

## The Symbiotic Relationship of a Chironomid with Its Ephemeropteran Host in an Arizona Mountain Stream

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**ABSTRACT:** Aspects of the symbiotic relationship of a species of *Nanocladius* (*Plecoptera*: *Plecoptera*: *acolutus*) associated with mayfly nymphs in a mountain stream in Arizona are described including its prevalence on three host species, infestation pattern, attachment site selection and feeding behavior. Larvae of this species, designated here as *N. (P.)* sp. 2, were found primarily on nymphs of *Thraulodes* sp. (Leptophlebiidae) and occasionally on *Choroterpes* sp. (Leptophlebiidae) and *Leucrocota* sp. (Heptageniidae). Sixty-seven of 116 (58%) *Thraulodes* sp. nymphs from one collection site harbored from 1–5 *N. (P.)* sp. 2 larvae (abundance: 0.99 larvae/nymph; mean intensity: 1.72 larvae/host nymph). The range of larval attachment sites on the host became narrower as larvae matured. Most 3rd and 4th instar larvae constructed tubes on *Thraulodes* sp. that extended from the thorax between the middle and hind coxae posteriorly to the 7th abdominal segment along the lateral margin of the tergites. Pupation occurred within this tube with the pupa oriented posteriorly. The gut contents of larvae and presence of scars at sites of larval attachment on hosts indicate *N. (P.)* sp. 2 fed upon host hemolymph. Larval feeding and tube construction resulted in gill reduction, or deformation as well as scarring of gill and other body surfaces. However, parasitized *Thraulodes* sp. nymphs were capable of metamorphosis to adulthood. Dispersion patterns of larvae and pupae across the host population imply intraspecific competition or parasite-induced mortality may limit the parasite's population size. The life stage structure of *N. (P.)* sp. 2 from this stream suggested the population was not synchronized, but was likely comprised of at least two overlapping generations at the time of collection. Early instars were most abundant on large, late-instar nymphs and on nymphs harboring later midge instars. Late instar larvae of the older cohort tended to be more prevalent on less mature nymphs, suggesting the parasite and/or the host may affect the others' development. Because collections were limited to only one date, the hypotheses presented should be regarded as speculative and tentative until a more extensive study can be performed.

Chironomid species whose larvae live in obligatory symbiotic relationships with other aquatic animals represent some of the more unusual of the many life strategies that have evolved within the family. Steffan (1967a), Bottorff and Knight (1987), Tokeshi (1995), and Jacobsen (1995) offer reviews and lists of symbiotic Chironomidae and their associations with other animals. The Nearctic Region has a relatively rich fauna of obligate symbiotic chironomids with at least 8 named species. However, despite their peculiarity and conspicuous habit, many Nearctic species and associations remain partially or entirely undescribed.

In her description of the nymphs of *Thraulodes speciosus* Traver (Ephemeroptera: Leptophlebiidae) from the Chiricahua Mountains of Arizona, Mayo (1969) noted and illustrated a purported commensalistic association between these nymphs and larvae of a midge she identified as "*Symbiocladius* sp." Hynes (1976) speculated that this chironomid belonged instead to the genus *Nanocladius* (*Plecoptera*: *Plecoptera*: *acolutus*) based upon its reported commensal habit and larval morphology. Examination of reared

specimens of this species obtained during a collecting trip to the Chiricahua Mountains confirmed that it was a new *Nanocladius* (*Plecopteracoluthus*), tentatively designated as *N. (P.)* sp. 2 (Jacobsen, 1995). Larvae were occasionally found on nymphs of *Choroterpes* (*Choroterpes*) sp. (Ephemeroptera: Leptophlebiidae) and *Leucrocuta* sp. (Ephemeroptera: Heptageniidae) as well as on *Thraulodes* sp. collected in kick samples and picked from substrates (Fig. 1). After obtaining sufficient associated material for a forthcoming description of this species, additional nymphs of the three host species were collected and examined in order to describe the prevalence of *N. (P.)* sp. 2 on these host species and, for their association with *Thraulodes* sp., to describe the relationship of midge larvae with nymphs, their attachment site selection and the magnitude and patterns of their infestation on this host population. This paper reports on these collections and observations.

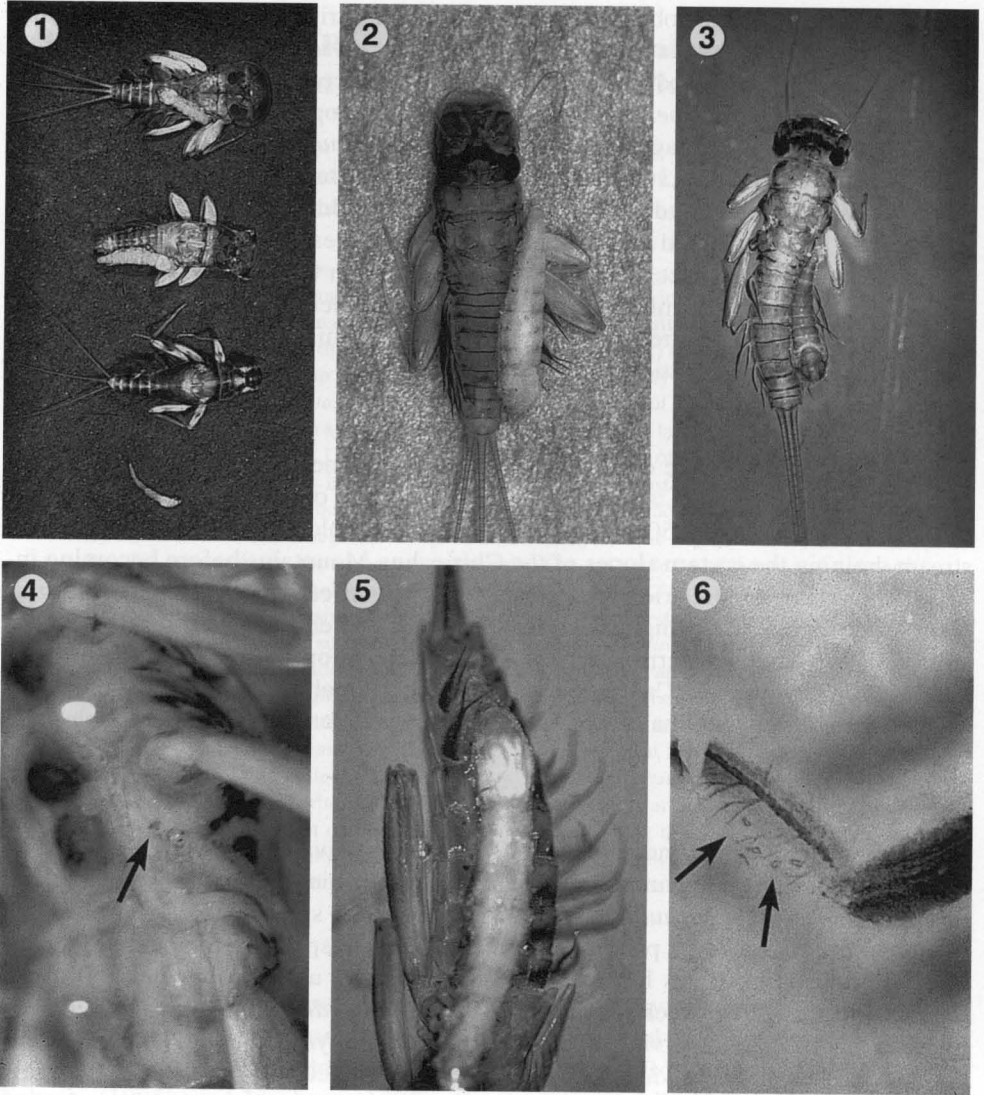
### Study Area

All mayfly nymphs were collected from Cave Creek near Idlewilde Campground, Coronado National Forest, approximately 2 miles west of Portal, Arizona, at an elevation of approximately 5000 feet. Cave Creek is a cold-cool water, high gradient stream draining the eastern slopes of the Chiricahua Mountains before becoming intermittent at lower, more arid plains near Portal. This stream has a steep thermal gradient and freezes over at higher elevations during winter. However, reaches downstream of the Southwestern Research Station (elevation 5400 feet) remain open year-round. At Idlewilde Campground, Cave Creek is about 5–10 m wide, equally divided into riffles and shallow ( $\leq 50$  cm) pools with coarse boulder, cobble, and gravel substrates.

### Methods

Preliminary collecting indicated that *Thraulodes* sp. was the principal host for *N. (P.)* sp. 2. In order to determine larval infestation densities and patterns on the three observed hosts, 116 *Thraulodes* sp., 42 *Choroterpes* sp. and 62 *Leucrocuta* sp. nymphs were collected by picking all nymphs of these species encountered on cobble and boulder substrates haphazardly sampled along an approximately 50 meter long segment of Cave Creek on July 15, 1992. *Thraulodes* sp. nymphs were placed immediately in individual vials upon collection while nymphs of the other two genera were placed 4–5 in a vial. Attachment site preferences for each instar of *N. (P.)* sp. 2 on *Thraulodes* sp. were determined from the above series of 116 nymphs and from an additional 43 nymphs bearing larvae, pupae, and empty puparia that were collected between July 11–14. All specimens, including those that were later dry weighed, were preserved in 70% ethanol in the field.

In the laboratory, the following were recorded for each nymph: body length from the anterior margin of the clypeus to the posterior margin of the last abdominal tergite (to nearest 0.1 mm), head width (to nearest 0.02 mm), sex and developmental maturity, as well as the number, developmental stage and site of attachment of any associated *N. (P.)* sp. 2. The maturity of each nymph was scored according to its wing pad length and color. The specific criteria for scoring were as follows: 1 = wing pads not extending to the first abdominal tergite; 2 = wing pads extending to the first abdominal segment; 3 = wing pads reaching the second abdominal segment; 4 = wing pads extending to the third abdominal segment but are not dark; 5 = wing pads dark (about to emerge).



Figs. 1-6. 1. *Nanocladius* (*P.*) sp. 2 4th instar larvae attached to a *Leucrocuta* sp. nymph (upper), *Thraulodes* sp. nymph (middle), and detached from a *Choroterpes* sp. nymph (lower). 2. Larva attached to a *Thraulodes* sp. nymph. 3. Pupa attached to *Thraulodes* sp. nymph. 4. Second instar larva detached from a scarred feeding site (arrow) on a *Thraulodes* sp. nymph. 5. Fourth instar larva attached to abdomen of *Thraulodes* sp. nymph. Note the absence of gills on abdominal segments 1-5. 6. Gill of *Thraulodes* sp. nymph showing scarring from larval activity.

Statistical terminology to describe midge infestation on mayfly nymphs follows that recommended by Margolis et al. (1982) for parasitic infestation on a host population: *Prevalence* is the total number of parasitized mayfly nymphs divided by the total number of all mayfly nymphs of a given taxon; *abundance* is the total number of parasites divided by the total number of nymphs; *intensity* is the number of parasites on an individual host nymph; *mean intensity* is the total number of parasites divided by the total number of parasitized mayfly nymphs.

The dispersion patterns of all immature stages and life-stage subsets of *N. (P.)* sp. 2 across the series of 116 *Thraulodes* nymphs collected on 15 July were assessed by comparing the indices of dispersion (i.e., variance/mean ratios) of the number of parasites per nymph against that of a Poisson distribution null model (variance/mean = 1) using a two-tailed Chi-square test (i.e., Index of Dispersion test) and the normal approximation for large sample size as described in Krebs (1989). Variance to mean ratios or indices of dispersion significantly less than 1 or significantly greater than 1 indicate distributions are uniform or aggregated, respectively. Kendall's tau was calculated to examine whether numbers of different life stages and groupings of life stages of *N. (P.)* sp. 2 on individual nymphs were correlated with nymph body length or wing pad development. Significance of tau was determined by using the normal approximation to test the null hypothesis that tau = 0 as outlined in Sokal and Rohlf (1981). A Wilcoxon sign-ranked nonparametric test was used to compare numbers of 1st and 2nd instar larvae on hosts with and without later instar larvae and pupae.

To determine what larvae were feeding upon, the gut contents of 15 larvae taken from *Thraulodes* sp. nymphs were examined either mounted alive in water or mounted in Hoyer's solution. The size relationship between mature larvae and pupae and their hosts was measured by dry weighing 4 mature 4th instar larvae (pre-pupae) and two pupae and their respective hosts to the nearest microgram with a Mettler UM3 electrobalance. All nymphs were checked for any noticeable scarring, gill deformities, gill loss or wing pad asymmetry. Wilcoxon sign-ranked nonparametric tests were used to test for differences in frequency of scarring and gill damage between unparasitized and parasitized nymphs and between sides of parasitized nymphs unoccupied and occupied by 3rd instar and older immature stages of *N. (P.)* sp. 2 for the series of 116 *Thraulodes* sp. nymphs collected on June 15.

## Results

*Thraulodes* sp. was the principal host of *N. (P.)* sp. 2 in Cave Creek. Sixty-seven of 116 nymphs collected (58% prevalence) harbored 1–5 chironomid larvae and/or pupae (abundance: 0.99 midges/nymph; mean intensity: 1.72 midges/host nymph). Proportions in each life stage were as follows: 1st instars 48.7%, 2nd instars 20.9%, 3rd instars 10.4%, 4th instars 17.4% and pupae 2.6%. Only 2 of 42 *Choroterpes* sp. nymphs and 2 of 62 *Leucrocota* sp. nymphs examined had attached larvae, all of which were either 1st or 2nd instars.

Larvae and pupae attached to nymphs by constructing silken tubes against the host's body. Only 9 larvae (6 1st instar and 3 2nd instar larvae) of the 181 immature *N. (P.)* sp. 2 collected on 159 nymphs became detached after preservation in alcohol. The range of attachment sites on *Thraulodes* sp. used by larvae becomes narrower as larvae mature (Table 1). First and 2nd instar larvae constructed tubes in a variety of positions on nymphs with the thoracic pleura, nota underneath the wing pads, gill lamellae and lateral margins of the abdominal tergites most often colonized. Larvae also were observed on the neck, the head (including beneath the labium) and legs of mayfly nymphs. Three 1st instar larvae and six 2nd instar larvae occupied tubes in contact with tubes of later instar larvae. Most 3rd and 4th instar larvae constructed tubes extending from the thorax between the middle and hind coxae posteriorly to the 7th abdominal segment along the lateral margin of the tergites dorsomesad of the gills (Figs. 2, 5). Pupation occurs within this tube with the

Table 1. The percent usage of attachment sites by 172 *Nanocladius* (*P.*) sp. 2 larvae and pupae found on 159 *Thraulodes* sp. nymphs. Pupal data includes 9 empty puparia found on nymphs.

Position on host	<i>N. (P.)</i> sp. 2 developmental stage				
	1st instars	2nd instars	3rd instars	4th instars	Pupae
Head & neck	12	21	0	0	0
Thoracic sterna & pleura	30	34	22	0	4
Wing pads	19	7	4	0	0
Legs	0	3	0	0	0
Gills	22	10	0	0	0
Host abdomen	13	21	41	10	0
Host abdomen (on other larvae)	4	0	0	0	0
Lateral thorax-abdomen	0	3	33	90	96
No. of larvae or pupae	69	29	27	21	26

pupa oriented posteriorly (Fig. 3). One puparia out of the 26 observed was constructed on the dorsolateral surface of the thorax.

The gut contents of larvae examined suggest *N. (P.)* sp. 2 larvae feed primarily on their host's hemolymph. The gut was either empty or filled with an amber-colored fluid and only small amounts of sediment, algae or detritus were observed when present. Tube construction and maintenance, which often was observed when larvae were examined alive, could explain the presence of particulate material in the gut. Small dark scars were usually found on hosts in the vicinity of the larval head capsule (Figs. 4, 6). Scarring was observed on body surfaces at both ends of larval tubes (6 instances out of 146 observations of attached larvae), suggesting midge larvae may reverse their orientation within tubes and feed from either end. Scarring was significantly more frequent on host nymphs than on unparasitized nymphs (Table 2).

Removal of live larvae from nymphs was somewhat difficult because larvae vigorously apply salivary secretions to the host and/or the forceps and use these secretions as an anchoring substrate which they clasp with their posterior parapods. When removed, larvae move about rapidly by quickly applying salivary secretions to the substrate, hooking into these secretions with their posterior parapods by arching the body, and then extending the body forward.

Table 2. Comparisons of mean ( $\pm 1$  SE) numbers of scars and deformed or missing gills observed on *Thraulodes* sp. nymphs without *Nanocladius* (*P.*) sp. 2 larvae and nymphs with only a single 1st or 2nd instar larva, only a single 3rd or 4th instar larva or pupa, and for all nymphs with larvae or pupae. Statistically significant differences are for Wilcoxon sign-ranked nonparametric comparisons of distributions in each host category versus distributions on unparasitized nymphs (\* =  $P < 0.05$ , \*\*\* =  $P < 0.001$ ).

Type of injury or deformation	All nymphs without midges	Nymphs with only one 1st or 2nd instar larva	Nymphs with only one 3rd or 4th instar larva or pupa	All nymphs with midges
Scars	0.26 $\pm$ 0.10	1.39 $\pm$ 0.65	1.73 $\pm$ 0.44*	2.69 $\pm$ 0.49***
Misshapen gills	0.04 $\pm$ 0.03	0	0.20 $\pm$ 0.14	0.06 $\pm$ 0.04
Reduced gills	0.35 $\pm$ 0.10	0.52 $\pm$ 0.20	0.73 $\pm$ 0.30	0.60 $\pm$ 0.13
Missing gills	1.00 $\pm$ 0.33	0.74 $\pm$ 0.45	0.87 $\pm$ 0.29	0.54 $\pm$ 0.17
No. of nymphs	49	23	15	67

Table 3. Comparisons of mean ( $\pm 1$  SE) numbers of scars and deformed, reduced or missing gills between sides of 35 parasitized *Thraulodes* sp. nymphs unoccupied and occupied by 3rd or 4th instar larvae or pupae of *Nanocladius* (*P.*) sp. 2. Statistically significant differences are for Wilcoxon sign-ranked nonparametric comparisons of distributions for the parasitized sides versus distributions for the unparasitized sides of nymphs (\* =  $P < 0.05$ , \*\*\* =  $P < 0.001$ ).

Type of injury or deformation	Side of nymph without larva or pupa	Side of nymph with larva or pupa
Scars	0.20 $\pm$ 0.11	2.28 $\pm$ 0.50***
Misshapen gills	0	0.06 $\pm$ 0.04
Reduced gills	0.14 $\pm$ 0.08	0.60 $\pm$ 0.17*
Missing gills	0.29 $\pm$ 0.11	0.23 $\pm$ 0.12

In addition to scarring, larval activity may cause deformation and size reduction of the gills (Fig. 5). Instances of gill size reduction and deformation tended to occur more often on parasitized nymphs, particularly those with later instar larvae and pupae (Table 2). However, host nymphs tended to have fewer missing gills than unparasitized nymphs. Numbers of misshapen gills were slightly higher and reduced gills and scarring were significantly higher on the sides of parasitized nymphs colonized by 3rd or 4th instar larvae or pupae than on the unoccupied sides of these hosts (Table 3). In two instances, misshapen and reduced gills were incorporated into puparia. Asymmetrical wing pad development was observed for two parasitized and one unparasitized nymph. Several host mayflies of midge larvae and pupae reared to adulthood were able to molt to the subimago stage but the percent eclosion success for hosts and unparasitized nymphs was not recorded.

Dry weights of the four mature 4th instar larvae ranged from 69–206  $\mu$ g (mean  $\pm 1$  SE: 150  $\pm$  29  $\mu$ g) and were equal to 8.6–23.3% (mean  $\pm 1$  SE: 13.8  $\pm$  3.4%) of the dry weight of their hosts. Weights for two female pupae with larval exuviae were 155  $\mu$ g and 162  $\mu$ g, or 11.7% and 20.0% of their respective host's weight.

Immature stages of *N. (P.)* sp. 2 were significantly aggregated across the series of nymphs collected (Table 4). Within each life stage, 1st instars were randomly distributed yet showed some aggregation while 2nd instars were significantly aggregated. Indices of dispersion for 3rd and 4th instar larvae and pupae shift towards values indicative of random distributions. When their distributions were pooled, these

Table 4. Results of Chi-square tests with normal approximation on the indices of dispersion of all life stages combined, individual life stages and grouped life stages of *Nanocladius* (*P.*) sp. 2 on the series of 116 *Thraulodes* sp. nymphs collected on July 15, 1992.

Life stage	No. larvae or pupae	No. larvae or pupae per nymph	Index of dispersion	$\chi^2$	$z$	$P^1$
All	115	0.991	1.377	158.35	2.663	0.0077
1st instars	56	0.483	1.205	138.71	1.523	0.1276
2nd instars	24	0.207	1.473	169.33	3.270	0.0011
3rd instars	12	0.103	0.913	104.00	-0.711	0.4774
4th instars	20	0.172	0.837	96.00	-1.276	0.2018
Pupae	3	0.026	0.962	113.00	-0.099	0.9208
1st & 2nd instars	80	0.69	1.512	163.60	2.956	0.0031
3rds, 4ths & pupae	35	0.302	0.704	81.00	-2.405	0.0162

<sup>1</sup> =  $P$  calculated for a two-tailed test; d.f. = 115.

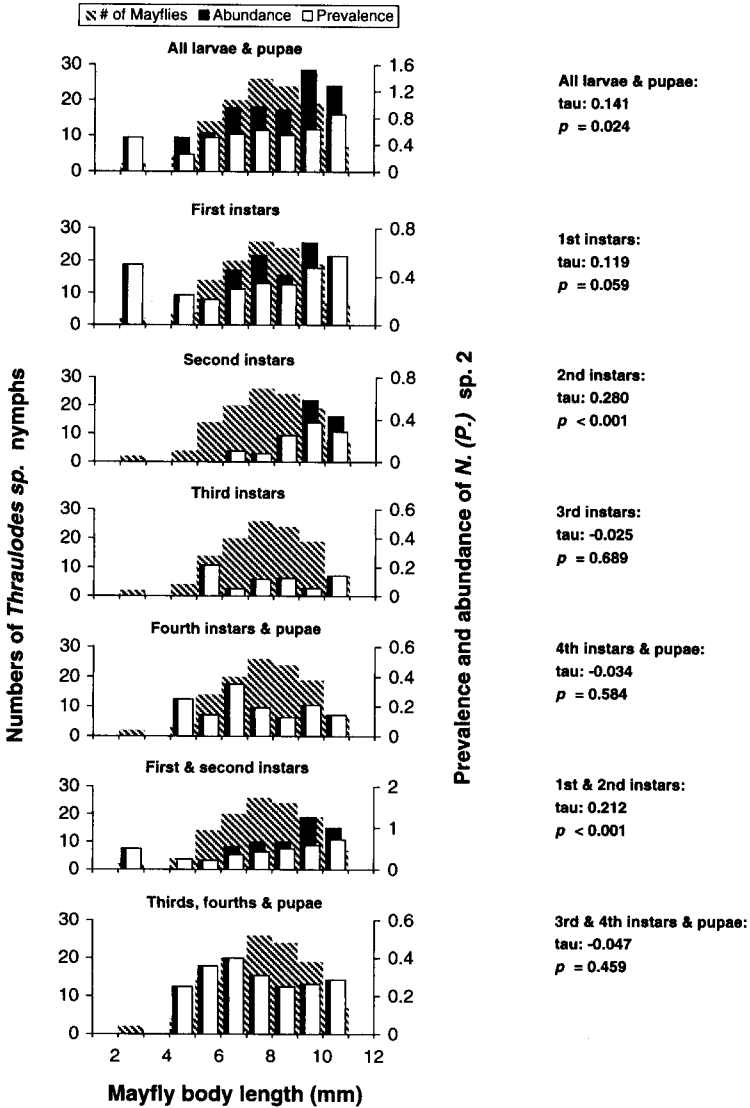


Fig. 7. The prevalence (frequency of nymphs which are parasitized) and abundance (numbers of parasitic larvae per nymph) of different life stages and groupings of life stages of *Nanocladius* (*P.*) sp. 2 across body length categories of *Thraulodes* sp. nymphs. A frequency distribution of *Thraulodes* nymph body lengths is shown as the striped histogram in the background. Kendall's tau and *P* values for the relationship between numbers of larvae per individual nymph and individual nymph body length are given to the right of each chart.

latter stages were significantly uniformly dispersed across nymphs with, at most, only one individual on a nymph. However, six out of 15 hosts with pupae collected earlier for rearing had an additional 3rd instar larva, and in one case, two 3rd instar larvae. None of these pupae, though, shared a nymph with a 4th instar larva nor were two 4th instar larvae found together on a host.

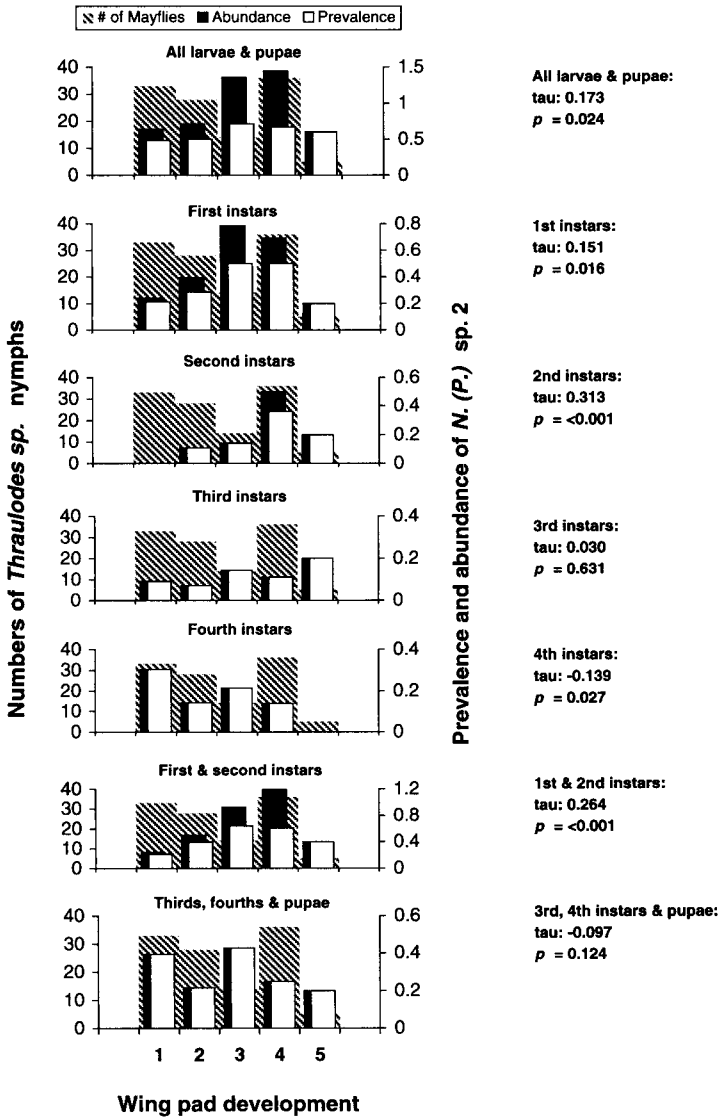


Fig. 8. The prevalence (frequency of nymphs which are parasitized) and abundance (numbers of parasitic larvae per nymph) of different life stages and groupings of life stages of *Nanocladius (P.) sp. 2* across wing pad development categories of *Thraulodes sp.* nymphs. A frequency distribution of *Thraulodes* nymph wing pad development is shown as the striped histogram in the background. Kendall's tau and *P* values for the relationship between numbers of larvae per individual nymph and individual nymph wing pad category are given to the right of each chart.

Abundance of all *N. (P.) sp. 2* life stages combined on individual nymphs increased significantly with increasing nymph body length (Kendall's tau: 0.141, *P* = 0.024) and wing pad development (Kendall's tau: 0.173, *P* = 0.006). When abundance patterns were examined for each instar the overall positive relationship for all life stages appeared to be due to 1st and 2nd instar larvae which comprised 69.6% of all life



stages collected (Figs. 7, 8). Abundances of later instars on individual nymphs showed no relationship with nymph size but tended to be higher on immature nymphs (Kendall's tau for 3rd and 4th instar larvae and pupae combined:  $-0.097$ ,  $P = 0.124$ ; for 4th instar larvae and pupae combined:  $-0.068$ ,  $P = 0.032$ ). These different patterns for early and later life stages might suggest early instar midges may be displaced by or avoid nymphs with older life stages. However, nymphs with 3rd instar or older life stages harbored significantly more 1st and 2nd instar larvae than nymphs without older midge life stages (0.97 versus 0.57 1st and 2nd instars/host;  $z = -2.03$ , two-tail  $P = 0.042$ ).

#### Discussion

*Nanocladius* (*P.*) sp. 2 larvae attached more frequently to *Thraulodes* sp. nymphs but were not necessarily restricted to this genus and were observed attached to other mayfly genera within and outside the Leptophlebiidae. Svensson (1979) and Bottorff and Knight (1987) suggested that midges associated with uni- and multivoltine host species utilize a larger range of host species than midges associated with semi- or mero-voltine species as a result of the need for 1st instar larvae to find suitably sized hosts after hatching. For example, Wiens et al. (1975) reported *Symbiocladius equitans* to be bivoltine, with successive generations selecting alternating host species on the basis of their availability when larvae emerged from eggs. The extent to which *N. (P.)* sp. 2 selects, and can exploit *Thraulodes* sp. nymphs year round or whether *N. (P.)* sp. 2 exhibits differential use of hosts based on their availability to newly hatched larvae requires further study.

*Nanocladius* (*P.*) sp. 2 larvae do not show a marked tendency to occupy certain positions on nymphs until they reach the 3rd instar. Even 4th instars and pupae exhibited a degree of variability in positioning. Strong site preferences in symbiotic Chironomidae have been cited as evidence of significant adaptation by the midge to their host (Steffan, 1967b; Jacobsen, 1998). On the other hand, site selection by some symbiotic species may simply represent instances where the physical features of a given locale on the host best coincide with microhabitat requirements of the larva or pupa. For *N. (P.)* sp. 2, site selection by later instar larvae is likely constrained by their great size relative to the host, the low, flat hydrodynamic profile of *Thraulodes* sp. nymphs, meeting oxygen requirements and reducing the risk of dislodgement.

Until recently, larvae of *Nanocladius* (*Plecopteracoluthus*) spp. were all thought to be phoretically or commensally associated with their hosts (e.g., Cranston et al., 1989; Tokeshi, 1995), perhaps because the first species described, *N. (P.) downesi* (Steffan), is a commensal. However, most species that I am aware of are actually parasitic. Aside from *N. (P.)* sp. 2, other parasitic species include *N. (P.) branchicolus* Sæther on Perlidae (Plecoptera), *N. (P.)* sp. 1 on *Ephemera guttulata* Pictet (Ephemeroptera: Ephemeridae) (Jacobsen, 1995), *N. (P.)* sp. 3 on *Pteronarcys* spp. (Plecoptera: Pteronarcyidae) (= *N. (P.)* undescribed sp., nr. *branchicolus* of Giberson et al., 1996) and *N. (P.)* sp. on *Kamimuria* (Plecoptera: Perlidae) in Nepal (Roback and Coffman, 1987). The morphological similarity of *N. (P.)* sp. 2 larvae to those of *N. (P.) bubrachiatus* Epler (Epler, 1986), particularly the presence of large dorsolateral fulcrum arms on the prementohypopharyngeal complex, suggests that *N. (P.) bubrachiatus* may be parasitic as well. Commensal or phoretic species of *Nanocladius* (*Plecopteracoluthus*) besides *N. (P.) downesi* include *N. (P.) asiaticus* Hayashi

on Asian Megaloptera (Hayashi, 1998) and *N. (P.)* sp. 5 on *Nigronia serricornis* Say (Megaloptera: Corydalidae), *Argia* sp. (Odonata: Coenagrionidae), and *Acroneuria* sp. (Plecoptera: Perlidae) (Pennuto, 1997, 1998).

The association of only one parasitic chironomid species, *Symbiocladius rhithrogenae* (Zavrel), has been extensively investigated up to the present time (see Codreanu, 1939). *S. rhithrogenae* is a large ectoparasitoid on developing nymphs (i.e., a 'koinobiont' parasitoid of Askew and Shaw, 1986; Mackauer and Sequeira, 1993) of *Rhithrogena* and *Heptagenia* (Ephemeroptera: Heptageniidae). Wet weights of mature larvae and pupae are 15–40% of the wet weights of their host nymphs. The size and maturity of the host mayfly nymph greatly affects *S. rhithrogenae* larval development time and adult size. Larvae that colonize small nymphs do not begin to grow until the nymph reaches about 4.0–4.5 mm in body length. In turn, developing larvae retard and, in most cases, eventually halt host development and the maximum sizes achieved by parasitized final instar nymphs are significantly less than those of unparasitized nymphs (Codreanu, 1939; see also Hynes, 1976). Superparasitism by 1st instar larvae occurs (Codreanu, 1939; Wiens et al., 1975), but only one larva will successfully develop on a host. This suggests some form of intraspecific interaction, and most likely territoriality, helps to regulate population size in *S. rhithrogenae*.

Though not as large as *S. rhithrogenae*, *N. (P.)* sp. 2 also attain considerable size compared to that of their host (mature larval and pupal dry weights were 8–24% of the dry weight of the host). Parasitized mayfly nymphs are capable of metamorphosis to adulthood. However, the large size of mature *N. (P.)* sp. 2 larvae implies their impact on nymphs is likely to be substantial and may produce effects similar to those seen in other ectoparasitic arthropod-aquatic insect associations. These effects include increased mortality, reduced fecundity, delayed development, and asymmetrical morphological development (Lanciani, 1975; Smith, 1988; Bonn et al., 1996). Gill damage, deformation and immobilization from larval feeding and tube-building activity may impose a degree of respiratory stress on infected nymphs. Further work is needed to determine to what extent parasitism by *N. (P.)* sp. 2 affects their host's fitness.

Parasites typically exhibit aggregated distributions across host populations as a result of heterogeneity in host exposure and resistance to infestation (Anderson and May, 1978) and, in most cases, the relatively small spatial and nutritional requirements of the parasite relative to the availability of these resources provided by their host. Occasionally, random or uniform distributions can occur in situations where resources (usually space) for parasites are limited and/or significant parasite-induced host mortality occurs (Smith, 1988).

The large size of *N. (P.)* sp. 2 relative to that of *Thraulodes* sp. nymphs also suggests resources provided by nymphs are limited and intraspecific competition among *N. (P.)* sp. 2 larvae for hemolymph or space is potentially high. Dispersion of *N. (P.)* sp. 2 across nymphs changed from a contagious pattern to a random pattern as larvae matured (or a uniform pattern if 3rd and 4th instars and pupae are pooled) and may reflect increased intraspecific competition for resources in older instars rather than mortality of high intensity infestations. The change in dispersion occurs in the 3rd instar and coincides with changes in attachment site preferences in which larvae begin to assume the longitudinal orientation on the posterior thorax and abdomen

that is maintained until emergence. Intraspecific interactions such as territoriality might be expected to occur at this stage and in 4th instar larvae due to their increased size. In addition, larvae exhibit considerable vagility such that changing hosts may be a viable option for stressed or displaced larvae, an option which is generally not available to helminth parasites or water mites on insects. Larval movement to escape intraspecific competition may also reduce the effect that the parasite population has on the host population and serve to stabilize their association.

Similar changes in variance to mean ratios with larval development have been observed in other symbiotic chironomids. Among parasitic species, *Symbiocladius rhithrogenae* achieve a maximum uniform distribution (one larva per host) through apparent territoriality during the 1st instar (Codreanu, 1939) while *Nanocladius* (*P.*) sp. 1 associated with *Ephemera guttulata* reach this uniformity by the 2nd or 3rd instar (Jacobsen, 1995, 1998). Declines in variance to mean ratios also occur as larvae mature for infestations of *N. (P.)* sp. 3 on *Pteronarcys proteus* Newman (Jacobsen, unpubl. data). Movement of larvae between hosts has been reported or suspected to occur for all three species (Codreanu, 1939; Giberson et al., 1996; Jacobsen, 1998 and unpubl. data). Commensal species such as *N. (P.) downesi* on *Acroneuria abnormis* (Newman) and *Epoicocladius* sp. 3 on *Ephemera guttulata* also show uniform distributions on their host populations and evidence of territorial interaction in the selection of attachment sites on their hosts (Bottorff and Knight, 1987; Jacobsen, 1998). On the other hand, mortality of heavily infested hosts, which comprise only a small fraction of the total nymph population, could shift variance to mean ratios from aggregation toward uniformity. Closely monitored rearing is necessary to determine whether shifts from aggregated to uniform distributions with increasing larval maturity represent the outcome of intraspecific interactions between larvae or parasite-induced mortality.

The life stage structure of *N. (P.)* sp. 2 on the series of *Thraulodes* sp. nymphs collected suggests the population was not synchronized at that time, but was likely comprised of two overlapping generations. Fourth instar larvae and pupae, which probably represented the remaining individuals of an earlier cohort, tended to be more prevalent on immature nymphs whereas 1st and 2nd instars of a subsequent generation were most numerous on large mature nymphs and nymphs harboring later instar larvae. The higher prevalence of 4th instar larvae and pupae on less mature nymphs suggests the host or parasite may delay the development of the other, much like what has been observed for *Symbiocladius rhithrogenae* associated with Heptageniidae (Codreanu, 1939). Higher numbers of early instar larvae on large nymphs and nymphs harboring later life stages of *N. (P.)* sp. 2 may reflect both their greater attractiveness to colonizing larvae by virtue of their size (Tokeshi, 1986) and the greater retentiveness of these nymphs which presumably molt less frequently than small and unparasitized nymphs. However, the extent to which larval movement between nymphs might influence larval distributions across host size and development classes also needs to be examined.

Since the data presented here represents only one sampling date, the hypotheses offered are quite speculative and should be regarded as tentative until a more extensive investigation can be undertaken. A year-round sampling program as well as monitored rearing and manipulative experimentation in microcosms is needed to better resolve the life history of *N. (P.)* sp. 2 and its associations with ephemeropteran nymphs.

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