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Description of Adults of *Acentrella nadineae* McCafferty, Waltz & Webb, 2009 (Baetidae: Ephemeroptera) with notes on rearing and madicolous behavior of nymphs

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Abstract

Acentrella nadineae McCafferty, Waltz, & Webb, 2009 is broadly distributed in cool, clean, swift rocky streams across parts of the southeast, northeast, and midwestern United States, but currently its adult stages are unknown. The difficulty associated with successfully rearing nymphs of *Acentrella* species is mostly responsible for our lack of knowledge of adult life stages. New field observations of *A. nadineae* nymphs from the Hubbard River, Massachusetts, USA were used to modify an already successful rearing system for baetid nymphs to obtain a reared series of male and female imagos for study. Both life stages were photographed alive and are described herein for the first time. In addition, details of the modified rearing system and notes on the unusual madicolous behavior of near final instar nymphs are presented.

Key words: *Acentrella,* taxonomy, imagos, rearing, madicolous nymphs

Introduction

Adults of *Acentrella* species are poorly known and nymphs have been difficult to rear resulting in an incomplete knowledge of the adult life stages of the 9 known North American species (Jacobus & McCafferty 2023, McCafferty 1996). In North America, *Acentrella nadineae* McCafferty, Waltz, & Webb, 2009 is one such species for which the adult stages are unknown. Nymphs of *A. nadineae* seem to be restricted to cool, clean, swift, rocky streams and have been reported from a few sites scattered across the southeastern, northeastern, and midwestern United States (McCafferty *et al.* 2009, Webb and Burian 2017). The discovery of a sustaining population of *A. nadineae* on the CT/MA state line (Webb and Burian 2017) has provided the opportunity to collect and rear specimens for adult association and to improve our understanding of the behavior and habitat requirements of nymphs. The primary purpose of this work is to provide complete and comparable descriptions of male and female imagos with particular attention to differences between living and ethanol preserved specimens.

Material and methods

All specimens were observed for morphological characters, coloration, and color patterns under Leica MS5 stereomicroscope and Bausch & Lomb phase contrast compound light microscope (maximum of 400x magnification). Specimens were photographed using a Nikon D300s DSLR camera and Nikon Camera Control Pro2® software. Scale bars were added to images using Image-J software (Schneider *et al.* 2012). All measurements of specimens were made using a calibrated ocular micrometer (nearest 0.10 mm) and specimens were held as flat as possible (without inducing distortions) using pieces of carefully broken glass microscope slides and coverslips.

Male genitalia were examined and photographed intact. Adult wings were examined intact and photographed from a dry-mount slide. Standard terminology for adult anatomy and morphology followed Kluge (1994). Conventions for standard measurements were Hubbard's (1995). Means and Standard Deviations (SD) were calculated for all

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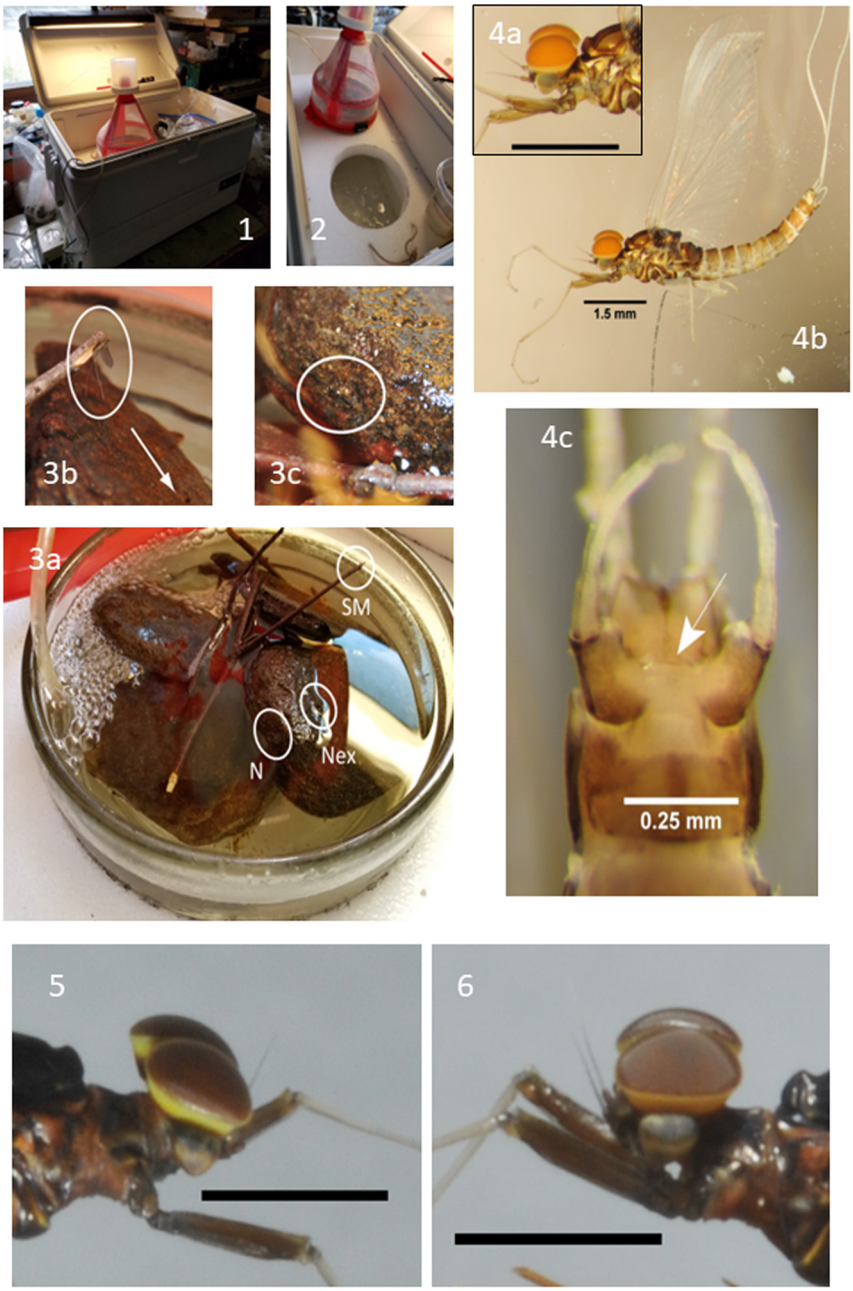
continuous data. Lengths of the foreleg segments of the male imago were standardized to the length of the foretibia by dividing the mean value of each segment by the mean value for the tibia. The resulting ratio values represent the proportional length of a leg segment to the length of the tibia. Colors were referenced to standard color tiles for natural history specimens given by Smithe (1975). In text of descriptions standard color tiles are noted as "close to NCG# …–name of color". Colors beyond the standard range of values were described as simply as possible. Color values for live specimens were recorded as soon as possible after molt to imago stage (usually within 2–4 hr.). Color values for preserved specimens were recorded anywhere from 1 day to a week after preservation.



FIGURES 1–6. *Acentrella nadineae* laboratory rearing setup and male imago. (1) Commercial 5-day temperature holding cooler setup for rearing cool-water stream mayflies; (2) Rearing dish, inverted funnel enclosure, foam insert and ice-bath compartment within rearing cooler; (3a) Circular rearing dish and arrangement of substrate, nymphs, N = location of madicolous nymph, Nex = shed nymphal exuviae at air/water interface, SM = location of subimago shown in 3b; (3b) Male subimago clinging to twig in rearing dish; (3c) Nymph in madicolous film of water at edge of stone in rearing dish; (4a) Head of imago (in ethanol); (4b) Left lateral view of male imago (in ethanol); (4c) Male genitalia, ventral; (5) Compound eyes of male imago (alive); (6) Head and pronotum of male imago (alive).

Field Collections. Nymphs for this study were collected from the Hubbard River near the MA state line in August of 2021. Previous trips to this site showed a very specific microhabitat association for *A. nadineae* nymphs. Nymphs were only found along the edges of large cobble and boulders that constricted the flow into narrow chutes and small boulders that protruded from the water along the edges of shaded pools. Live nymphs were transferred from field sites to the laboratory in large transparent plastic cups with dome-shaped plastic lids. Each cup had screen mesh glued over openings cut in the sides to allow water to circulate through the cup; the dome lid was also screened. Each cup was individually immersed halfway into a 1000 ml clear plastic circular container. Each plastic container was filled about three-quarters full with stream water and a single airstone was inserted between the cup screen window and the larger container. This arrangement allowed for vigorous aeration and without over agitating the specimens inside. The screened cups also allowed enough space for any specimens that may emerge while in transit to escape the water surface. Transport systems were placed into a thick-walled foam cooler and a separate quart-sized Ziploc® bag of ice was used to maintain water close to stream temperatures (~ 17 ºC) during transport in the transport containers.

Rearing Methods. An ice bath cooled rearing system capable of maintaining temperature in rearing dishes within ±2 ºC of stream temperatures for multiple specimens was used to rear nymphs of *A. nadineae* (Figs. 1–4). Nymphs were placed in a large circular glass dish (Fig. 3a) that fit tightly into a 5.0 cm thick closed-cell foam support that separated the dish from the ice bath in the bottom of the 5-day commercial-quality cooler (Figs. 1–2). Ice in the cooler maintained water temperature close to stream conditions and was easily replaced as necessary. A plastic inverted funnel with screen mesh and an apical capture container was placed over the rearing dish. This basic system was used successfully to rear a wide range of baetids both in the field as well as the laboratory. In this instance the substrate contents of the rearing dish were modified specifically for *A. nadineae* nymphs based on the observation that nymphs require substrates that allow them to reside at the waters' edge partially covered by a thin film of water adjacent to the main flow (Figs. 3a–c). Flow in the circular dishes was produced by an aerator with bubbles forcing the water around the sides of the circular dish. In addition, twigs extending from the water above where nymphs congregated provided places for emerging subimagos to rest above the water (Figs. 3a–b). Dishes were monitored frequently and emerging subimagos (Fig. 3b) were removed along with their shed exuviae (Fig. 3a).

Material examined. USA: Massachusetts: Hampden Co., Hubbard River, along Hartland Hollow Rd., from edges of large rocks upstr. of bedrock outcrop, 42.038462/-072.940517, S.K. Burian, 1 August 2021, 12M(+Nex), 13F(+Nex), 5SM(+Nex), 3SF(+Nex), 23N(died in rearing) [NEL].

Results

Although mortality of nymphs was high with many specimens failing to complete the molt to the subimago, a total of 33 imagos (M = male, F = female) and subimagos(SM=subimago male, SF=subimago female) were obtained for study (12 M, 13F, 5 SM, & 3 SF). Descriptions of male and female imagos are given based on observations of specimens preserved in 80% ethanol as well as alive. In figures accompanying the descriptions, scale bars are 1.00 mm unless otherwise stated. Descriptions of the preserved life stage of each sex included both structural details as well as persistent color patterns, whereas the description of live specimens focuses mostly on life colors and patterns not maintained after preservation. All measurements and statistics are from specimens after preservation.

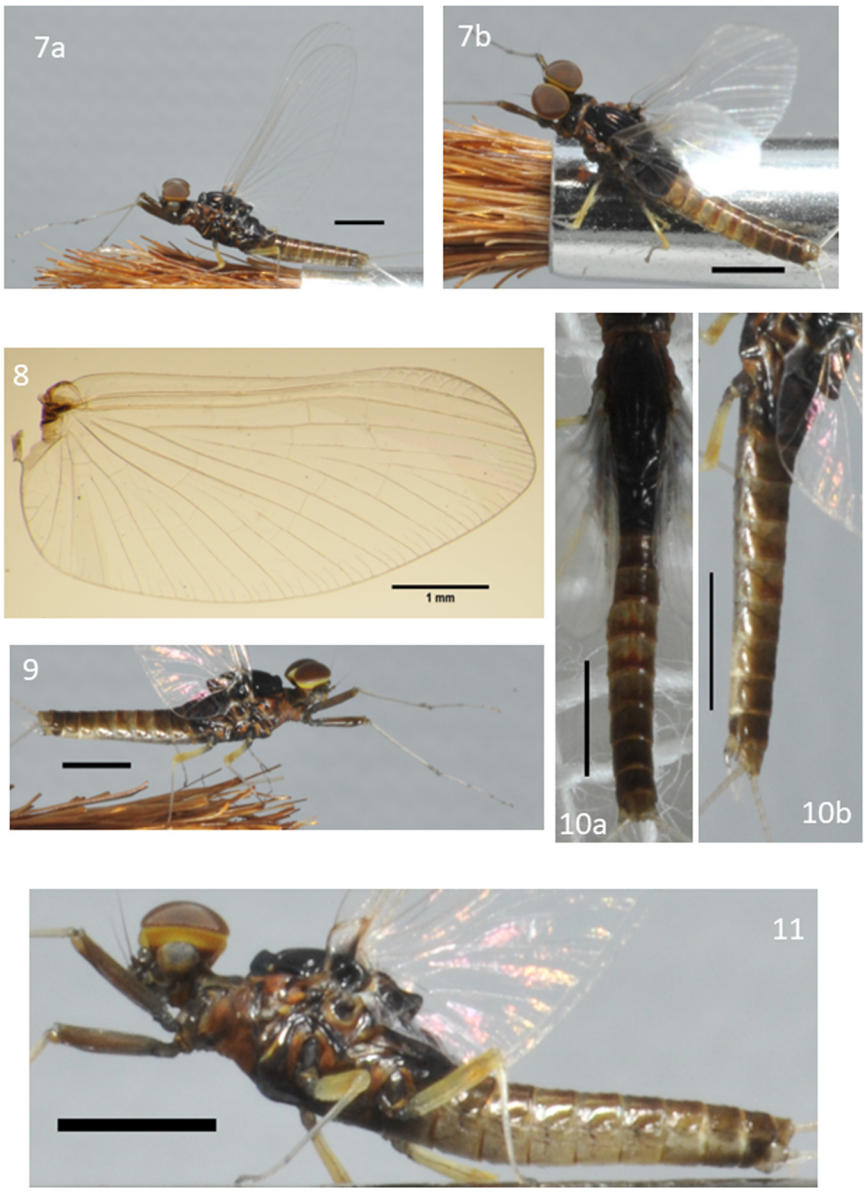
Acentrella nadineae McCafferty, Waltz, & Webb, 2009

(Figs. 4–18)

Male Imago: Body length: 4.88–5.76 mm (5.20±0.26, n=10); Forewing length: 4.88–5.28 mm (5.09±0.13, n=10).

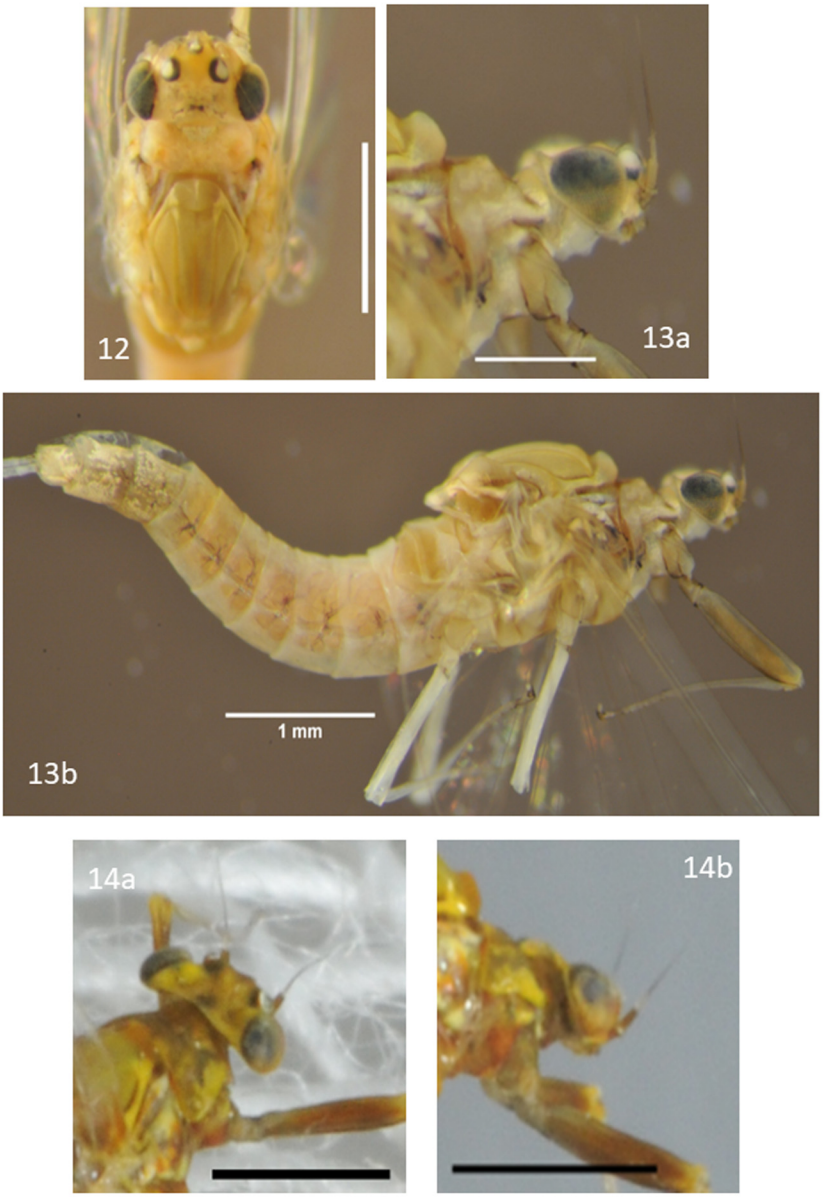
Head [**In Ethanol**]. Medium brown (close to NCG# 123—Raw Umber) with thicker ridges and edges darker brown (Fig. 4a). Antennae brown with scape slightly darker than pedicel and flagellum becoming progressively lighter toward apex. Frons below median ocellus with small ridge terminating in small point. Compound eyes turbinate and diverge slightly from their bases such that upper faceted surfaces are separated by distance equal to or greater than width of median ocellus. Upper portion of turbinate compound eyes brownish-orange (close to NCG# 17—Spectrum Orange) and stalk yellow (close to NCG# 53—Buff Yellow) with light brown band at base

(band progressively fades to lighter color over time) (Fig. 4a). Lower portion of compound eyes with grayish facets across upper and lower parts of eyes separated by pale horizontal band with upper edge of pale band slightly tinted reddish-brown (Fig. 4a). [**Alive**]. Color extremely dark brown almost black (close to NCG# 119—Sepia) on all well sclerotized parts of head capsule (Fig. 5). Antennae scape and pedicel similarly dark brown, almost black and flagellum only slightly lighter. Upper portion of turbinate compound eyes dark cranberry red (close to NCG# 132b—Mahogany) and stalk lemon yellow with dark cranberry colored band at base (Fig. 5). Lower portion of compound eyes with facets of upper and lower parts dark gray (almost black) separated by pale yellow horizontal band with light brown tinted edges (Fig. 6).



FIGURES 7–11. *Acentrella nadineae* male imago (alive). (7a) Left lateral view of body an wings; (7b) Dorsal-lateral view of thorax and abdomen; (8) Right forewing (dry-mounted, slight difference in appearance of wing membrane due to its contact with glass coverslip);.(9) Right lateral view of body and legs; (10a) Dorsal view abdomen; (10b) Left lateral view of abdomen; (11) Left ventral-lateral view of thorax and abdomen.

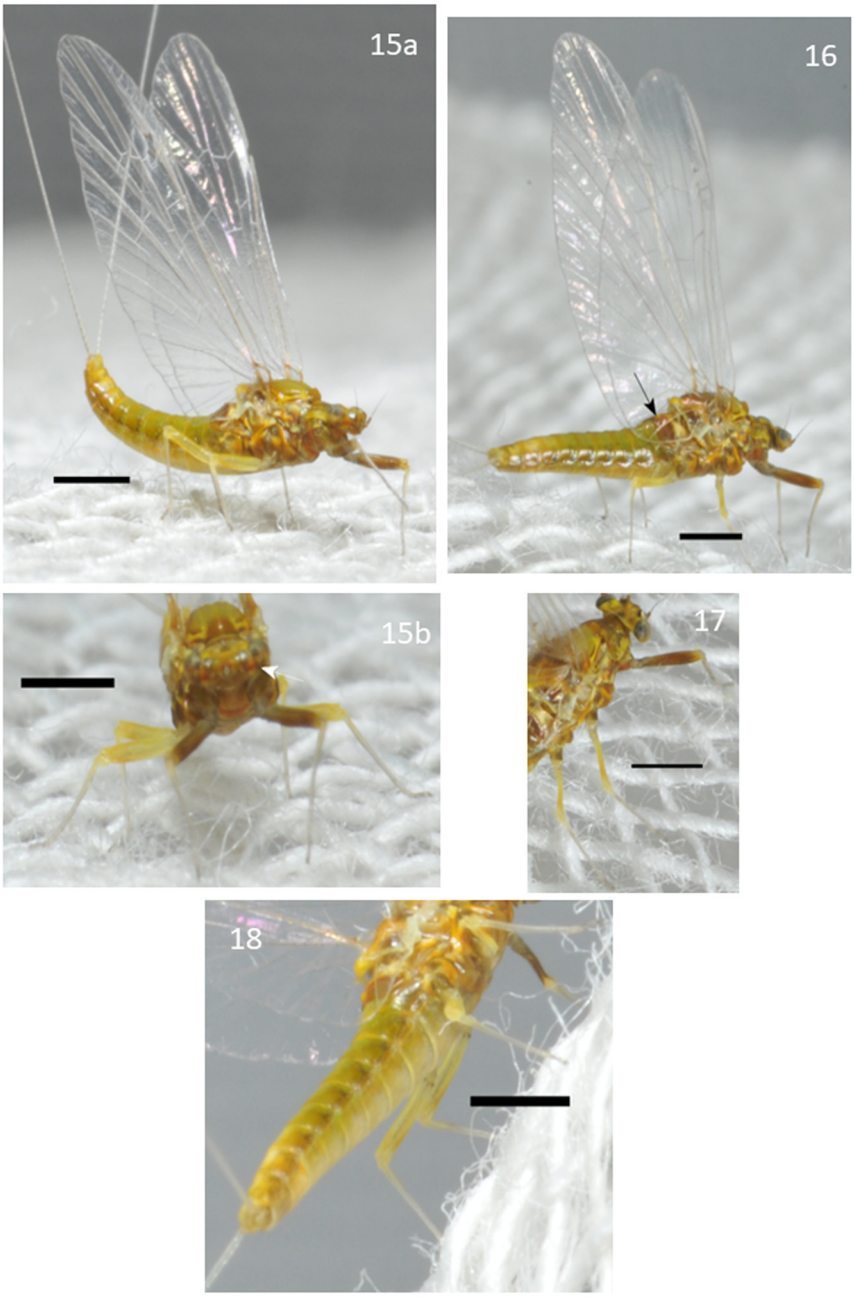
Thorax [**In Ethanol**]. Mostly medium brown with more heavily sclerotized areas dark brown (close to NCG# 223—Raw Umber) (Fig. 4b). Less well sclerotize area lighter brown (close to NCG# 223B—Verona Brown) and membranous areas white or cream colored (Fig. 4b). Pronotum rectangular and well developed with somewhat raised areas laterally. Middle of pronotum medium brown, with some areas of dark brown posteriorly. Mesonotum with medioscutum (MS) and submedioscutum (SMS) mostly uniform medium brown with some dark brown along



FIGURES 12–14. *Acentrella nadineae* female imago (in ethanol & alive). (12) Dorsal view of head and mesonotum (in ethanol); (13a) Right lateral view of head and antennae (in ethanol); (13b) Right lateral view of thorax, legs, and abdomen (in ethanol); (14a) Dorsal view of head and pronotum (alive); (14b) Right lateral view of head, pronotum, and antennae (alive).

their edges (Fig. 4b). Anteronotal protuberance (ANp) and anteronotal transverse impression (ANi) slightly darker brown than adjacent areas of SMS. Prelateposcutum (PLS) uniform dark brown (Fig. 4b). Apex of scutellum (SL) and surface of infrascutellum (ISL) much darker brown than rest of mesonotum (close to NCG# 221—Vandyke Brown) (Fig. 4b). Area of ISL large and distinctly indented below SL. Area of parascutellum (PSL) slightly lighter yellowish-brown than rest of surrounding mesonotum. ANp subconical with apex varying from acute to blunt. Metanotum mostly medium brown, SL slightly darker reddish-brown compared to lateral areas (Fig. 4b). Lateral margins of metanotum above bases of hind legs pale yellow to cream colored. Pleural sclerites of pro- , meso- and metanotum similar in color to well sclerotized dorsal areas, but membranous areas pale yellow to cream colored (Fig. 4b). Basiradiales of forewings, bases of associated wing veins, and axillary sclerites dark brown. Sternites medium to dark brown, similar to general color of thoracic nota and pleural sclerites. [**Alive**]. All sclerotized areas extremely dark, mostly blackish-brown to black (Fig. 7a). Pronotum blackish-brown to black with only slightly lighter brown areas anteriorly beneath compound eyes and along posterior margin (Fig. 7b). All of meso- and metanotum extremely dark, with no obvious color differences as occur on preserved specimens. Bases of forewings

and axillary sclerites dark grayish-brown to darker blackish-brown. All pleural sclerites similarly dark blackish-brown, but membranous areas vary from medium to light brown with few edges tinted with pale yellow (Fig. 7a). On some specimens lighter colored parts of pleural region appear tinted with orangish-brown. Sternites extremely dark blackish-brown, similar to other sclerotized parts of thorax.



FIGURES 15–18. *Acentrella nadineae* female imago (alive). (15a) Right lateral view of body and wings; (15b) Frontal view of head, mesonotum, and legs (arrow indicates reddish-orange stripe across frons); (16) Right lateral view of abdomen and thorax from posterior perspective (arrow indicates dark reddish-brown of posterior part of metanotum); (17) Right lateral view of legs; (18) Ventral lateral view of abdomen (specifically showing sternites).

Wings [**In Ethanol**]. Forewing as in Fig. 8. Forewing membrane unpigmented, except for extreme base where dark brown pigment stains bases of major longitudinal veins (i.e., costa, subcosta, and radius), as well as brace vein and membrane adjacent to base of cubital and anal veins. Stigmatic area semi-transparent with about 5–6 crossveins, with 4–5 crossveins forming anastomose connections with costa (Fig. 8). First marginal space below stigma usually with 1 unattached intercalary vein (on 1 specimen studied this space lacked an intercalary vein). Pairs of unattached

marginal intercalary veins occur in marginal spaces between all succeeding major longitudinal veins and attending intermediate veins, except for last anal vein space (Fig. 8). All major longitudinal veins and crossveins pale. Hind wings absent. [**Alive**]. Color same as on preserved specimens. When at rest individuals hold forewings above body (Fig. 7a). However when preserved, about half of imagos studied died with forewings in down position.

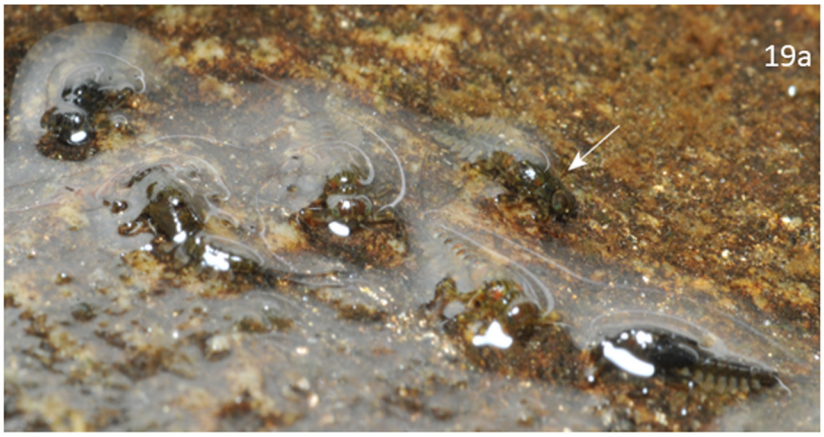


FIGURE 19 a–b. *Acentrella nadineae* nymphs (alive). (19a) Final instar black wing pad nymphs in madicolous habitat on edge of stone in rearing dish (arrow indicates partially exposed head and thorax of nymph); (19b) Shows nymphs with and without black wing pads congregating in madicolous habitat in rearing dish.

Legs [**In Ethanol**]. Ranges, means, and ratio values of segments of forelegs as follows: Femur 0.81–0.94 mm (0.88± 0.04 [0.55], n = 10) (mean ± SD[ratio to tibia], n); Tibia 1.50–1.70 mm (1.61±0.06[1.00], n = 10); $Tarsus_1$ 0.04–0.06 mm (0.05±0.005[0.03], n = 10); $Tarsus_2$ 0.40–0.62 mm (0.54±0.07[0.34], n = 10); $Tarsus_3$ 0.40–0.47 mm (0.42±0.03[0.26], n = 10); $Tarsus_4$ 0.17–0.25 mm (0.21±0.02[0.13], n = 10); $Tarsus_5$ 0.11–0.15 mm (0.13±0.01[0.08], n = 10). Coxa and trochanters medium brown with darker brown edges, color similar to pleural sclerites (Fig. 4b). Darker color of trochanters tends to bleed over onto bases of femora. Forefemora yellow-brown with gray shading along ventral edge and at joints with foretibiae. Foretibiae pale except for area adjacent to joint with forefemora, which is shaded yellow-brown and with darker color at points of articulation. Foretarsus mostly pale, with light brown shading near apex of each tarsal segment. No spines or knobs present on foretibae or foretarsi. Tarsal claws pale similar to foretarsi and both blunt and sharp. Segments of mid and hind legs mostly pale, only femora have faint yellow-brown shading along dorsal edge (Fig. 4b). Mid and hind tarsi appear 4 segmented with tarsal segments from claws to tibiae coded as: T_5, T_4, T_3, and T_{1+2} (T_{1+2} apparently fusion of 2 tarsal segments). Tarsal segments T_3 and T_{1+2} have distinct long spines on ventral apical margins. Tarsal segment T_4 has ventral margin extended forward on base of T_5 and terminates with an acute spine-like tip. Tarsal claws of mid and hind legs similar to those of forelegs. [**Alive**]. Coxa and trochanters extremely dark blackish-brown (Fig. 9), similar to pleural sclerites and thoracic nota. Dark color of coxa and trochanters tends to bleed over onto bases of femora. Forefemora dark grayish-brown with slightly lighter reddish-brown or yellow-brown color near joints with foretibia (Fig. 9). Foretibiae pale with slight

gray shading that is darker at joints with forefemora and foretarsi (Fig. 9). Foretarsus pale with faint gray shading as on foretibia with similar concentration of gray color near joints of each tarsal segment. Foretarsal claws also with faint grayish shading (Fig. 9). Segments of mid and hind legs mostly pale, only femora have noticeable light yellow-brown shading with faint reddish-brown tint near apex. Coloration of mid and hind tibiae and tarsi also pale, but with less gray shading near joints in contrast to forelegs (Fig. 9).

Abdomen [**In Ethanol**]. Tergites as in Fig. 4b, light brown suffused with areas of medium brown on tergites I–III and VI–IX. Tergites IV and V with darker brown restricted to posterior margins and posterolateral surfaces (Fig. 4b). Posterior margins of tergites II–IV with reddish-orange band of variable width, most well developed on tergites III and IV. On some specimens reddish-orange color of posterior margins can extend anteriorly along midline for about half length of tergite. On some specimens tergites IV and V are semi-transparent with limited brown color making reddish-orange posterior band more distinctive. Only on 1 specimen studied were tergites IV and V more heavily shaded with brown muting reddish-orange color of posterior band. On 2 specimens studied reddish-orange color faded extensively after preservation. Tergite X light brown with pale areas along anterior margin, medially, and laterally. Tergite X usually with 2 pairs of small brown submedian spots that often tend to merge into paired streaks. On tergites VII–IX one pair of small brown submedian spots usually occur. On some more lightly pigmented specimens subcuticular white soft tissue can make all of abdominal segments VII–X appear much paler than noted above. On tergites II–VI spiracles, tracheal branches, and trunk trachea distinctly outlined in black. Sternites mostly pale with light brown shading restricted to anterior half of sternites I–VII. Sternites VIII and IX with more extensive medium brown shading. Sternite IX with posterolateral areas adjacent to forceps bases shaded darker brown compared to rest of sternite (Fig. 4c). Genitalia as in Fig. 4c., forceps 3 segmented. Forceps bases (i.e., segment 1) mostly medium brown, except for small pale areas near concave inner edges. Forceps segments 2 and 3 pale except for slight brownish shading near constriction of segment 2 and near apex of segment 2 near joint with segment 3. Forceps segment 2 with basal portion somewhat cylindrical up to point of constriction with narrow apical portion (Fig. 4c). Forceps segment 2 does not appear to broaden before joint with terminal segment 3. Forceps terminal segment 3 about 2x as long as wide and lacks pigmentation. Sclerotized penes cover broad and somewhat trapezoidal or sub-rectangular, usually extending completely across gap between forceps bases (Fig. 4c). Penes cover usually light yellow-brown to cream colored. [**Alive**]. Tergites as in Figs. 10a–b. Tergites base color light brown, but heavily suffused with dark brown on tergites I and VII–X. Tergites II–VI semi-transparent with distinct reddish-orange band across posterior margin much more distinctive than on other tergites. On most specimens reddish-orange color along posterior quarter to one-third of tergites extends anteriorly along midline of tergites for as much as half length of each tergite (Fig. 10a). Tergites VII–X mostly dark brown and reddish-orange color usually heavily muted. Tergites VII–IX usually with thin pale line along extreme edge of posterior margin. Tergite X dark brown, small paired brown spots usually indistinct against darker background color. On tergites II–VII spiracles, tracheal branches, and trunk trachea distinctly outline with black. Sternites mostly pale with slight light brown shading, posterior margins of sternites I–VIII with distinctive thin brown line (Fig. 11). Sternites VIII and IX with more extensive brown shading, sternite IX with areas lateral to forceps bases dark brown. Genitalia as in Figs. 4c and 10b. Forceps bases usually grayish-brown, except for small pale areas along inner concave edges and occasionally middle of outer edges. On some specimens middle of outer edges of forceps bases can lack gray pigment and appear as pale or light brown, which are distinctive because of flanking areas of darker color. Forceps segment 2 with cylindrical basal section shaded gray up to constriction with narrow upper section. Upper section of segment 2 and terminal segment 3 pale. Sclerotized penes cover usually light brown.

Caudal Filaments [**In Ethanol**]. Cerci pale white lacking any dark bands (Fig. 4b). Median terminal filament vestigial and transparent with only tip of minute stub extending beyond posterior margin of tergite X. [**Alive**]. Cerci pale white with faint light brown shading forming bands on basal 8–10 annuli (Figs. 7a, 10b). Median terminal filament light yellow with slight grayish shading.

Female Imago: Body length: 4.24–5.28 mm (4.87±0.34, n=13); Forewing length: 5.20–6.00 mm (5.59±0.253, n=13).

Head [**In Ethanol**]. Base color brownish-yellow (close to NCG# 53—Buff Yellow), white soft tissue within head capsule visible through vertex (Fig. 12). Antennae bicolored, light brown scape slightly darker than pedicel and distinctly darker flagellum with pale basal annuli (Fig. 13a). Frons below median ocellus with faint remnant of reddish-orange horizontal band, but otherwise color same as vertex. Frons with small medial ridge terminating

in small point as in male imago. Compound eyes small and widely separated. Dorsal facets of compound eyes dark gray and separated from lighter colored lower facets by light greenish-yellow horizontal band (Figs. 13a–b). Apparent lighter color of lower facetted part of compound eyes changes if viewed from different angles. Color of lower facets seems similar to those of upper part of eye, but facets seem to have more space between them revealing more light-colored cuticle—hence yielding an overall lighter appearance to lower part of compound eyes. [**Alive**]. Base color varies from dark olive-yellow to lighter yellow-green. Near inner edges of each compound eye are chartreuse colored spots (close to NCG# 158—Chartreuse) (Fig. 14a). Antennae distinctly bicolored with reddish-brown scape and pedicel darker flagellum shaded with gray with pale basal annuli (Fig. 14b). Frons beneath compound eyes and ocelli mostly dark toned olive-yellow with reddish- orange horizontal stripe (Figs. 15a–b). Upper portion of compound eyes varies from dark gray to blueish-gray and separated from lower greenish-yellow portion of compound eye by narrow horizontal band of slightly darker greenish-yellow facets with faint reddish-brown tinted upper edge (Fig. 15a).

Thorax [**In Ethanol**]. Mostly cream colored, but varies in places to light brownish-yellow (close to NCG# 53—Buff Yellow) (Fig. 13b). Pronotum rectangular with anterior median notch and slightly raised center (Figs. 12, 13a). Sides of pronotum with slight depressions with light brown shading. Mesonotum with MS, ANp, SMS, PLS and SL all slightly darker yellow than less sclerotized areas, but overall little difference in color across most of mesonotum (Figs. 12, 13b). Structurally, all general features of mesonotum similar to that of male imago. Anterior portion of metanotum, which included SL and surrounding areas, pale cream colored, but posterior postscutellum shaded slightly darker brownish-yellow (Fig. 13b). Basiradiales of forewings, bases of associated wing veins, and axillary sclerites pale cream colored or lack color. Brace vein often has traces of light brown shading. On some specimens parascutellar lateral concavity (PSLccv) where base of forewing attaches to mesonotum can have traces of light brown. Sternites pale cream colored to light brownish-yellow, similar to general color of thoracic nota and pleural sclerites (Fig. 13b). Generally, some preserved specimens studied retained faint remnants of life colors or patterns (especially along edges of heavier sclerites and surfaces), but most seemed to lose almost all evidence of complex life colors and patterns. [**Alive**]. Thorax complexly colored as in Fig. 15a, with light yellow base color (close to NCG# 55—Spectrum Yellow), but varying in places to yellow-green. Pronotum with most of its midsection shaded medium brown, but outer edges of lateral depressions with dark brown crescent-shaped marks that border broad light olive-yellow bands of lateral margins (Fig. 14a). Mesonotum with ANp and Ani orange-rufous (NCG# 132c), MS and SMS light lime green (Fig. 15a). Lower edge of SMS and anterolateral scutal costa (ALSC) dark reddish-brown and area between these edges, which extend to base of ANp, golden-yellow (close to slightly amber version of NCG#55—Spectrum Yellow) (Fig. 15a). Mesonotum SL yellow and posterior scutal protuberance (PSp) yellow tinted with reddish-brown. Deeply incised ISL of mesonotum dark reddish-brown with posterior edge leading into axillary cord (Axc) of forewing pale yellow to white. Metanotum essentially bicolored with SL golden-yellow and surrounding anterior area pale cream colored, which contrasts with dark reddish-brown posterior postscutellum (Fig. 16). Basiradiales of forewings and bases of associated wing veins light reddish brown (Fig. 16). Axillary sclerites light brownish-yellow with various pale or yellow edges (some margined with reddish-brown). On most specimens PSLccv stained with dark brown. Sternites brownish-yellow similar to pleural sclerites of thoracic segments.

Wings [**In Ethanol**]. Forewing as in Fig. 8. Membrane of forewings, basiradiales, and major longitudinal veins usually unpigmented. Only brace vein has faint traces of brown shading. Occasionally costa, subcosta, and radius veins appear slightly darker than other longitudinal veins. Stigmatic area semi-transparent and clouded region includes apical interspaces between both costa and subcosta veins, as well as between subcosta and radius veins from first bullae to apex of forewing. Stigmatic area of costa and subcosta veins with 5–6 simple crossveins, with only 1 or 2 crossveins forming anastomose connections with costa vein. First marginal spaces below costa/subcosta stigmatic area lack unattached marginal intercalary veins (rarely second marginal space also lacks unattached marginal intercalary veins). Pairs of unattached marginal intercalary veins occur in all succeeding marginal interspaces, except for last space of anal vein. All crossveins lack pigment. Hind winds absent. [**Alive**]. Forewing as in Fig. 15a. Forewing membrane unpigmented except for extreme base where dark brown stains bases of longitudinal veins (costa, subcosta, and radius) and surrounding membrane. In addition, wing membrane adjacent to bases of cubital and anal veins appears opaque white. Brace vein shaded with light reddish-brown. On some specimens studied entire length of costa, subcosta, and radius veins shaded light brown, otherwise these veins lack color.

Legs [**In Ethanol**]. Legs as in Fig. 13b, with coxa light brownish-yellow similar to pleural sclerites. Trochanters and femora of all legs pale yellow to cream colored. Forefemora slightly darker brownish-yellow compared to

much paler mid and hind femora (Fig. 13b). Tibiae of all legs about same pale yellow to cream colored. Foretarsi 5 segmented with segment T_1 fused to apex of foretibia. Foretarsal segments T_2 and T_3 with minute apical spine on ventral apical margins. Foretarsal segment T_4 with ventral apical margin extended up onto ventral surface of segment T_5 , but has blunt apex. Tarsal claws of all legs both blunt and sharp. Foretarsi shaded with light gray, which is most distinctive on segment T_5 and claws (Fig. 13b). Mid and hind tarsi appear 4 segmented with tarsal segments from claws to tibiae coded as: T_5, T_4, T_3, and T_{1+2} (T_{1+2} apparently fusion of 2 tarsal segments). Tarsal segments T_3 and T_{1+2} have distinct long spines on ventral apical margins. Tarsal segment T_4 has ventral margin extended forward on base of T_5 and terminates with an acute spine-like tip. Tarsal claws of mid and hind legs similar to those of forelegs. Gray shading noted on forelegs also evident on mid and hind tarsi. [**Alive**]. Coxa of all legs dark brown infused over yellow base color (Fig. 17). Trochanters shaded with light gray over yellow base color. Forefemora reddish-brown over yellow base color, but apex remains mostly yellow (Figs. 16, 17). Foretibiae pale white with light gray to light brown shading basally (Figs. 15a–b, 17). Foretarsi shaded similar to foretibiae, but segment T_5 and foreclaws more darkly shaded with gray than rest of segments.

Abdomen [**In Ethanol**]. Tergites as in Fig. 13b, pale yellow to cream colored. Tergites VII–IX with faint light brown shading over pale base color, otherwise no distinctive darker color patterns on tergites. On tergites II–VII spiracles, tracheal branches, and trunk trachea distinctly outline with black. Sternites pale, essentially same color as tergites. [**Alive**]. Abdominal tergites as in Figs. 15a, 17. Tergites base color lime green (close to NCG# 159—Lime Green), color darker toned on tergites I–VII and tending to be lighter with greater yellow tint on tergites VIII–X. Posterior margins of tergites I–VI with thin dark brown line (Fig. 16). On tergites I–IX faint reddish-orange shading along posterior margins extends anteriorly only about one quarter length of each tergite (Fig. 16). Tergites VII–IX with much less green color allowing orangish tint along posterior margin to become more distinctive. Lateral margins of tergites I–IX with reddish-orange shading around area of pleural fold, especially distinctive on tergites with black spiracular marks. On tergites II–VII spiracles, tracheal branches, and trunk trachea distinctly outline with black. Sternites I–VII pale white medially, but with lime green color of tergites bleeding onto lateral margins near pleural fold (Fig. 18). Sternites VIII and IX base color light brownish-yellow and sternite X shaded with dark brown medially (Fig. 18).

Caudal Filaments [**In Ethanol**]. Cerci pale white lacking any dark bands. Median terminal filament vestigial and transparent with only tip of minute stub extending beyond posterior margin of tergite X. [**Alive**]. Cerci pale white lacking any evidence of faint brown shading on basal annuli as noted for male imagos (Figs. 15a). Median terminal filament pale with slight grayish shading.

New Provisional Couplets For Key To Nearctic Acentrella Adults:

The following are couplets to include the male imago in the key to Nearctic *Acentrella* (Burian and Meyers 2011), characters based on ethanol preserved specimens:

4. Abdominal terga I–VI mostly olive brown, reddish brown, or light brown with reddish-orange brown posterior margins; genitalia usually with broad sub-rectangular sclerotized penes cover that can have brown shading medially 5a

5a. Abdominal terga II–V with distinct reddish orange band on posterior margins as in Fig. 4b (on terga IV & V reddish-orange color can extend anteriorly along midline for about half length of segments line), terga III–V often semi-hyaline with lateral brown triangular shading (Fig. 4b); genitalia as in Fig. 4c with extensive brown shading on forceps bases and anterior half of sternite IX, basal portion of forceps segment 2 somewhat cylindrical and usually lacks dark pigmentation except near constriction, penes cover broad extending completely across gap between forceps bases and appears somewhat trapezoidal or sub-rectangular (Fig. 4c) . . . . . *A. nadineae*

5a'. Abdominal terga mostly olive brown or reddish brown with posterior portion occasionally darker than anterior, but lacks reddish orange marks as above, terga III–V sometimes pale as a result of preservation in alcohol, but usually not semi-hyaline as above; genitalia without extensive brown shading on forceps bases or sternite IX and basal portion of second segment of forceps more distinctly conical, penes cover does not extend across entire gap between forceps bases and is usually rectangular (occasionally with slightly arched center) and can have brown shading medially or is inconspicuous . . . . . *A. turbida*

Madicolous Behavior of A. nadineae Nymphs. Following the first record of *A. nadineae* in Connecticut from the Hubbard River in 2012, initial attempts to rear *A. nadineae* in 2013 were unsuccessful. Although the nymph of

A. nadineae was noted to be associated with rocks at the edge of swift chutes of water, nothing was known about the behavior of nymphs prior to emergence. During the first attempt to rear this species in 2013, a few nymphs were observed to be clinging to the vertical sides of the glass rearing dishes at the water contact line, when dishes were disturbed nymphs retreated below the water. Only 2 female imagos and 5 male subimagos were obtained from these first attempts. Initial observations of nymphs concerning their behavior on the sides of rearing dishes were confirmed to be natural behaviors of near final instar nymphs by observing nymphs in the field at the Hubbard River. It was observed that near last instar nymphs move to the edges of some hard (usually mineral) substrate at the edge of a swift flow (large rocks with gradually sloping surfaces at the edges of shaded pool inlets yielded the greatest number of nymphs in collections) and crawl to the point where they are only covered by a thin layer of water—thus occupying a type of madicolous habitat (Figs. 19a–b). Early to mid-instar nymphs (i.e., those with forewing pads not reaching 3rd abdominal segment) tend to occupy hard substrates at the edges of swift flows below the surface air/water interface.

Places where *A. nadineae* nymphs congregate coincidentally seem to be places that facilitate emergence to the subimago stage. In rearing dishes, upon emergence from these madicolous resting sites, subimagos were sheltered out of the main flow and abandoned nymphal exuviae were always found firmly clinging tightly to the rock surface further providing support at the waters' surface for rafting subimagos prior to flight. In addition, subimagos seemed to survive best if provided sticks that protrude from the water above the sites where final instar nymphs congregate (Figs. 3a–c).

Discussion

With the successful rearing of *A. nadineae* only the adults of *A. feropagus* Alba-Tercedor and McCafferty (2000) remain unknown among the Nearctic species of *Acentrella*. With the couplets included here, using largely coloration of abdominal tergites and structure of the male genitalia, it should be possible to separate the male imago of *A. nadineae* from those of most other species presented in the key by Burian and Myers (2011). Currently, only the imagos of *A. turbida* could possibly be confused with those of *A. nadineae.* The possible confusion of these two species was first addressed by McCafferty *et al.* (2009) in the description of the nymph of *A. nadineae* because nymphs of both species co-occur throughout their ranges and as nymphs have some superficial morphological similarities.

Although this paper is concerned with the adult stages, it became clear during this study that there are several unique morphological features of nymphs of *A. nadineae* that were not mentioned by McCafferty *et al.* (2009) that could easily separate the nymphs of *A. turbida* and *A. nadineae* and deserve further study. As for adult characters of *A. nadineae,* once imagos were obtained it immediately became clear that body lengths, wing lengths, colors and color patterns, and the structure of the male genitalia differed between these species. In addition, colors and color patterns of *A. nadineae* differed greatly between preserved and live states. This was especially true for *A. nadineae* female imagos, which had striking and complex colors and color patterns that all but vanish after preservation. Extremely dark colors of the head, thorax, and abdomen of male imagos faded extensively to lighter shades of brown, but colors did not change to the extent as occurred in female imagos. Although body and wing lengths of *A. nadineae* ranged from 0.88–1.75 mm greater than body and wing lengths known for *A. turbida*, the most diagnostic characters of male imagos of *A. nadineae* were the reddish-orange bands along posterior portion of abdominal tergites (Fig. 4b) and structure of the genitalia with regards to the structure of segment 2 and 3 of genital forceps (Fig.4c). Despite some similarities concerning the dark body and abdominal coloration of *A. turbida* with regards to *A. nadineae*, the latter is overall much lighter brown across most tergites and consistently more lightly pigmented brown on abdominal segments III–VI and expresses the reddish-orange posterior band on tergites I–IX. In addition, subgenital plate and forceps bases (i.e., forceps segment 1) of *A. nadineae* are shaded much more extensively with brown compared to *A. turbida* and the basal portion of forceps segment 2 of *A. nadineae* is cylindrical and shaded with light gray in contrast to that of *A. turbida* , which has a conically shaped basal portion to forceps segment 2 and seems to lack gray or dark shading.

Currently there is little known about female imagos of the Nearctic *Acentrella*, especially concerning life colors. Female imagos of *A. nadineae* have similar body and wing lengths to male imagos making them distinctly larger than female imagos of *A. turbida*. The following comments are given for the purpose of facilitating future

comparisons when female imagos of other species of Nearctic *Acentrella* are better known. Female imagos of *A. nadineae* have distinctive and complex coloration of the head and thorax. Distinctive head characters include a reddish-orange horizontal stripe across the frons (Fig. 15b) and the bicolored antennae (Fig. 14b). On the thorax, the overall olive-yellow color with contrasting golden-yellow bands edged with reddish-brown along the sides of the mesonotum seems to be a particularly distinctive pattern. In addition, the reddish-brown ANp is distinctive and contrasts with the olive-yellow color of the rest of the mesonotum (Fig. 15a–b). Finally, the lime green color of the abdominal tergites tinted with reddish-orange across their posterior margins, in conjunction with colors of the head and thorax, make female imagos well equipped to blend in on similarly colored leaf surfaces. Thus, the overall greenish appearance may be adaptive for the brief periods between mating, dispersal, and oviposition when females may need to rest on vegetation. With the description of the female imago of *A. nadineae*, which is likely the primary adult dispersal stage, it may now be possible to track female movements within drainage networks and thus evaluate dispersal potential and what this may mean regarding this species ability to move within its current range or expand its range boundaries.

Conclusions

The difficulty in rearing certain species of baetid mayflies has been a long standing problem for mayfly systematists. The successful rearing of *A. nadineae* extends our knowledge of its life stages and behavior of nymphs within its aquatic habitat. Beyond North America there are several species of *Acentrella* for which the adults are unknown. It is hoped that the discovery of the rearing conditions that allowed nymphs of *A. nadineae* to be reared may provide a roadmap for rearing other *Acentrella* species.

The description of the adult life stages of *A. nadineae* adds to our knowledge of one of the most distinctive Nearctic *Acentrella*. The ability to identify and rear adults of *A. nadineae* means that it is now possible to conduct detailed studies of its life history from the perspective of all its primary life stages, which is important with regards to expanding our knowledge of the aquatic biodiversity of North America. Nymphs of *A. nadineae* are already known to be associated with some of the highest quality and perennially cool stream habitats in North America, but we have no information on the ability of adults to disperse or track changes in these habitats that may result from anthropogenic disturbances or climatic changes. In theory, with the ability to identify adults of *A. nadineae* much information could be gained in this regard. Further, the observation of madicolous behavior of nymphs and new adult morphological characters may prove useful in future phylogenetic analyses of *Acentrella* species groups.

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