Notes on Empididae (5), Bergenstammia pulla sp.n.

by

F. VAILLANT (Montbonnot) and R. WAGNER (Schlitz)

In 1964 Vaillant reported the occurence of *Bergenstammia nudipes* (Loew) in the West Alpa. The species was originally described by Loew (1858) from "Ober Italien". Several populations from the eastern Alps were inspected and found to be identical with the type material in size and shape of the inner appendage of the gonostyle, but very distinct from the West Alpine specimens. This justifies the description of a new species:

Bergenstammia pulla sp.n.

Material: holotype 3, France, Col du Galibier, 26.VIII.1981, paratype 3, together with holotype, both in coll. R. Wagner at the Limnologische Flußstation Schlitz.

Description: Head dark brown with a single row of 7-9 post-ocular bristles. Thorax brown. Pronotum with a pair of long bristles, mesonotum with 2 rows of 14-16 dorsocentral bristles. Hind margin of scutellum with 6 bristles. Legs dark brown, front femora distally at the inner side with 3 prominent spines. Wing with an elongate stigma, wing length 6.0-6.3 mm.

Genitalia: hypandrium and aedeagus without specific features. Gonostyle approximately triangular in lateral view. The lateral appendage of the dististyle is elongate triangular, the inner appendage distally bipartite, the front part longer than the rear (Figs 1-2).

Relations: B. nudlpes (Loew) (Figs 3-4) is the closest relative of the new species. The main differences are the proportions of the lateral appendage of the dististyle, being more elongate in the new species, and in the extension of the front part of the inner appendage, being bigger in B. nudlpes.

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Figs. 1-2. Bergenstammia pulla sp.n., Figs 3-4: B. nudipes (Loew). 1, 3 lateral views of male genitalia; 2, 4 styles and cerci, inner lateral views. 0165-0424/89/1104-0201 \$ 3.00 • Swets & Zeitlinger

Behaviors of the Midge, *Cricotopus* (Diptera: Chironomidae) Related to Mutualism with *Nostoc parmelioides* (Cyanobacteria)

by

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Behaviors of Cricotopus nostocicola (Wirth) related to its mutualistic interaction with Nostoc parmelioides Kutzing were explored. The late instars and pupae probably always remain inside their Nostoc colony until emergence. Laboratory observations of 4th instar larvae removed from Nostoc colonies showed that only 65% of the midges were able to re-enter their colony after 20 hours in still water, although placed less than 5 cm away, and midge larvae could move at only 8.9 mm h⁻¹ in still water. This suggests larvae would not be able to survive if they left their colony. Midge larvae attached their colonies tightly to rocks and may use this ability to orient their colony with respect to flow. Pupation occurred at the same time host Nostoc colonies formed hormogonia (the dispersal stage of Nostoc), and at the same time many new, uninhabited colonies appeared. The replication of both taxa occurred simultaneously, and may have been beneficial to both.

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INTRODUCTION

The midge larva Cricotopus nostocicola (Wirth) is only found inside the cyanobacterium, Nostoc parmelioides Kutzing from which it emerges as an adult (Brock, 1960). Nostoc colonies containing Cricotopus have been found attached to rocks in streams throughout western North America in addition to Connecticut, West Virginia, Tennessee, and Ohio (Boesel 1980, Ward 1985, Wirth 1957). Nostoc colonies which do not contain the midge are spherical with a diameter usually less than 5 mm. Nostoc colonies which contain Cricotopus larvae are ear shaped with a thickness of up to 5 mm, length to 10 mm and width to 20 mm.

The interaction between the midge larva and *Nostoc* is mutualistic, with the midge gaining nutrition from eating *Nostoc* cells (Ward et al., 1985) and possibly gaining refuge from predators (Brock, 1960). Benefits to the alga are less obvious, but Ward et al. (1985) have shown higher photosynthetic rates for *Nostoc* colonies containing the midge larvae than for colonies without. It has also been suggested that grazing by the midge facilitates nutrient regeneration

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and that the altered morphology could enhance nutrient uptake by Nostoc (Ward et al., 1985).

We were interested in further documenting behavioral interactions with *Nostoc* since Gregory (1983) noted that the interaction between *Cricotopus* and *Nostoc* is one of the few cases from streams of an obligate mutualistic interaction between an aquatic invertebrate and a primary producer.

METHODS

Field observations and most collections were from McRae creek, a 3rd order stream in the H. J. Andrews Experimental Forest in the Cascade Mountains of western Oregon, USA (44°11'N, 122° 14' W). The stream bed is mainly cobble in the collection area. Colonies were attached to rocks within the stream. Collections for larval activity experiments and *Nostoc* cell morphology measurements were taken from Hyalite creek, Gallatin County, Montana, USA (45° 32' N, 111° 0' W).

All behavioral experiments were done with late 3rd or early 4th instar larvae as determined using pigmentation and headcapsule width (Brock, 1960). Detached *Nostoc* colonies or rocks with attached *Nostoc* colonies were transported in stream water. Inhabited colonies could be kept alive for several weeks in an aquarium with circulating water (maximum velocity 10 cm s⁻¹) at 14°C under a 14 hour light, 10 hour dark cycle, under cool white fluorescent lights (99 μ E m⁻² sec⁻¹ as measured by a Biospherical Instruments QSL-100 quantum meter).

Larvae were gently removed from the colonies after the edge of the colony was torn. The larvae were induced to crawl out of the tear by gently pushing on the outside of the *Nostoc* colony immediately above the posterior prolegs of the larva with a pair of forceps. Midge larvae were used for experiments immediately after they were removed from the colonies.

Experiments were conducted to determine if a midge larva prefers its own colony over another. A midge larva was placed in the center of an open 10 cm petri dish midway between its own colony and one inhabited by another larva. The larva was observed after 20 hours to determine whether it located and/or entered either colony. Light versus dark colony location and entry experiments were used to determine if midges were only active in the dark. There were 28 light and 28 dark trials. Rate of movement in midge larvae was measured repeatedly over 5 minute periods in light and dark petri dishes to check if light simply increased *Cricotopus* activity.

To ascertain the mechanism of *Nostoc* attachment, colonies were removed from rocks and allowed 2 days to reattach to coverslips. The colonies were examined with epi-polarized illumination to study structural detail at the point of attachment. *Nostoc* attachment strength was measured in the field on spherical and ear shaped colonies. The force necessary to tear a colony from its rock was measured by attaching a handscale to the colony and pulling the colony of the rock with the handscale. To measure the area of the site of attachment, it was pressed against an ink pad and then pressed against a piece of white paper. The area of the ink spot was then determined by cutting and weighing the spot.

Field data on 77 colonies were collected on 24 February 1987 and 20 March 1987 to determine if *Nostoc* position and current velocity were correlated. Current velocity was measured at the site of colony attachment with a thermistor flow meter (LaBarbera and Vogel, 1976). The current direction and degrees of the colony plane up from the rock surface in the downstream direction were also noted.

Newly hatched adults, which may transport viable *Nostoc* cells, were ground in a sterile 2 ml tissue grinder with 0.5 ml sterile 10% ND cyanobacterial culture medium (Castenholz, 1981) and the resulting suspension was transferred to 75 ml sterile 10% ND medium and incubated for 6 weeks at 23°C under cool white fluorescent lights. This combination of medium, temperature and light supported growth of *N. parmelioides* from single trichomes isolated from McRae Creek colonies which had been ground in a 2 ml tissue grinder.

RESULTS AND DISCUSSION

Larval specificity to Nostoc colonies

Experiments were done with only one of the two species of midge larvae known to inhabit *Nostoc*. Twelve adults were reared, mounted, and identified as *C. nostocicola* by J. Furnish. In addition, of 32 adults reared, none had the light abdomen characteristic of *Cricotopus fuscatus Wirth*, another midge which is known to inhabit *N. parmelioides* (Wirth, 1957).

Co-habitation of a single *Nostoc* colony by two late instar larvae or pupae probably never occurred. In the over 300 colonies collected which contained midge larvae, cohabitation of late instars or pupae was not observed. When two 4th instars were placed in a colony, antagonistic pinching with the mandibles was observed and one or both individuals were dead within 24 hours. The same result occurred all 5 times the experiment was done. Brock (1960) never observed two fourth instar larvae in the same colony, but observed many earlier instars inhabiting a single colony. These data imply intra-specific competition for space or food with *Nostoc* as the sole resource.

Larvae can not easily re-enter a colony once removed and do not appear to distinguish between inhabited and uninhabited colonies. When larvae in the laboratory were given 20 hours to choose between their own and another colony in 25 trials, 6 were at least touching their own colony (4 of those entered their own colony), 7 found neither colony, and 12 found the colony containing another midge (7 entered). The larvae which do locate colonies may use sight. In the light, 63% of larvae were able to locate a colony (n = 53) and only 35% were

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able to do so in the dark (n = 42). This represented a significant improvement in location ability (P < 0.01, chi square test). This did not represent a light induced increase in locomotion. Larvae observed repeatedly for 5 minute periods in the light moved 8.6 mm h⁻¹ (standard deviation 11.5 mm h⁻¹, n = 30), not significantly different (P > 0.25) than the repeated dark rate measurements (6.9 mm h⁻¹, standard deviation 9.5 mm h⁻¹, n = 30). The slow locomotion rates and poor ability to re-enter colonies show that it is unlikely a larva could survive if it ever left its colony.

The ability of Cricotopus larvae to attach Nostoc to rocks

Brock (1960) observed that midge larvae were able to re-attach colonies which had been removed from rocks, and that they sometimes pulled colonies into an upright position before re-attachment. Our re-attachment observations showed a fibrous material is present in the area of reattachment which exhibited birefringence, and may be silk secreted by the larva. Other members of the genus *Cricotopus* are known to spin silk cocoons (Oliver, 1971), and the site of attachment is generally near holes which lead into the midge chamber at the base of the colony, further evidence that *C. nostocicola* attaches colonies with silk.

Morphological observations indicated that *Cricotopus* also changes cellular morphology of *Nostoc*. Mean *Nostoc* cell diameter in an inhabited colony in the area of attachment was $4.75 \,\mu\text{m}$ (std. dev. = $1.26 \,\mu\text{m}$, n = 100) and $5.43 \,\mu\text{m}$ (std. dev. 1.86, n = 100) near the midge channel. In an uninhabited colony mean cell diameter was $4.97 \,\mu\text{m}$ (std. dev. 1.33, n = 100). Pairwise comparisons showed significant differences between all these values (pooled *t* test, P < 0.005). The alteration in cellular morphology may be a result of macroscale changes in morphology (i.e. protruding *Nostoc* has different diffusional characteristics), chemical effects of the larvae, grazing effects of the larvae, or some other variable.

If a detached colony containing a midge larva is placed in a circulating aquarium near a rock, the midge will reattach the colony to the nearest available substrate, either the floor of the aquarium or the bottom of the rock. Midges were never observed to reattach colonies to the tops of rocks which would be better for continued *Nostoc* growth than attachment to the bottoms of rocks. Since colonies were never observed attached to the bottoms of rocks in the field, and the larvae were unable to pull colonies to the tops of rocks under ideal laboratory conditions, it is unlikely that midges in flowing water are able to reattach colonies in areas receiving adequate light for photosynthesis. Therefore, the ability to reattach colonies which have been scoured from rocks under natural circumstances is probably not important to algal survival and may be of limited importance to midge survival.

However, it is likely that colonies are secured to rocks more strongly by midge larvae. Even though the surface area of attachment is not significantly greater in the presence of larva, the force to pull colonies off rocks is significantly higher for colonies which contain a midge than for those which do not (Figure 1). McRae Creek has turbulent flows, and water spates remove a significant portion of the periphyton (Rounick and Gregory, 1981). It is of clear advantage to a midge larva to secure its host colony and reattach it if it is partially torn off. Fourth instar midge larvae are much larger than any uninhabited colonies in McRae Creek and require larger colonies which experience greater drag. Therefore, if larvae are to survive winter spates, they need to increase attachment strength of the colony they inhabit.



Fig. 1. Force per unit area to remove *Nostoc* colonies from rocks and surface area of attachment with and without a midge larva. Error bars represent 1 standard deviation. Open bars represent attachment area, closed bars show attachment strength. Values were significantly different for attachment strength (P < 0.01) but not for attachment area (P < 0.10 pooled *t* test). With midge, n = 11, without midge, n = 13.

Midge larvae may position colonics with respect to flow. Inhabited colonies can either be attached to the rock by the narrow end and protrude from the rock or be attached by the flat side of the oval disk to the surface of the rock. More appressed colonies were found in regions of higher current velocity (regression analysis, P < 0.01, 77 total colonies, 44 of which were appressed). Also, colonies which were attached by the narrow end were more upright in regions of lower current velocity ($P < 0.05 r^2 = 0.43$, n = 77). The correlation between colony placement and current velocity may also be because protruding colonies grow faster than appressed colonies but tend to be torn off in areas of high flow. Even though Brock (1960) noted midge larvae were able to position smaller colonies, it is still uncertain if midge larvae place the colonies with regard to flow, if *Nostoc* grows in response to flow, both, or neither.

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Nostoc dispersal by Cricotopus

Midge behavior which increases Nostoc dispersal upstream or to other new habitat could be of advantage to both the Nostoc and the midge. Freshwater invertebrates have been shown to transport viable algal cells in their guts (Porter, 1976). However, no flasks inoculated with suspensions of newly hatched adults (n = 5) showed Nostoc growth, indicating that emergent adults do not transport viable Nostoc trichomes to a site of oviposition.

Midge pupation was synchronous with a drastic change in *Nostoc* morphology (Brock, 1960). The colony in the region of the pupa became thicker and much softer, with a cotton like appearance (Figure 2). Softening of the tough outer layer of *Nostoc* may make it easier for the midge to emerge. It is not known if pupae or late 4th instar larvae induce this change. External cues which trigger pupation are probably not responsible for this change since *Nostoc* colonies which were less than 1 cm away from colonies with pupae, and colonies which were uninhabited or contained younger instars, never exhibited this change.



Fig. 2. Cross sections of *Nostoc* colonies inhabited by a 4th instar *Cricotopus* larva (A) and a pupa (B). The scale is in 1 mm increments. The midge larva channel is the open space on the edge of the colony. The dark spot in B is a pupa's head. The colonies were attached by the thinner portion at the bottom.

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Replication of new Nostoc colonies may be aided by the morphological change induced by pupation. The trichomes around the midge pupa are short (8 - 10 cells long) and lack heterocysts. They are also able to glide upon agar and thus are motile hormogonia, the dispersal form of Nostoc (Lazaroff, 1973, Robinson and Miller, 1970). In McRae Creek, on 24 February 1987, 6 of 26 colonies (23%)) had pupae or recently emerged midges, and uninhabited colonies were extremely rare. On 20 March 1987, 42% of 51 colonies had pupating or recently emerged midges, and small unoccupied colonies were moderately abundant. By 7 May 1987, many rock surfaces were entirely covered by small uninhabited colonies, and inhabited colonies could not be found. These observations suggested that *Cricotopus* emergence is synchronous with an increase in the number of new colonies. This flush of new colonies may be triggered by an external cue, by *Cricotopus*, or by the lowered velocity in late spring which allows the less tightly attached uninhabited colonies to re-establish position.

Cricotopus nostocicola, as an obligate mutualist of *Nostoc*, displays several behaviors related to this mutualism. Late instars do not leave their colony. *Cricotopus* can aid in *Nostoc* attachment to rocks, and may orient colonies with respect to current. Pupation is synchronous with formation of motile hormogonia in *Nostoc* and with a flush of new, uninhabited colonies, which may increase available habitat for first instar larvae.

These data do not complete the story on midge interactions with Nostoc, and should be viewed with caution, since they are based in part on laboratory experiments. Important questions remain as to mechanical and biochemical aspects of the larval relationship with Nostoc in terms of alteration of morphology and primary production, and how gravid females or newly hatched larvae colonize Nostoc. We hope our study will encourage more interest in this unusual stream mutualism.

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New Data on the Genus *Amphinemura* Ris (Plecoptera: Nemouridae) from the Iberian Peninsula, with a Description of a New Species

by

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PARDO, I.: New Data on the Genus Amphinemura Ris (Plecoptera: Nemouridae) from the Iberian Peninsula, with a Description of a New Species. Aquatic Insects, Vol. 11 (1989), No. 4, pp. 209-216.

Amphinemura hibernatarii sp.n. from Galicia, Spain, is described and illustrated. Specific status is proposed for the subspecies Amphinemura sulcicollis guadarramensis Aubert, 1952, and a key for the Spanish species of this genus is provided.

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Four taxa belonging to the genus Amphinemura Ris, 1902, have been cited to date from the Iberian Peninsula: A. standfussi (Ris, 1902); A. sulcicollis sulcicollis (Stephens, 1835); A. sulcicollis gundarramensis (Aubert, 1952) and A. triangularis (Ris, 1902) (Sanchez, 1986).

In 1952, Aubert described the subspecies *A. sulcicollis guadarramensis*, from several females, taken in the Sierra de Guadarrama and Sierra de Gredos, which differred from *A. sulcicollis* in their pregenital and subgenital plates, which were more extended and sclerotized (Aubert, 1952, Figs. 9 and 10).

In 1954, the same author raised it to species rank, *A. guadarramensis*, based on the coexistence of both subspecies in the Sierra de Gredos. However in 1963, Aubert returned this species to subspecific status. According to Berthélemy and Whytton da Terra (1980), a definitive choice between specific and subspecific status will not be possible until a more detailed study in the contact area is made. According to Aubert (1956) this important area would comprise the Sil basin River, near Villablino (León).

In 1988 several collections of *Amphinemura* adults were made in the Invernadeiro mountains, Southeast of Galicia (Orense). These included a species new to science, *A. hibernatarii* sp.n. and specimens of the subspecies *A. s.* sulcicollis and *A. s. guadarramensis*. The former of these has not been cited previously in Galicia (Spain). Since both subspecies were found not only in the same geographic area, but also within the same fluvial basin, a detailed morphological study of these samples was carried out. As a result of these studies, specific status for *A. guadarramensis* is proposed.

The descriptive terminology for male and female genitalia follows Baumann (1975).