Zootaxa 2069: 59–68 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Differentiation of larvae and pupae of aquatic genera of Nearctic Hemerodromiinae (Diptera: Empididae)

COLIN A. BRAMMER[1], J. ROBERT HARKRIDER[2] & JOHN F. MACDONALD[3]

[1]*North Carolina Museum of Natural Sciences, Raleigh, North Carolina, 27601 USA. E-mail: flygene@peacemail.com*
[2]*Cadiz, Kentucky 42211 USA. E-mail: jhark@ix.netcom.com*
[3]*Department of Entomology, Purdue University, West Lafayette, Indiana 47907 USA. E-mail: macdonaj@purdue.edu*

Abstract

Larvae and pupae representing four genera of aquatic dance flies, *Chelifera* Macquart, *Hemerodromia* Meigen, *Metachela* Coquillett, and *Neoplasta* Coquillett (Diptera: Empididae: Hemerodromiinae) are differentiated, diagnosed, illustrated, and keyed. Results are based on: limited rearing associations of larvae, pupae, and adults; external morphology of larvae, pupae, and pupal exuviae; and fragment sizes of ribosomal DNA extracted from larvae, pupae and adults of Nearctic Hemerodromiinae. Fragment size of ribosomal DNA is diagnostic at the generic level.

Key words: *Chelifera*, *Hemerodromia*, *Metachela*, *Neoplasta*, pupal exuviae, ribosomal DNA, PCR

Introduction

Adults of species assigned to four Nearctic genera of the subfamily Hemerodromiinae (Diptera: Empididae) are associated with aquatic habitats with immature stages either known or presumed to develop in water, primarily flowing water. The genera involved are *Chelifera* Macquart, *Hemerodromia* Meigen, *Metachela* Coquillett, and *Neoplasta* Coquillett. Adults have been described by Melander (1928), MacDonald (1989, 1994, 1998), and MacDonald and Turner (1993), but inability to differentiate larvae and pupae of all genera has hindered their inclusion in ecological research and in calculations of biotic indices based on diversity of insect taxa in streams.

Larvae of aquatic Hemerodromiinae possess seven pairs of abdominal prolegs, including one pair on the last abdominal segment (Steyskal & Knutson 1981; MacDonald & Harkrider 1999), distinguishing them from larvae of known Clinocerinae (Sinclair 1995; Sinclair & Harkrider 2004) and *Oreogeton* Schiner (Courtney *et al.* 1996) which possess eight pairs of abdominal prolegs, including one pair of the last abdominal segment.

Hemerodromia larvae are the most structurally distinct of the Hemerodomiinae and are well illustrated (McCafferty 1981, Steyskal & Knutson 1981). Larvae of *Metachela* and *Neoplasta* are described, illustrated, and keyed by MacDonald and Harkrider (1999). The first published account of a larva of *Chelifera* (Brocher 1909) pertains to a Palearctic species, but it lacked a detailed illustration and is of no diagnostic value. The first published account of larvae of *Chelifera* accompanied by detailed illustrations are based on two Palearctic species (Vaillant 1965), and appear to be the basis for distinguishing larvae of *Chelifera* in identification keys subsequently developed for the Nearctic region (Steyskal & Knutson 1981; Courtney *et al.* 1996).

Pupae of aquatic Hemerodromiinae are characterized by the presence of a pair of long, lateral processes on each of seven abdominal segments (Figs. 5–8) (Steyskal & Knutson 1981), structures that are lacking on pupae of known aquatic Clinocerinae (Steyskal & Knutson 1981; Sinclair & Harkrider 2004) and *Oreogeton* (Sommerman 1962).

Accepted by B. Sinclair: 19 Mar. 2009; published: 10 Apr. 2009

Hemerodromia pupae are the most structurally distinct of the Hemerodromiinae and are well illustrated (McCafferty 1981; Steyskal & Knutson 1981). Knutson and Flint (1971) described and illustrated two pupae of Neotropical *Neoplasta*, but the figures do not show all structures that are used in identification. Pupae of Nearctic *Chelifera* and *Metachela* have not been differentiated and diagnosed. A pupa illustrated and labeled as *Hemerodromia* in Steyskal and Knutson (1981) appears to be a specimen of *Metachela*, based on results reported below.

The present study of immature Hemerodromiinae is based largely on external morphology of larvae, pupae, and pupal exuviae together with variation in ribosomal DNA fragment sizes derived from larvae (except *Hemerodromia*), pupae, and adults. The combination of results allowed differentiation, diagnosis, and illustration of pupae of *Chelifera* and *Metachela* for the first time and facilitated an identification key to genera of pupae of Nearctic Hemerodromiinae. We also include a revised identification key to genera of larvae in which we differentiate those of *Chelifera* and *Metachela*, which previously was not possible (MacDonald & Harkrider 1999).

Materials and methods

The present study is based primarily on mature larvae, pupae, pupal exuviae, and adults collected at one Utah (USA) study site in 2002 located near the Blacksmith Fork River in Cache County (elevation approximately 1, 800 m) near 41.41 N, - 111.34 W. It is supported by examination of specimens collected at a California (USA) study site in San Bernardino County (elevation approximately 1, 300 m) near 34.14 N, - 117.39 W (Harkrider 2000a) and at an earlier Utah (USA) study site on Pleasant Creek in Capitol Reef National Park (elevation from 2, 120 to 1, 630 m) near 38.18 N, - 111.18 W (MacDonald & Harkrider 1999). The more recent Utah site is approximately 15 km upstream from the mouth of the Left-Hand Fork of the Blacksmith Fork River in Cache County, southeast of Logan, Utah. Adults were collected by sweeping riparian vegetation along a feeder spring (Lime Springs) that runs into the Blacksmith Fork River. Larvae, pupae, and pupal exuviae were collected from depositional substrates in Lime Springs using a kick net on 19 May, 31 May, 30 June, and 31 June in 2002, and were preserved directly in 95% ethanol.

The following accounts provide indirect evidence of the species composition of larvae and pupae in our study streams. Collections of adults along Lime Springs included the following species: *Chelifera caliga* Lavalle, *Metachela collusor* (Melander), *Neoplasta hansoni* MacDonald and Turner, *N. octoterga* MacDonald and Turner, *N. paramegorchis* MacDonald and Turner, and *N. scapularis* (Loew). Collections of adults made along Pleasant Creek both within and upstream from Capitol Reef National Park in Utah included the following species: *Hemerodromia burdicki* MacDonald, *Metachela collusor*, *Neoplasta concava* MacDonald and Turner, *N. hansoni*, *N. octoterga*, *N. paramegorchis*, and *N. scapularis*. Collections of adults made along San Antonio Creek in the San Gabriel Mountains of California included the following species: *Chelifera lovetti* Melander, *C. neangusta* MacDonald, *Metachela albipes* (Walker), *Neoplasta parahebes* MacDonald and Turner, and *N. scapularis*.

Last instar hemerodromine larvae with fully inflated abdominal segments and fully protruded prolegs and crochets are required for identification (MacDonald & Harkrider 1999), with the possible exception of *Hemerodromia* larvae. Although some larvae killed in 70% ethanol are adequate for identification, dropping live specimens into sub-boiling water (ca 85°C) for ca 1 min prior to their placement in 70% ethanol produces much better specimens. Pupae collected in the field and placed directly in 70% ethanol usually are preserved well enough to facilitate identification. However, first killing live specimens in sub-boiling water for ca. a minute is recommended and is especially important in preserving teneral adults inside pupal cases.

DNA was extracted from hemerodromine larvae (except *Hemerodromia*), pupae, and adults preserved in 95% ethanol using a Roche High Pure PCR template preparation kit (Roche Molecular Biochemicals). 25µl polymerase chain reactions (PCR) were prepared, using the primers ITS 5 and RNA 2 (MacDonald & Harkrider 1999). Primer sequences are 5'-3':

ITS 5 – GGAAGTAAAAGTCGTAACAAGG
RNA 2 – CACGAGCCGAGTGATCCACCGCTAAGAGT

The PCR mix for each sample consisted of 0.2mM dNTP, 2.5µL 10x buffer A (containing 15mM $MgCl_2$) (Roche Molecular Biochemicals), 0.2µM of each primer pair for the appropriate gene region, and 0.25µL *Thermus aquaticus* BM (5U/µL) (Roche Molecular Biochemicals). Each tube received 22.5µL of the PCR mix and 2.5µL of 10ng/µL template DNA.

An ISC Genemate thermocycler was programmed for the following procedure: 95° C initial denature for 3 minutes; 35 cycles with the parameters: 94° C denature for 1 minute, 56° C anneal for 1 minute, 72° C extend for 1 minute, with a final extension at 72° C for 10 minutes. PCR products were observed on a standard 1.5% agarose gel.

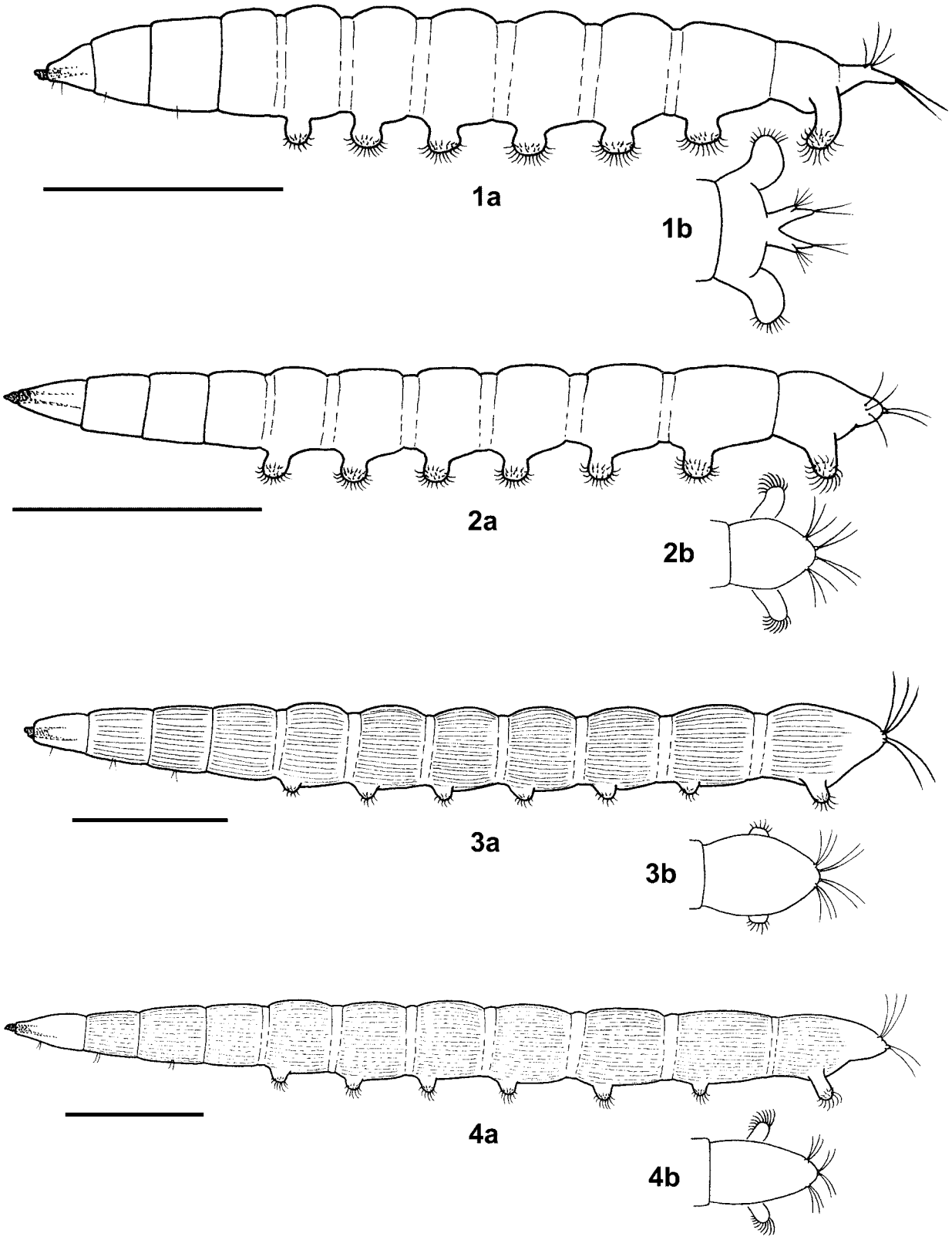
Results

The collection of hundreds of adults of *C. caliga* at the Utah study site in 2002 was a "breakthrough," suggesting productive immature developmental sites in which we focused efforts to collect larvae and pupae. Hemerodromine larvae collected at the Utah study site in 2002 that did not match specimens of *Hemerodromia* or *Neoplasta,* and thus hypothesized to be either *Chelifera* or *Metachela* (see MacDonald & Harkrider 1999), initially were sorted into two distinct forms based on degree of development of terminal setae arising from the last abdominal segment and degree of development of longitudinal cuticular striations on abdominal and thoracic segments. Similarly, pupae and pupal exuviae that did not match specimens of *Hemerodromia* or *Neoplasta,* and thus hypothesized to be either *Chelifera* or *Metachela,* initially were sorted into two distinct forms based on degree of development of spines located on the last and next to last abdominal segments. More detailed examination of pupae subsequently revealed the presence of a teneral male of *C. caliga* inside several pupal cases on which there was greater development of spines on the last and next to last abdominal segments, thus allowing identification of *Chelifera* pupae, based on this one species.

DNA amplification using the primers for the rDNA ITS 1 produced fragments of distinctly different sizes between the two unidentified forms of larvae and pupae (Fig. 9). The rDNA ITS 1 fragments from larvae with relatively greater development of terminal abdominal setae and stronger longitudinal cuticular striations matched those from adult *C. caliga*. The rDNA ITS 1 fragments from larvae with weaker development of terminal abdominal setae and less prominent longitudinal cuticular striations matched those from adult *M. collusor.* Similarly, the rDNA ITS 1 fragments from pupae with relatively greater development of spines on the last and next to last abdominal segments matched those from adult *C. caliga.* The rDNA ITS 1 fragments from pupae with relatively weaker development of spines on the last and next to last abdominal segments matched those from adult *M. collusor.*

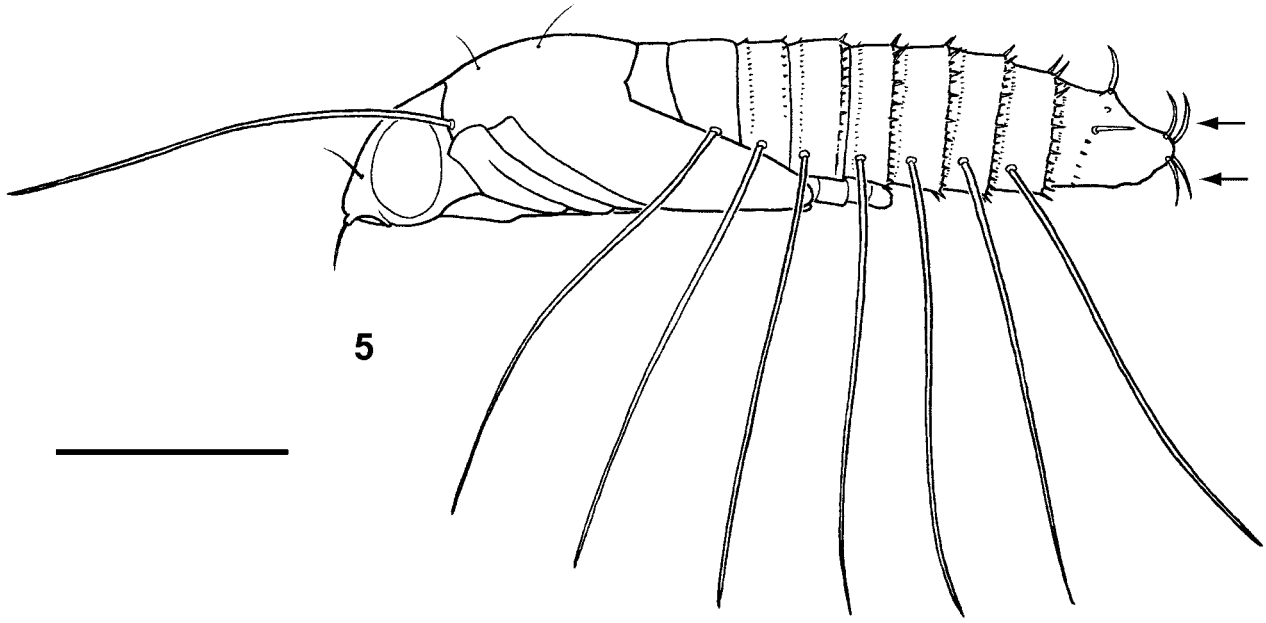
Differentiation of Hemerodromiinae larvae (Figs. 1–4). *Hemerodromia*: Well-preserved larvae are distinct from those of *Chelifera*, *Metachela*, and *Neoplasta*. The most prominent features of *Hemerodromia* larvae are two elongate, setae-bearing lobes that arise from the apex of the abdomen (Figs. 1a, 1b). Similar lobes are lacking on larvae of the other genera. In addition, the length of the terminal pair of abdominal prolegs of *Hemerodromia* larvae is at least twice that of the preceding six pairs, in contrast to all prolegs being of sub-equal length on larvae of the other genera.

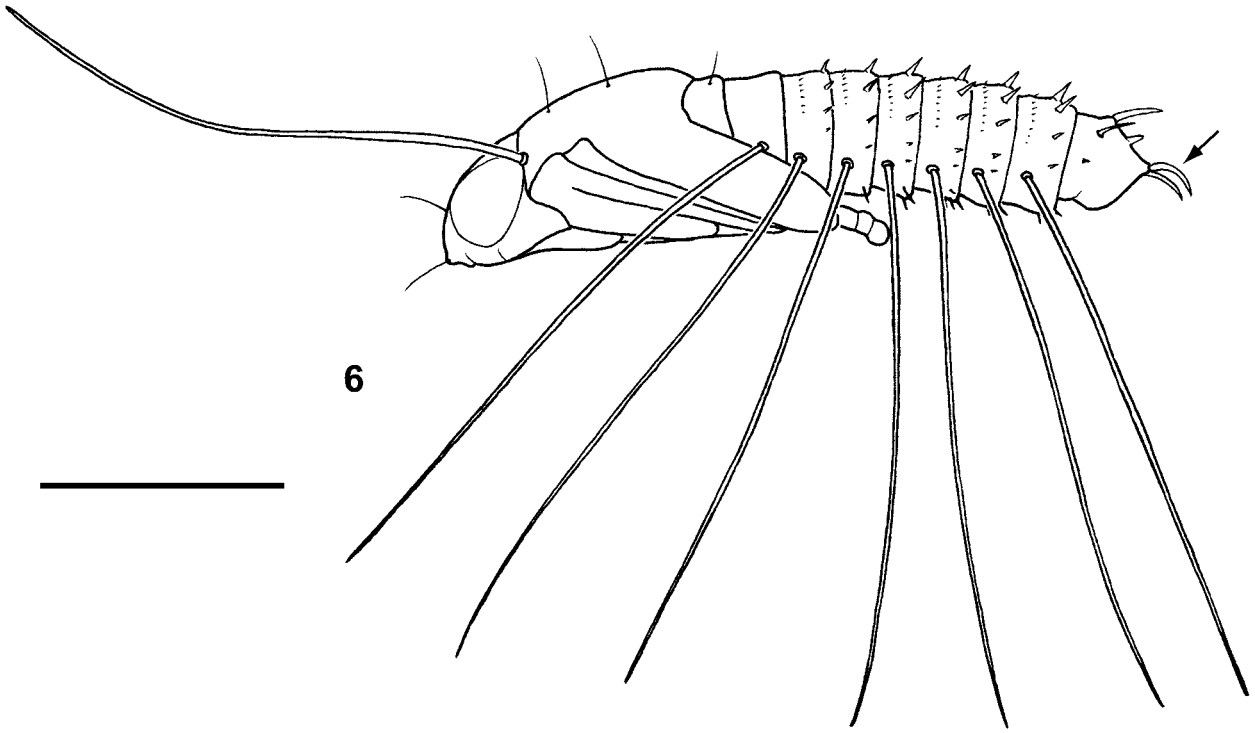
Neoplasta: Larvae are best differentiated from larvae of *Chelifera* and *Metachela* by making measurements of body length and body diameter together with proleg length and diameter, followed by calculating the following ratios (MacDonald & Harkrider 1999). Last instar larvae with fully inflated abdominal segments and fully protruded prolegs and crochets are required. Measurements and calculations based on our four best *Neoplasta* larvae include: body length 4.5 mm, body diameter 0.5 mm, ratio of body length to diameter 9.2; proleg length 0.2 mm, proleg diameter 0.15, ratio of proleg length to length of body segment 0.4; ratio of proleg diameter to length of body segment 0.3. However, separation of larvae is possible



FIGURES 1–4. Hemerodromiinae larvae. **1a.** *Hemerodromia* sp., lateral view (cuticular striations very weak, not included). **1b.** *Hemerodromia* sp., dorsal view of terminal abdominal segment. **2a.** *Neoplasta* sp., lateral view (cuticular striations similar to *Metachela*). **2b.** *Neoplasta* sp., dorsal view of terminal abdominal segment. **3a.** *Chelifera* sp., lateral view (cuticular striations emphasized). **3b.** *Chelifera* sp., dorsal view of terminal abdominal segment. **4a.** *Metachela* sp., lateral view (cuticular striations emphasized). **4b.** *Metachela* sp., dorsal view of terminal abdominal segment. Scale bars = 1.0 mm.

based on general morphology, especially when specimens are collected concurrently and available for direct comparison. Larvae of *Neoplasta* are distinct in being more robust and in possessing more prominent prolegs and crochets (Fig. 2a). In addition, *Neoplasta* larvae possess a pair of very small, but distinctly divided terminal abdominal processes, each of which bears a pair of setae (Fig. 2b).

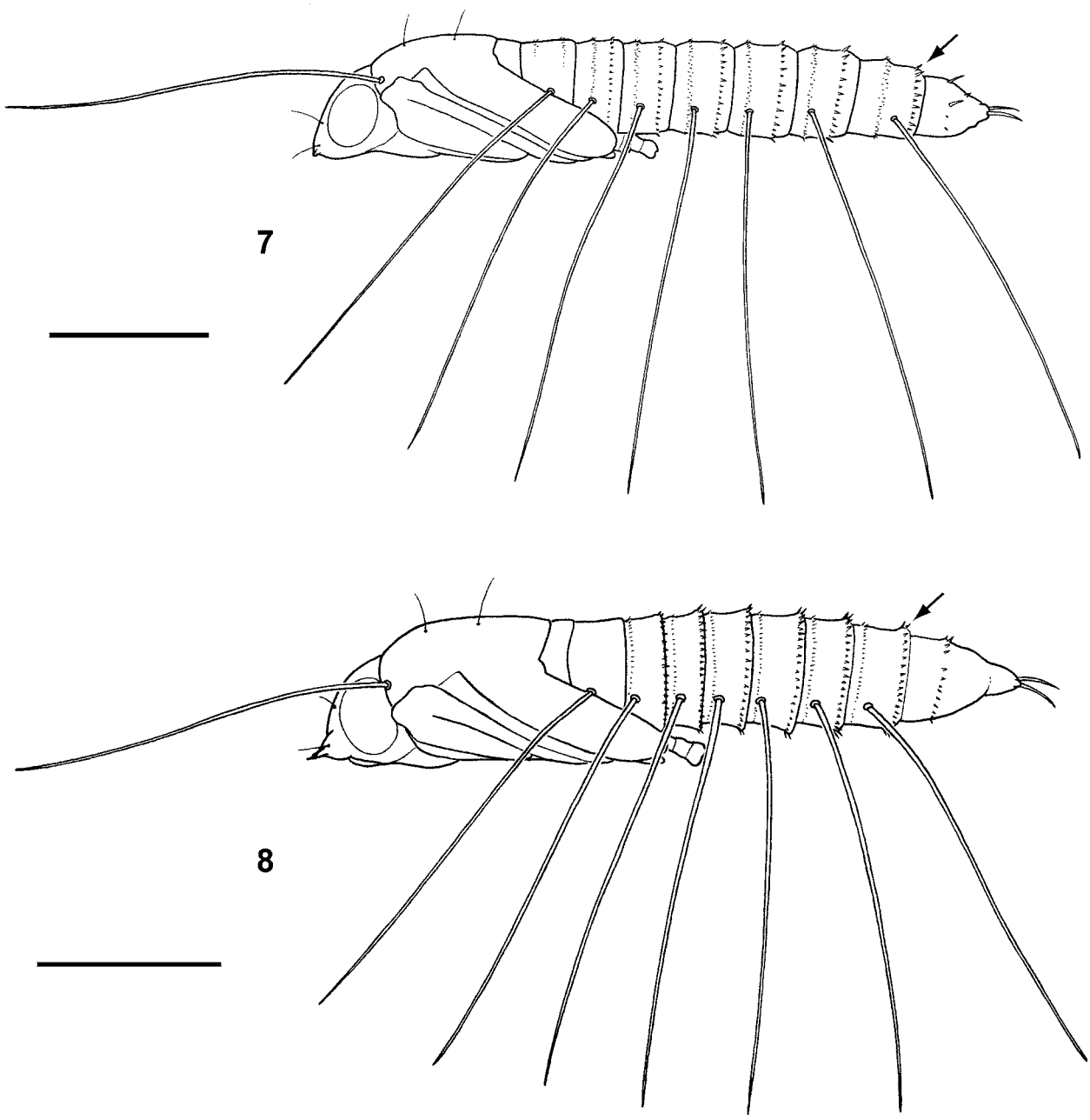




FIGURES 5–6. Hemerodromiinae pupae, lateral view. **5.** *Neoplasta* sp. **6.** *Hemerodromia* sp. Scale bars = 1.0 mm.

Chelifera and *Metachela*: Larvae of *C. caliga* and *M. collusor* are similar in form and size, and share the following measurements based on *M. collusor* larvae (MacDonald & Harkrider 1999). Last instar larvae with fully inflated abdominal segments and fully protruded prolegs and crochets are required. Measurements and calculations based on our four best *Metachela* larvae include: body length 5.5 mm, body diameter 0.45 mm, ratio of body length to diameter 12.2; proleg length 0.15 mm, proleg diameter 0.1 mm, ratio of proleg length

to length of body segment 0.3; ratio of proleg diameter to length of body segment 0.2. Compared to *Neoplasta* larvae, larvae of *Chelifera* and *Metachela* are relatively slender and possess less prominent prolegs and crochets, and the two pairs of terminal abdominal setae do not arise from distinctly divided terminal abdominal processes (Figs. 3b, 4b). We were unable to discover measurable features that differentiate larvae of *C. caliga* and *M. collusor*, but noted consistent differences in degree of development of terminal abdominal setae and longitudinal cuticular striations (Figs. 3a, 4a). Both features are more prominent on *C. caliga* larvae, but direct comparison with known specimens is necessary in order to accurately separate larvae of the two genera.



FIGURES 7–8. Hemerodromiinae pupae, lateral view. **7.** *Chelifera* sp. **8.** *Metachela* sp. Scale bars = 1.0 mm.

Differentiation of Hemerodromiinae pupae and pupal exuviae (Figs. 5–8). *Neoplasta*: Pupae and pupal exuviae are distinct from those of the other hemerodromine genera. The distinguishing features are the existence of two pairs of bristle-like, apical spines on the terminal abdominal segment and relatively thin femur of the fore leg, which is subequal in width to the femur of the middle and hind legs (Fig. 5). Specimens

of *Chelifera*, *Hemerodromia*, and *Metachela* possess a single pair of bristle-like, apical spines on the terminal abdominal segment and the femur of the fore leg is approximately twice the width of the femur of the middle and hind legs (Figs. 6–8).

Hemerodromia: Pupae and pupal exuviae are distinguished from those of *Chelifera* and *Metachela* by more robust form and greater pigmentation of the paired lateral processes of the thorax and abdomen, but side-by-side comparison is often required. Characters employed in the key that distinguish *Hemerodromia* pupae and pupal exuviae include a pair of apical spines on the terminal abdominal segment that project posteroventrally and the more strongly developed mid-dorsal spines on each abdominal segment (Fig. 6).

Chelifera and *Metachela*: Pupae and pupal exuviae possess a pair of apical spines on the terminal abdominal segment that project posterolaterally. The two genera, at least based on *C. caliga* and *M. collusor*, are separated by differences in the development of dorsal spines on each abdominal segment. They are best seen on the next to last segment, with the posterior row of dorsal spines of *C. caliga* consisting of alternating longer and shorter lengths (Fig. 7), while those of *M. collusor* are short and of nearly uniform length (Fig. 8). Dorsal spines of the terminal abdominal segment of *C. caliga* are more prominent than those of *M. collusor*, but the former often are broken or dislodged on specimens.

Key to genera of aquatic larvae of Nearctic Hemerodromiinae

1 Terminal abdominal segment ending in a pair of prominent, divided apical lobes; terminal pair of prolegs at least 2 x longer than preceding 6 pairs (Figs. 1a, 1b) ..... ***Hemerodromia*** Meigen
- Terminal abdominal segment lacking apical lobes; terminal pair of prolegs 1.5–2 x longer than preceding 4 pairs (anterior 2 pairs may be shorter yet) (Figs. 2–4) 2
2 Body robust, prolegs long and robust (Fig. 2a), crochets prominent relative to couplet 2- below (see description for actual measurements and ratios); terminal abdominal segment ending in two very small, but distinctly separated processes, each of which bears a pair of setae (Fig. 2b) ..... ***Neoplasta*** Coquillett
- Body slender, prolegs short and slender (Figs. 3a, 4a), crochets less prominent relative to couplet 2 above (see description for actual measurements and ratios); terminal abdominal segment ending in two pairs of setae that do not arise from distinctly divided processes (Fig. 3b, 4b) 3
3 Setae on terminal abdominal segment and longitudinal cuticular striations strongly developed relative to couplet 3- below (see diagnosis section for explanation and interpretation) (Fig. 3a) ..... ***Chelifera*** Macquart
- Setae on terminal abdominal segment and longitudinal cuticular striations weakly developed relative to couplet 3 above (see diagnosis section for explanation and interpretation) (Fig. 4a) ..... ***Metachela*** Coquillett

Key to genera of aquatic pupae of Nearctic Hemerodromiinae

1 Four bristle-like apical spines on terminal abdominal segment, 2 projecting dorsolaterally and 2 projecting ventrolaterally; femur of fore leg (visible through the pupal cuticle) thin, subequal in width to femur of middle and hind leg (Fig. 5) ..... ***Neoplasta*** Coquillett
- Two bristle-like apical spines on terminal abdominal segment, each projecting either posteroventrally or posterolaterally; femur of fore leg thick, width 2 x that of femur of middle and hind leg (Figs. 6–8) 2
2 Two bristle-like apical spines on terminal abdominal segment projecting posteroventrally; pre-terminal abdominal segments with posterior row of several large spines; dorsal spines of terminal abdominal segment ca. 1/2 length of bristle-like apical spines (Fig. 6)..... ***Hemerodromia*** Meigen
- Two bristle-like apical spines on terminal abdominal segment projecting posterolaterally; pre-terminal abdominal segments with posterior row of numerous small spines; dorsal spines of terminal abdominal segment no more than 1/5 length of bristle-like apical spines (Figs. 7–8)..... 3
3 Pre-terminal abdominal segments with posterior row of spines of nearly alternating longer and shorter lengths (Fig. 7); dorsal spines of terminal abdominal segment ca. 1/5 length of bristle-like apical spines (tend to get broken or dislodged on specimens in alcohol vials) ..... ***Chelifera*** Macquart
- Pre-terminal abdominal segments with posterior row of short spines of uniform length (Fig. 8); dorsal spines of terminal abdominal segment ca. 1/10 length of bristle-like apical spines ..... ***Metachela*** Coquillett

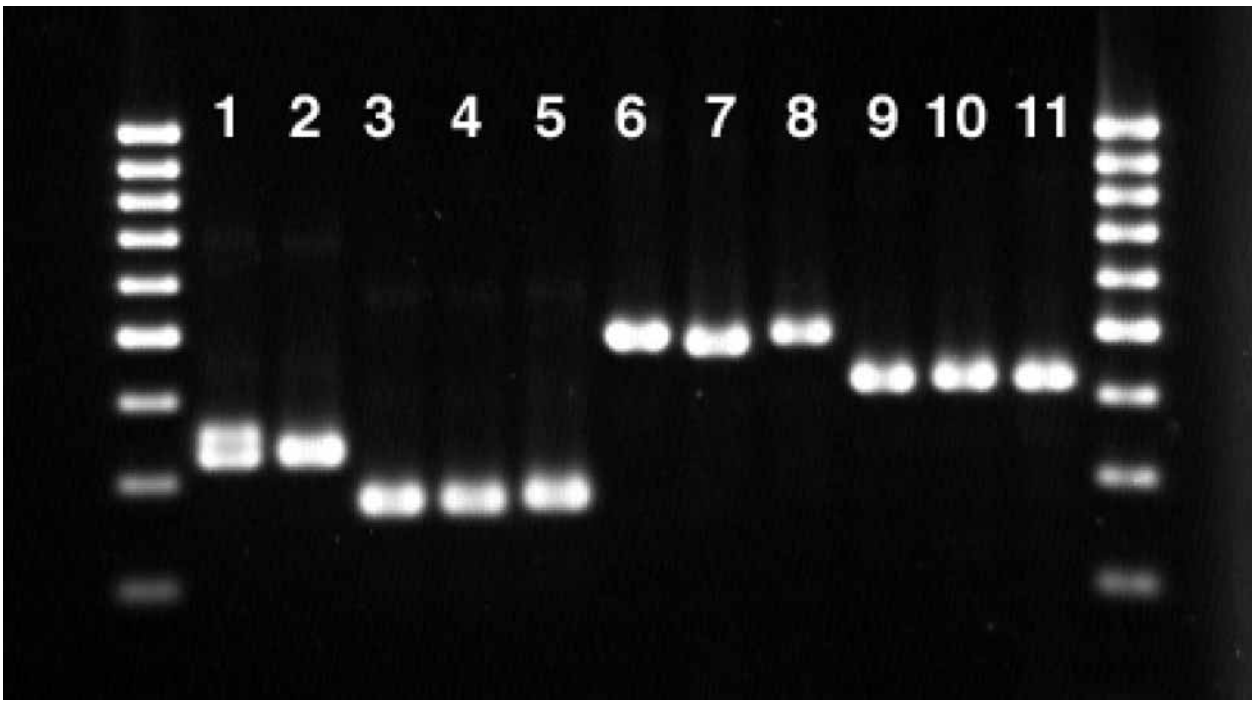


FIGURE 9. DNA fragments amplified from rDNA ITS 1 primers for the following Hemerodromiinae: 1 = *Hemerodromia sp.* pupa, 2 = *Hemerodromia burdicki* adult male, 3 = *Neoplasta sp.* larva, 4 = *Neoplasta sp.* pupa, 5 = *Neoplasta paramegorchis* adult male, 6 = *Metachela sp.* larva, 7 = *Metachela sp.* pupa, 8 = *Metachela collusor* adult male, 9 = *Chelifera sp.* larva, 10 = *Chelifera sp.* pupa, 11 = *Chelifera caliga* adult male. Electrophoretic gel is 1.5% agarose with 100 base pair markers. For reference, numbers 3-5 = ca. 300 base pairs and numbers 6-8 = ca. 500 base pairs.

Natural history notes

Merritt and Cummins (1996, p. 541) provide a table that summarizes developmental sites and includes aspects of the biology of larvae of *Chelifera*, *Hemerodromia*, *Metachela*, and *Neoplasta*, together with an extensive list of references. Based on this table, it appears larvae of most aquatic hemerodromine species develop in lotic habitats, but those of a few species have been associated with lentic habitats.

Most collections of immature hemerodromines have been made in flowing water with the aid of kick nets, mostly in depositional substrates among cobble of various sizes. Larvae also have been collected in moss at and just above water level. Additional collections include pupal cases of a South American species of *Neoplasta* found inside cocoons of caddisflies (Trichoptera) (Knutson & Flint 1971). Larvae of Nearctic *Neoplasta* have been collected inside tubes of midges (Diptera: Chironomidae) picked off of cobble removed from a California stream (Harkrider 2000b). Indirect evidence of developmental sites comes from the collection of adult hemerodromines in emergence traps placed directly over lotic and lentic habitats in Canada (Harper 1980; Landry & Harper 1985).

Observations made in the field summarized in Merritt and Cummins (1996, p. 541) revealed that larvae of several hemerodromine species are predacious, with the most commonly mentioned prey being larvae of black flies (Diptera: Simuliidae). Laboratory studies by Harkrider (2000b) documented the predatory behavior of *Neoplasta* larvae on midge larvae (Diptera: Chironomidae).

Chelifera: Larvae of several species have been reported from fast-flowing streams in Europe (e. g., Brindle 1969, Vaillant 1965). There is indirect evidence that larvae of a Nearctic species, *C. palloris*, develop in lentic habitats, based on collection of adults in emergence traps placed in a bog (Landry & Harper 1985). Using kick nets, we collected large numbers of larvae and pupae of *C. caliga* in the Blacksmith Fork River

southeast of Logan, UT, USA. The substrate consisted almost entirely of small pieces of flat, shale-like cobble that was dislodged during relatively deep kicking.

Hemerodromia: Most collections of larvae have been made using kick nets in lotic habitats in a wide range of currents or by picking them out of mosses growing on stream cobble (Merritt & Cummins 1996, p. 541). Surprisingly, despite extensive use of kick nets in several streams in the western USA, we have collected very few larvae and pupae, including Pleasant Creek in Utah, along which hundreds of *H. burdicki* adults were collected in 1994 and 1995. Adults of this species are relatively small and it is possible larvae and pupae passed through the mesh of kick nets.

Several field observations have revealed that *Hemerodromia* larvae prey on black fly larvae, usually associated with moss growing on cobble (e. g., Vaillant 1953). There is additional suggestion of predation by *Hemerodromia* larvae based on discoveries by Harkrider (2000b). Larvae belonging to the *H. empiformis* complex were collected inside tubes built by a midge (Diptera: Chironomidae; *Rheotanytarusus*) in three different streams in southern California. Also, last instar larvae and pupae of *H. brevifrons* Melander were found inside black fly cocoons removed from rocky substrate in Walnut Creek, Los Angeles County (Harkrider, unpubl. obs.).

Metachela: Numerous larvae and pupae were collected in kick net samples made in Pleasant Creek in Utah, most commonly in substrate consisting of gravel to small cobble. Surprisingly, only a small number of *Metachela* larvae and pupae were collected together with those of *C. caliga* in the Blacksmith Fork River in Utah along which large numbers of adults of both species were collected concurrently when sweeping streamside foliage.

Neoplasta: The first account of *Neoplasta* immatures (Knutson & Flint 1971) was based on intact pupae and larval exuviae found inside pupal cocoons of a species of *Cailloma* (Trichoptera: Rhyacophiloidea, Hydrobiosidae) collected near Santiago, Chile. The caddisfly cocoons contained remnants of the host pupa, suggesting that *Neoplasta* larvae were predators. Field and lab research in southern California by Harkrider (2000b) revealed that *Neoplasta* larvae prey on tube-making larvae of a chironomid midge (Diptera: Chironomidae) in the genus *Rheotanytarsus*. Midge tubes were picked from cobble removed from a first-order stream and dissected in lab. Several *Neoplasta* larvae that were fed midge larvae eventually were reared to adults identified as *N. parahebes*. Recent research by Harkrider (unpubl. obs.) in southern California provides further evidence of the developmental sites and feeding behavior of *Neoplasta* larvae. Dissections of small diameter pieces of saturated, decaying wood submerged in the streams revealed numerous *Neoplasta* larvae feeding on midge larvae within the tunnels of a wood-boring species of *Orthocladius*. *Neoplasta* pupae also were found in these tunnels. These discoveries of *Neoplasta* immatures inside structures built by their hosts or within midge tunnels in submerged wood may explain the paucity of *Neoplasta* larvae and pupae in kick nets used in streams along which we have collected hundreds of adults of several *Neoplasta* species in the western USA.

Acknowledgements

Bradley Sinclair (Canadian Food Inspection Agency) kindly reviewed an early draft of the manuscript and two anonymous reviewers provided suggestions that improved the final manuscript. The illustrations of larvae and pupae were prepared by Arwin Provonsha, Department of Entomology, Purdue University. We thank Dr. Carol von Dohlen (Utah State University) for the use of lab equipment and supplies to extract DNA and run the agarose gels.

References

Brindle, A. (1969) Taxonomic notes of the larvae of British Diptera. No. 27, A revised note of the subfamily Hemerodromiinae (Empididae). *The Entomologist*, 102, 35–39.

Brocher, F. (1909) Metamorphoses de l' *Hemerodromia precatoria* Fall. *Annales de Biologie Lacustre*, 4, 44–45.
Courtney, G.W., Teskey, H.J., Merritt, R.W. & Foote, B.A. (1996) Aquatic Diptera Part One. Larvae of aquatic Diptera. *In*: Merritt, R.W. & Cummins, K.W. (Eds.) (1996) *An Introduction to the Aquatic Insects of North America.* 3rd. Edition. Kendall/Hunt, Dubuque, Iowa, pp. 484–514.
Harkrider, J.R. (2000a) Phenology of aquatic dance flies (Diptera: Empididae; Hemerodromiinae) along a stream in southern California. *Pan-Pacific Entomologist*, 76, 170–175.
Harkrider, J.R. (2000b) Predation of *Neoplasta* Coquillett larvae (Diptera: Empididae) on larval midges in the genus *Rheotanytarsus* Bause (Diptera: Chironomidae). *Pan-Pacific Entomologist*, 76, 176–183.
Harper, P.P. (1980) Phenology and distribution of aquatic dance flies (Diptera: Empididae) in a Laurential watershed. *American Midland Naturalist*, 104, 110–117.
Knutson, L.V. & Flint, O.S. (1971) Pupae of Empididae in pupal cocoons of Rhyacophilidae and Glossosomatidae (Diptera-Trichoptera). *Proceedings of the Entomological Society of Washington*, 73, 314–320.
Landry, B. & Harper, P.P. (1985) The aquatic dance fly fauna of a subarctic river system in Quebec, with the description of a new species of *Hemerodromia* (Diptera: Empididae). *The Canadian Entomologist* 117, 1279–1386.
MacDonald, J.F. (1989) Review of the Nearctic *Metachela* Coquillett, with description of a new species (Diptera: Empididae; Hemerodromiinae).*Proceedings of the Entomological Society of Washington*, 91, 513–522.
MacDonald, J.F. (1994) Review of the genus the Nearctic species of the genus *Chelifera* Macquart (Diptera: Empididae; Hemerodromiinae). *Proceedings of the Entomological Society of Washington*, 96, 236–275.
MacDonald, J.F. (1998) Review of the genus *Hemerodromia* Meigen of America north of Mexico (Diptera: Empididae; Hemerodromiinae). *Proceedings of the Entomological Society of Washington*, 100, 792–823.
MacDonald, J.F. & Harkrider, J.R. (1999) Differentiation of larvae of *Metachela* Coquillett and *Neoplasta* Coquillett (Diptera: Empididae: Hemerodromiinae) based on larval rearing, external morphology, and ribosomal DNA fragment size. *Journal of the North American Benthological Society*, 18, 414–419.
MacDonald, J.F. & Turner, W.J. (1993) Review of the genus *Neoplasta* Coquillett of America north of Mexico (Diptera: Empididae; Hemerodromiinae). *Proceedings of the Entomological Society of Washington*, 95, 351–376.
McCafferty, W.P. (1981) *Aquatic Entomology.* Science Books International, Boston, 448 pp.
Melander, A.L. (1928) Diptera, Fam. Empididae. *In*: Wytsman, P. (Ed.). *Genera Insectorum*, 185 (1927), 1–434.
Merritt, R.W. & Cummins, K.W. (Eds.) (1996) *An introduction to the aquatic insects of North America.* 3rd. Edition. Kendall/Hunt, Dubuque, Iowa, 862 pp.
Sinclair, B.J., & Harkrider, R.H. (2004) The immature stages and rearing observations of the aquatic dance fly, *Roederiodes wirthi* Chillcott (Diptera: Empididae: Clinocerinae), with taxonomic notes of the genus. *Studia dipterologica*, 11, 51–61.
Sommerman, K.M. (1962) Notes on two species of *Oreogeton*, predacious on black fly larvae. *Proceedings of the Entomological Society of Washington*, 64, 123–129.
Steyskal, G.C. & Knutson, L.V. (1981) Empididae [Chapter] 47. *In*: McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood,, D.M. (Coords.), *Manual of Nearctic Diptera*, Volume 1. *Agriculture Canada Monograph*, 27, 607–624.
Vaillant, F. (1953) *Hemerodromia seguyi*, nouvel empidide d'Algérie destructeur de simulies. *Hydrobiologia*, 5, 180–188.
Vaillant, F. (1965) Revision des Empididae Hemerodromiinae de France, d'Espagne et d'Afrique du Nord (Dipt.). *Annales de la Société Entomologique de France*, 133, 143–171.