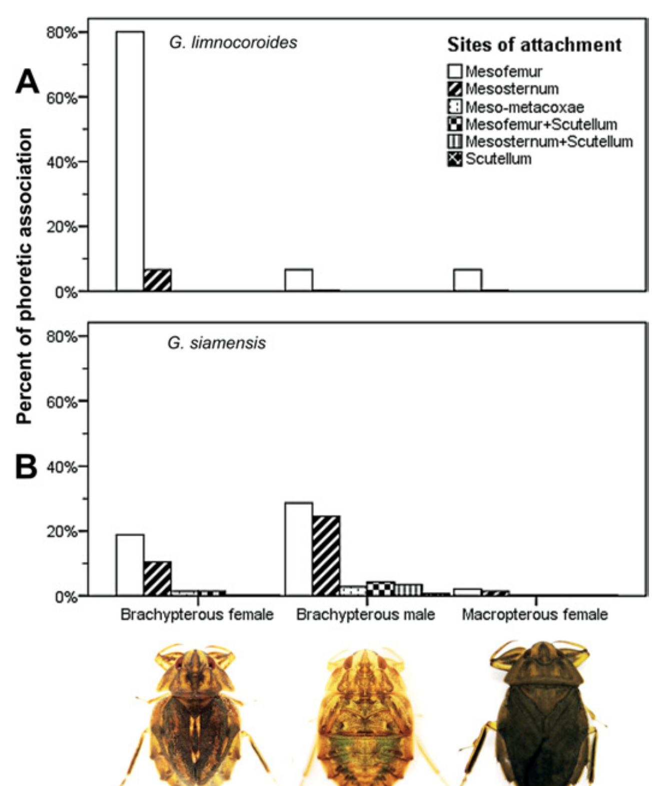


**Fig. 4.** Comparison between the attachment site of *N. asiaticus* on each form of *G. limnocoroides* (A) and *G. siamensis* (B).

($n = 15$) (Fig. 5). There was a positive correlation between body length of the chironomids and naucorid body length ($r = 0.389$, $P < 0.01$) (Fig. 6). This result implies that earlier instars of the chironomid occur on earlier instars of the naucorids.

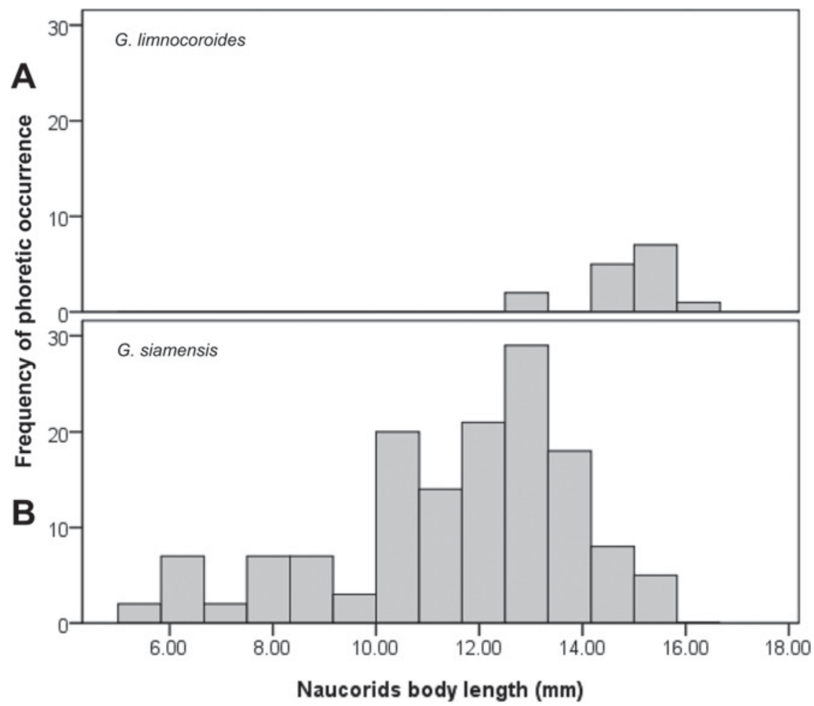


**Fig. 5.** Frequency histograms of body size distributions for *G. limnocoroides* (A) and *G. siamensis* (B).

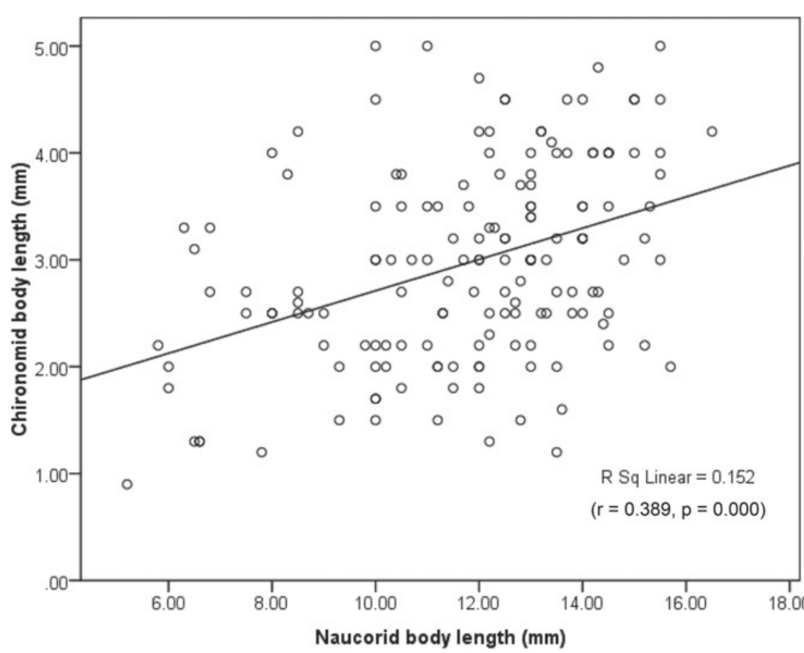


**Fig. 6.** Scatter plot between body length of *N. asiaticus* and two species of *Gestroiella*.

Twenty-eight euphaeid larvae were collected in the samples, and five odonates (18%) showed cases of phoresy by *N. asiaticus*. Both chironomid larvae and pupae were found to be associated symphoretically with nymphs of the damselfly *E. masoni* (Fig. 7). Chironomids were attached to the ventral sides of abdominal segments of damselfly nymphs. This is the first report of a symphoretic association between benthic invertebrates.

## Discussion

The larvae and pupae of the chironomid *N. asiaticus* were previously reported in megalopteran larvae in Japan, Taiwan and Malaysia (Hayashi, 1998). In addition, Polhemus *et al.* (2008) reported phoretic relationships between this species of chironomid and two species of naucorids, *G. siamensis* and *G. limnocoroides,* in Thailand.

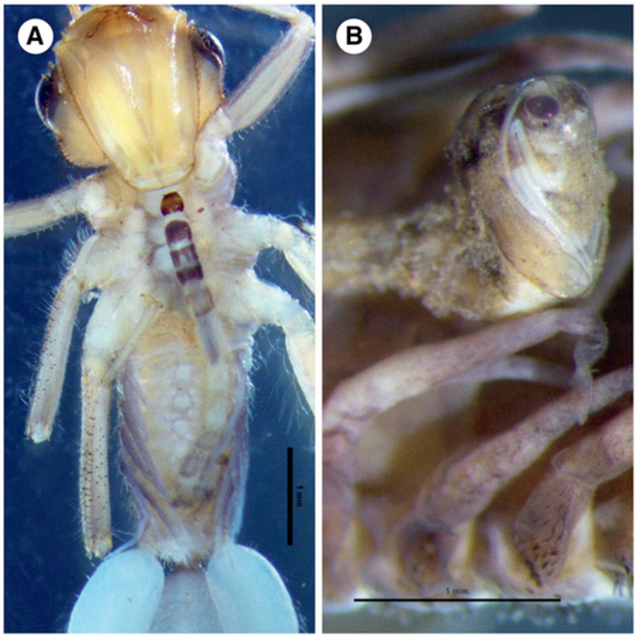


**Fig. 7.** Larva (A) and pupa (B) of *N. asiaticus* symphoretic on *E. masoni*.

In the present study, the percentage of phoretic relationships was close to the previously recorded data (Polhemus *et al*., 2008). However, *N. asiaticus* has not been known to be phoretic on corydalid larvae in Thai streams. The genus *Gestroiella* (Naucoridae) seems to be the preferred host for chironomids in Thai streams because this genus inhabits clear, rocky-bottomed streams with a relatively fast current velocity. The larva of *N. asiaticus* feeds on algae and detrital materials caught on their silken nets, or on the body surfaces of their hosts (Hayashi, 1998). There are four major benefits of symphoresis in the Chironomidae: (i) increasing stability in fast currents (increased mobility); (ii) reducing energy expenditure for relocation (reduced predation risk); (iii) reducing interspecific competition for food (better feeding opportunity); and (iv) obtaining a superior pupation site (protection from disturbance) (Dosdall and Parker, 1998; Tokeshi, 1993). The chironomids have always been under pressure from both limited mobility and high predation, due mainly to their morphological characteristics. Under these circumstances, commensalism is a convenient strategy to solve both the mobility and predation problems at the same time (Tokeshi, 1993). In this study, most chironomid larvae attached to the ventral side of the host's mesothorax. Hayashi (1998) reported that larvae of this species are also found on the ventral thorax of the megalopterans. The mesothorax is the most suitable attachment site for the ectosymbiotic chironomid, *N. asiaticus*, on its megalopteran host, *P. grandis* (Hayashi and Ichiyanagi, 2005). The ventral mesothorax may be a preferred attachment site because hosts groom around the head region (Hayashi, 1998). The chironomid *N. asiaticus* has long been considered to be a commensalistic dipteran without preference for a specific host, but the results of this study indicate a possible preference for naucorid hosts since they were found in the stream in a higher percentage than other potential hosts. Symphoresy appears to be obligatory for *N. asiaticus*, because free-living larvae have never been found, and the size of the chironomid has been found to be correlated with the size of the naucorid until pupation and emergence.

This study represents the first report of symphoresy involving Chironomidae with *E. masoni* (Euphaeidae). However, *N. asiaticus* was previously recorded in symphoretic associations with nymphs of the orders Megaloptera (Corydalidae, Hayashi, 1998) and Hemiptera (Naucoridae, Polhemus *et al*., 2008). Dudgeon (1989) reported seven chironomid genera (*Thienemannimyia*, *Cricotopus*, *Eukiefferiella*, *Thienemanniella*, *Polypedilum*, *Paratanytarsus* and *Rheotanytarsus*) that were found on the bodies of dragonfly larvae, *Zygonyx iris insignis* (Kirby), in Hong Kong streams. Among Odonata, such associations appear to be relatively rare, especially among the Zygoptera (Dosdall and Parker, 1998). In the present study, a relatively low percentage of phoretic relationships were observed (18%) between damselflies and chironomids. The association of other species of chironomids with damselflies has been observed in the families, Coenagrionidae (*A. moesta* (Hagen) and *N. branchicolus*; *Argia translata* (Hagen) and *N. downesi* (Steffan)), Calopterygidae (unidentified genus) and Megapodagrionidae (*Rheotanytarsus* sp. and *Heteragrion* sp.) (Dosdall and Parker, 1998; Vescovi Rosa *et al*., 2009). Euphaeid larvae live in lotic habitats and can be found in riffle areas. The establishment of the phoretic association between Chironomidae and Odonata, reported in the present work, may be related to the co-occurrence of the organisms in habitats with similar physical characteristics.

## Conclusion

In summary, this study found that *E. masoni* is a new host for *N. asiaticus*, which may be important for its population dynamics and dispersion. Moreover, *G. siamensis* was used by chironomid larvae as hosts more often than *G. limnocoroides*. The attachment site of the chironomid was frequently along the mesofemur and mesosternum of the naucorid host. There was a positive correlation of body length between chironomids and naucorids.

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