

## ***Calyxocephalus karyopera* g. nov., sp. nov. (Eugregarinorida: Actinocephalidae: Actinocephalinae) from the Ebony Jewelwing Damselfly *Calopteryx maculata* (Zygoptera: Calopterygidae) in Southeast Nebraska, U.S.A.: Implications for Mechanical Prey–Vector Stabilization of Exogenous Gregarine Development**

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**ABSTRACT:** *Calyxocephalus karyopera* g. nov., sp. nov. (Apicomplexa: Eugregarinorida: Actinocephalidae: Actinocephalinae) is described from the Ebony Jewelwing Damselfly *Calopteryx maculata* (Odonata: Zygoptera: Calopterygidae) collected along Turkey Creek in Johnson County, Nebraska, U.S.A. *Calyxocephalus* gen. n. is distinguished by the form of the epimerite complex: a terminal thick disk or linearly crateriform sucker with a distal apopetalus calyx of petaloid lobes and a short intercalating diamerite (less than half of the total holdfast length). The epimerite complex is conspicuous until association and syzygy. Association occurs immediately before syzygy and is cephalolateral and biassociative. Gametocysts are spherical with a conspicuous hyaline coat. Lacking conspicuous sporoducts they dehisce by simple rupture. Oocysts are axially symmetric, hexagonal dipyramic in shape with slight polar truncations, bearing 6 equatorial spines, 1 at each equatorial vertex and 6 terminal spines obliquely inserted at each pole, 1 at each vertex created by polar truncation. The ecology of the *C. karyopera*–*C. maculata* host–parasite system provides a mechanism for mechanical prey–vector stabilization of exogenous gregarine development and isolation.

**KEY WORDS:** Odonata, Zygoptera, *Calopteryx maculata*, damselfly, Apicomplexa, Eugregarinorida, Actinocephalidae, Actinocephalinae, *Calyxocephalus karyopera*, new genus, new species, parasite evolution, biodiversity, species isolation, vector, transmission.

The Ebony Jewelwing, *Calopteryx maculata* (Beauvois, 1805) (Odonata: Zygoptera: Calopterygidae), is a large (39–57 mm) and visually striking broadwing damselfly with a metallic green to blue body and ebony black wings (Westfall and May, 1996). The adult *C. maculata* is a characteristic resident along small shaded forest streams with patches of dappled sunlight where they are highly territorial; males often defend the same small territory from other males for most of their adult lives (Dunkle, 1990; Westfall and May, 1996). Although the family Calopterygidae is primarily pantropical in distribution, *C. maculata* is common in the eastern Nearctic, ranging from Nova Scotia, Canada to Ontario, Canada, down the eastern seaboard of the United States to Lake Okeechobee, Florida, and west across the great plains to Nebraska, Kansas, Oklahoma, and eastern Texas (Dunkle, 1990; Westfall and May, 1996; Catling and Brownell, 2002). During an ongoing biotic survey of the gregarine parasites of North American insects, an heretofore unknown gregarine species was discovered in populations of *C. maculata* collected from Turkey Creek and its tributaries in Johnson County in southeastern Nebraska, U.S.A. The gregarine populations recovered are taxonomically distinct from known gregarine genera and species and represent a new taxon. The structure of the oocyst unites this taxon with the

Acanthosporinae that are characterized by oocysts bearing spines or tufts of spines, but the form of the epimerite distinguishes the new taxon from all described species within the subfamily. The work presented herein delineates a basic extended gregarine morphometric set for the Acanthosporinae similar to that established for the Stylocephalidae (Clopton, 1999) and *Citellocephalus* by Clopton and Nolte (2002), establishes a new genus within Actinocephalidae, and describes that new taxon's type species.

### **MATERIALS AND METHODS**

*Calopteryx maculata* adults were collected using an aerial net from the following localities: Cedar Creek at the Paxton-Sarben Road, Keith Co., Nebraska, U.S.A. (41°11'02.8"N; 101°21'61.3"W) on 22 June 2001 ( $n = 21$ : 13 ♀, 8 ♂) and 1 August 2003 ( $n = 18$ : 7 ♀, 11 ♂); Clear Creek at Highway 92, Keith Co., Nebraska, U.S.A. (41°19'20.9"N; 102°02'24.9"W) on 17 June 1998 ( $n = 33$ : 18 ♀, 15 ♂) and 12 June 1999 ( $n = 51$ : 15 ♀, 36 ♂); Otter Creek at Highway 92, Keith Co., Nebraska, U.S.A. (41°18'08.3"N; 101°55'39.0"W) on 17 June 1996 ( $n = 30$ : 14 ♀, 16 ♂); Sand Creek at the Keystone-Paxton Road, Buckhorn Springs Ranch, Keith Co., Nebraska, U.S.A. (40°11'95.1"N; 101°27'57.8"W) on 21 June 2001 ( $n = 30$ : 18 ♀, 12 ♂); Turkey Creek in Johnson Co., Nebraska, U.S.A. (40°15'75.3"N; 96°21'40.4"W) on 30 July 2003 ( $n = 26$ : 14 ♀, 12 ♂), 6 August 2003 ( $n = 31$ : 28 ♀, 3 ♂), and 19 August 2003 ( $n = 20$ : 19 ♀, 3 ♂). *Calopteryx maculata*

naiads were collected using an aquatic dip net from the following localities: Cedar Creek at the Paxton-Sarben Road, Keith Co., Nebraska, U.S.A. (41°11'02.8"N; 101°21'61.3"W) on 1 August 2003 ( $n = 21$ ); Clear Creek at Highway 92, Keith Co., Nebraska, U.S.A. (41°19'20.9"N; 102°02'24.9"W) on 17 June 1998 ( $n = 27$ ); and Turkey Creek in Johnson Co., Nebraska, U.S.A. (40°15'75.3"N, 96°21'40.4"W) on 19 August 2003 ( $n = 31$ ). On postmortem examination, gregarine infections were found only in adult odonates sampled from Turkey Creek. No gregarine was found in a naiad. Odonates were examined for gregarines postmortem using protocols similar to those detailed below for the Turkey Creek samples. Adult damselflies were collected with an aerial net, placed in 3.785-liter plastic jars, and stored on ice for transportation to the laboratory at Peru State College. Two individuals were prepared as permanent voucher specimens. The remaining 77 damselflies were placed individually, abdomen down, in 10- × 75-mm disposable polystyrene tubes with 2 ml of 17-M $\Omega$  deionized water and held for ca. 24–36 hr to collect shed gametocysts. After gametocyst collection, individual damselflies were examined for gregarine infection. Damselflies were eviscerated and their alimentary canals dissected in insect muscle saline (Belton and Grundfest, 1962). Permanent parasite preparations were made using wet smears of gregarines and host gut tissues fixed by flotation on hot AFA (ethanol, formalin, and acetic acid), stained with either Semichon's acetocarmine or Harris' hematoxylin and eosin-xylol, and mounted in Damar balsam as described by Clopton (1996, 1999, 2000b). Subsamples of gregarines infecting 5 damselflies were preserved in 90% ethanol for future genetic analysis. Gametocysts were isolated from feces collected in disposable styrene tubes and from the hindgut and rectum during postmortem examinations, triple-rinsed in insect muscle saline, photographed for morphometric analysis, triple-rinsed in 17-M $\Omega$  deionized water, and transferred to individual 4- × 12-mm glass micro vials (BioQuip Products, Gardena, California, U.S.A.). Vials were sealed with white silicon stoppers after adding 50  $\mu$ l of 17-M $\Omega$  deionized water, and gametocysts were held for maturation and dehiscence. Gametocysts were observed daily, and any changes in structure, maturation, or dehiscence were noted. Oocyst structure and dimensions were taken from fresh preparations of oocysts in wet mounts and agar monolayer mounts (Clopton, 1999, 2000b).

Differences in the prevalence of gregarine infection between male and female damselflies were tested using  $\chi^2$  contingency table analysis. All analyses were conducted using SPSS Base 10.0 (SAS Institute, Carey, North Carolina, U.S.A.) with  $\alpha$  set at 0.05.

Observations were made using an Olympus B-Max 50 compound microscope with  $\times 4$ ,  $\times 20$ ,  $\times 40$ , and  $\times 100$  universal planapochromatic objectives and either phase contrast condensers or differential interference contrast prisms. Digital photographs were taken with an Olympus DP-11 digital camera through the aforementioned microscope. Measurements were taken from digitized images of preserved specimens using Image-Pro Express® v 4.0 image analysis software (Media Cybernetics, L.P., Silver Spring, Maryland, U.S.A.). Drawings were made using digitized images of live and fixed specimens. Photographic plates were processed and assembled using Adobe® Photoshop® 7.0.1 software (Adobe Systems, Inc., San Jose, California, U.S.A.).

An extended acanthosporine morphometric character set is delineated by Figures 1–4. The morphometric set is consistent with those proposed by Clopton (1999), Kula and Clopton (1999), and Clopton and Nolte (2002) but includes additional metrics particular to the genus of study. The structure of the mature trophozoite, particularly the epimerite, is diagnostic among Actinocephalidae. Fully mature trophozoites retaining functional epimerites typically persist until just before syzygy, precluding an extended gamontic stage and reducing the diagnostic importance of gamontic characters within the group. Thus, the morphometric set delineated herein differs from those of Clopton (1999), Kula and Clopton (1999), and Clopton and Nolte (2002) in that descriptive focus is placed on mature trophozoites rather than gamonts. As suggested by Filipponi (1949) and implemented by Clopton (1999), the holdfast of the taxon described herein is considered a compound structure composed of a terminal epimerite or holdfast proper and an intercalating diamerite. Measurements are presented in micrometers as mean values followed by range values, SDs, and sample sizes in parentheses. Terminology for parasite ontogenetic stages and anatomy largely follows that proposed by Levine (1971). Terminology for shapes of planes and solids is according to that of Clopton (2004). Additional terminology is derived from Harris and Harris (1994).

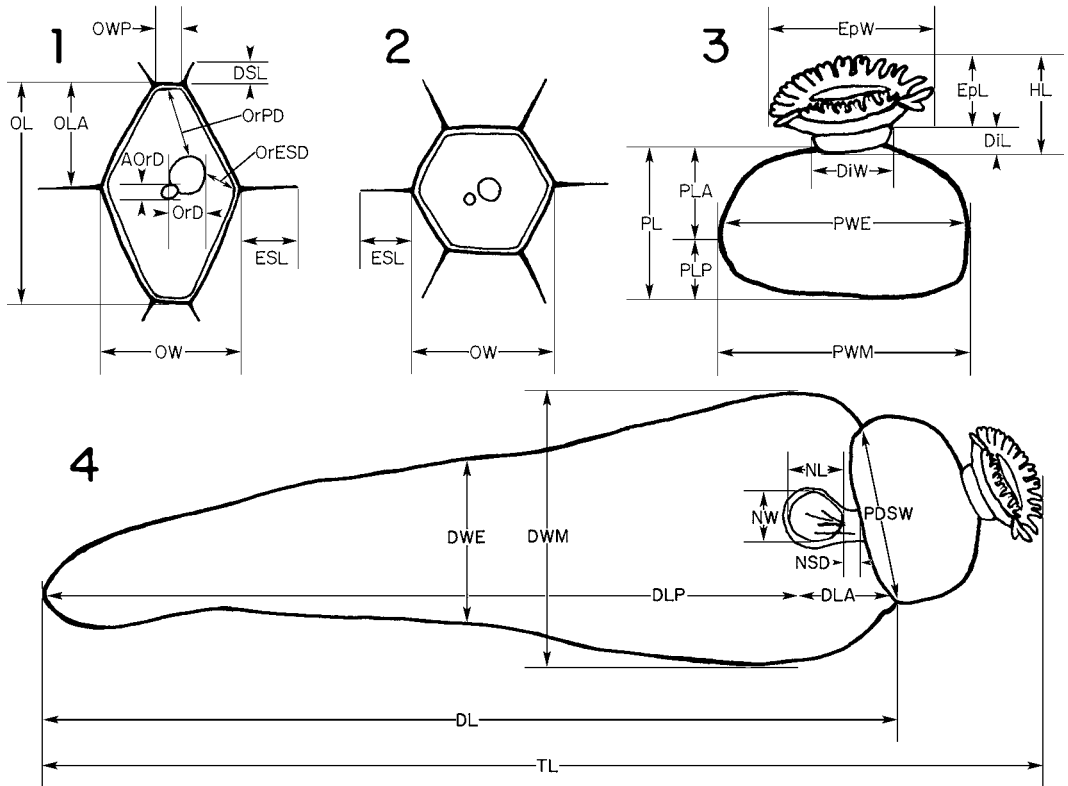
### ***Calyxocephalus* g. nov. (Figures 1–5, 13–27)**

*Diagnosis:* Eugregarinorida Léger, 1892, sensu Clopton (2000a); Septatorina Lankester, 1885, sensu Clopton (2000a); Stenophoricae Levine, 1984, sensu Clopton (2000a); Actinocephalidae Léger, 1892, sensu Clopton (2000a); Acanthosporinae Léger, 1892, sensu Clopton (2000a); with characters of the genus *Calyxocephalus* g. nov.: holdfast compound, comprising a terminal epimerite and intercalating diamerite, epimerite a thick disk or linearly crateriform sucker with distal apetalous calyx of petaloid lobes, diamerite short (less than half of the total holdfast length) very broadly obdeltoid; association late, cephalolateral, biassociative; gametocysts spherical, hyaline coat present, conspicuous sporoducts absent, dehiscing by simple rupture to release individual oocysts en masse; oocysts axially symmetric, hexagonal dipyramidic in shape with slight polar truncations, bearing 6 equatorial spines, 1 at each equatorial vertex, 6 terminal spines obliquely inserted at each pole, 1 at each vertex created by polar truncation.

### **Taxonomic summary**

*Type species:* *Calyxocephalus karyopera* sp. nov.

*Etymology:* The generic name *Calyxocephalus* is taken from the Greek root, *calyx*, meaning a cup. The name is given to mark the resemblance between



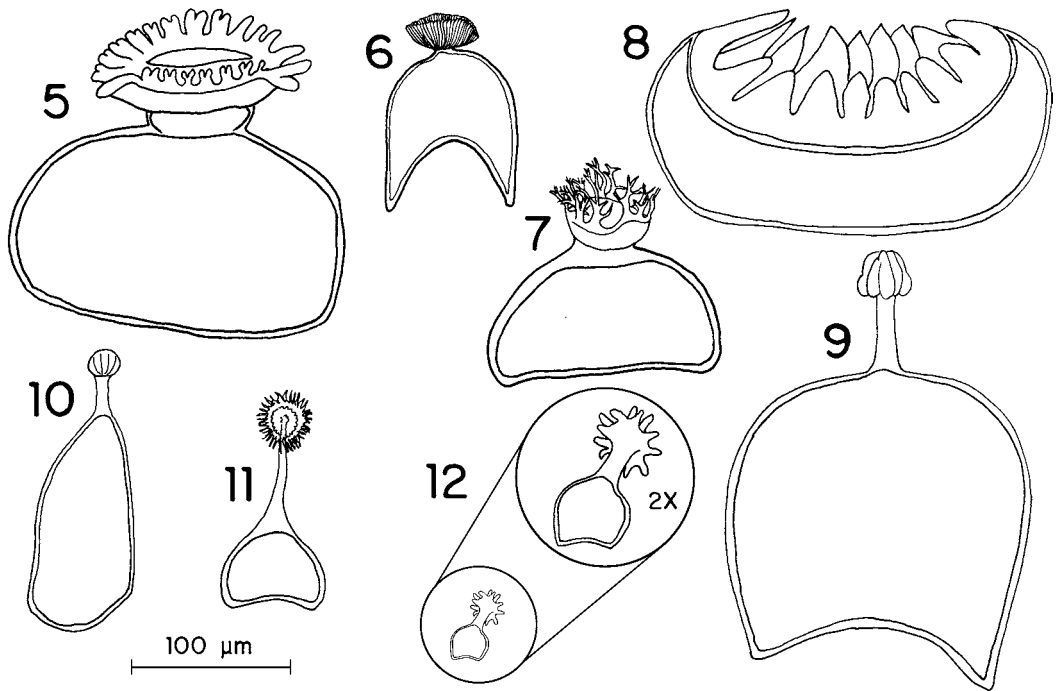
**Figures 1–4.** Diagrammatic delineation of the extended gregarine morphometric character set for *Calyxocephalus* g. nov. **1.** Oocyst, lateral aspect. **2.** Oocyst, polar aspect. **3.** Epimerite–protomerite complex, lateral aspect. **4.** Mature trophozoite, lateral aspect. AOrD, diameter of accessory oocyst residuum; DiL, length of diamerite; DiW, width of diamerite at widest transect; DL, length of deutomerite; DLA, distance from widest transect of deutomerite to protomerite–deutomerite septum; DLP, distance from widest transect of deutomerite to posterior tip of deutomerite; DSL, length of dorsal or polar spine; DWE, width of deutomerite at equator; DWM, width of deutomerite at widest transect; EpL, length of epimerite; EpW, width of epimerite; ESL, length of equatorial spine; HL, total length of holdfast; NL, length of nucleus parallel to axis of deutomerite; NSD, distance from nucleus to protomerite–deutomerite septum; NW, width of nucleus perpendicular to axis of deutomerite; OL, length of oocyst; OLA, distance from oocyst equator to oocyst pole; OrD, diameter of oocyst residuum; OrESD, distance from oocyst residuum to base of closest equatorial spine; OrPD, distance from oocyst residuum to oocyst pole; OW, width of oocyst at equator; OWP, width of oocyst at polar truncation; PDSW, width of protomerite at protomerite–deutomerite septum; PL, length of protomerite; PLA, distance from widest transect of protomerite to anterior end of protomerite; PLP, distance from widest transect of protomerite to posterior end of protomerite; PWE, width of protomerite at equator; PWM, width of protomerite at widest transect; TL, total length of trophozoite including epimerite complex.

the epimerite holdfast and the outer perianth whorl of a flower.

### Remarks

Gamontic association, the timing of syzygy, and the mode of gametocyst dehiscence in *Calyxocephalus* are consistent with the cardinal characters of Actinocephalidae. *Calyxocephalus* shares the cardinal oocyst characters of Acanthosporinae but is clearly distinguished from known acanthosporine genera by epimeritic structure.

Acanthosporinae comprise 23 genera parasitizing carnivorous arthropods, primarily aquatic insects (Clopton, 2000a). These taxa are distinguished primarily by differences in oocyst and epimerite structure although the relative weight of these characters at the generic level remains the subject of some debate (compare relative weight given these characters in the revisions of Amoji and Kori [1992], Sarkar [1995], and Clopton [2000a]). The oocysts of *Calyxocephalus* take the form of a hexagonal bipyramid bearing polar and equatorial spines morphologically similar to oocysts described for 7



**Figures 5–12.** Comparative epimerite structure among representative of 8 acanthosporine genera infecting odonates. **5.** *Calyxocephalus karyopera*, g. nov., sp. nov. **6.** *Munkundaella undulatus* (after Sarkar [1981]). **7.** *Ramiccephalus ozakii* (after Obata [1953]). **8.** *Rodgiella ceriagrioni* (after Ahamed Nazeer and Narasimhamurti [1979]). **9.** *Nubenocephalus nebraskensis* (after Clopton et al. [1993]). **10.** *Acanthospora bengalensis* (after Sarkar and Haldar [1981a]). **11.** *Tetrameridionospinospora karnatakii* (after Kori and Amoji [1985]). **12.** *Ancyrophora ischnurae* (after Sarkar and Haldar [1981b]), lower left figure drawn to scale for comparison, upper right inset figure drawn twice plate scale to reveal detail of epimerite structure.

Acanthosporine genera that also infect odonates: *Acanthospora* Léger, 1892 (e.g., *Acanthospora bengalensis* Sarkar & Haldar, 1981, q.v. Sarkar and Haldar [1981a]), *Ancyrophora* Léger, 1892 sensu Baudoin, 1971 (e.g., *Ancyrophora gigantea* Hoshide, 1953, q.v. Hoshide [1953, 1959]; *Ancyrophora ischnurae* Sarkar and Haldar, 1981, q.v. Sarkar and Haldar [1981b]; *Ancyrophora ovooides* Sarkar and Haldar, 1981, q.v. Sarkar and Haldar [1981b]), *Munkundaella* Sarkar, 1981 (e.g., *Munkundaella undulatus* Sarkar, 1981, q.v. Sarkar [1981]), *Nubenocephalus* Clopton, Percival and Janovy, 1993 (e.g., *Nubenocephalus nebraskensis* Clopton, Percival and Janovy, 1993, q.v. Clopton et al. [1993]), *Ramiccephalus* Obata, 1953 (e.g., *Ramiccephalus ozakii* Obata, 1953, q.v. Obata [1953]), *Rodgiella* Sarkar, 1995 (e.g., *Rodgiella ceriagrioni* (Ahamed Nazeer and Narasimhamurti, 1979) Sarkar, 1995, q.v. Ahamed Nazeer and Narasimhamurti [1979]), and *Tetrameridionospinospora* Kori and Amoji, 1985 (e.g., *Tetrameridionospinospora karnatakii* Kori and Amoji, 1985, q.v. Kori and Amoji [1985]). An eighth

acanthosporine genus infecting odonates, *Prismatospora* Ellis, 1914, is characterized by oocysts that are regular hexagonal prisms. The intercalation of rectangular faces between the opposing bipyramid poles clearly distinguishes this genus from *Calyxocephalus* and the 7 other acanthosporine genera mentioned above. *Calyxocephalus* is distinguished from known acanthosporine genera with similar oocyst morphology by clear differences in epimerite morphology (Figs. 5–9). The epimerite of *Calyxocephalus* is a broad disk or linearly crateriform sucker with an apopetalous margin of petaloid lobes that is attached to the protomerite by a short (less than half of the total holdfast length) very broadly obdeltoid intercalating diamerite (Fig. 5). Of the existing acanthosporine genera with dipyramidic oocysts, only *Munkundaella* and *Ancyrophora* possess a short intercalating diamerite (Figs. 6, 12). In *Ramiccephalus* (Fig. 7) and *Rodgiella* (Fig. 8) the epimerite is directly fused to the protomerite, whereas in *Nubenocephalus* (Fig. 9), *Acanthospora* (Fig. 10), and *Tetrameridionospinospora* (Fig. 11) the epimerite is

attached to the protomerite by a long (more than half of the total holdfast length) intercalating diamerite. The epimerite of *Calyxocephalus* is characterized by an apopetalous margin of petaloid lobes that is absent in *Mukundaella* and *Ancyrophora*. Conversely, the epimerite of *Mukundaella* is an unornamented broadly to depressed obdeltoid cup with numerous distinct axial folds or undulations extending the length of the epimerite, whereas that of *Ancyrophora* is subspherical to discoid with a terminal surface bearing numerous straight or crooked digitiform lobes. These epimeritic structures are distinct and clearly differentiate *Calyxocephalus*, *Mukundaella*, and *Acanthospora*.

***Calyxocephalus karyopera* sp. nov.**

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**(Figures 1–5, 13–27)**

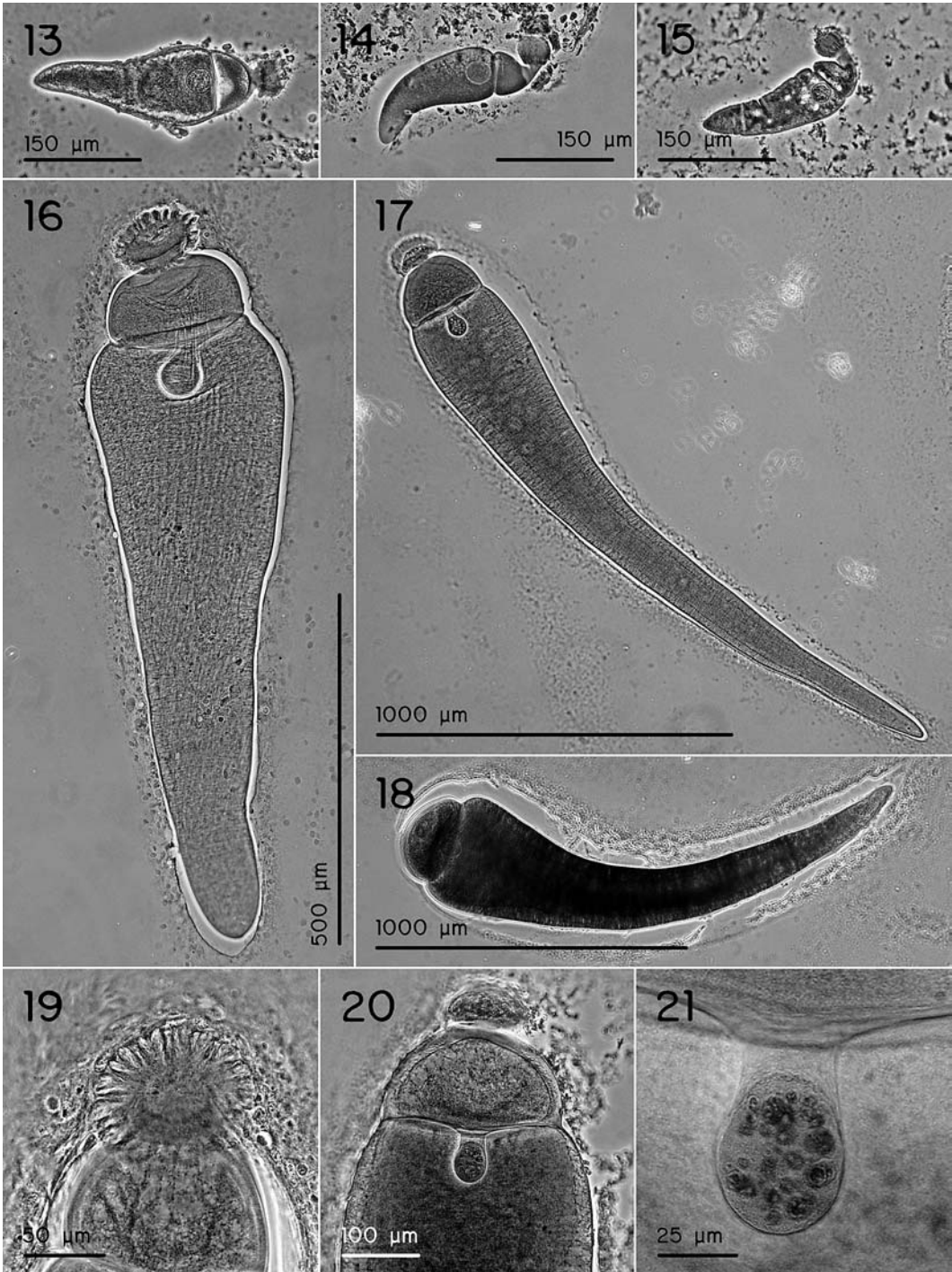
*Immature trophozoite* (Figs. 13–15): Developing trophozoites solitary, attached to host ventricular epithelium. Epimerite very broadly elliptoid with evidence of marginal petaloid lobe development, diamerite cylindrical, short, less than half of the holdfast length; protomerite deltoid with distinct constriction at protomerite–deutomerite septum; deutomerite finely obdeltoid, ca. 4 times the length of protomerite; nucleus spherical, placement axial, adjoining protomerite–deutomerite septum.

*Mature trophozoite* (Figs. 3–5, 16, 17, 19–21): Holdfast compound, comprising a terminal epimerite and intercalating diamerite, epimerite a thick disk or linearly crateriform sucker with distal apopetalous calyx of 17–21 obtuse bifid petaloid lobes, diamerite short (less than half of the total holdfast length) very broadly obdeltoid, total holdfast length (HL) 65.9 (47.7–99.5,  $\pm 13.21$ , 30), epimerite length (EpL) 50.6 (31.7–72.4,  $\pm 11.02$ , 30), epimerite width (EpW) 106.3 (77.2–146.1,  $\pm 16.47$ , 30), diamerite length (DiL) 15.1 (6.9–24.8,  $\pm 3.95$ , 30), diamerite width (DiW) 46.7 (28.1–62.9,  $\pm 9.05$ , 30). Protomerite depressed ovoid, length (PL) 109.4 (82.0–191.7,  $\pm 23.5$ , 30), width at equatorial transect (PWE) 200.2 (129.0–297.4,  $\pm 47.41$ , 30), width at widest transect (PWM) 214.7 (139.1–305.9,  $\pm 51.26$ , 30), distance from widest transect of protomerite to anterior end of protomerite (PLA) 76.9 (49.4–127.5,  $\pm 18.34$ , 30), distance from widest transect of protomerite to posterior end of protomerite (PLP) 30.8 (8.1–66.2,  $\pm 14.31$ , 30). Protomerite–deutomerite septum clearly marked, with conspicuous marginal constriction, width of protomerite at protomerite–deutomerite septum (PDSW) 210.8 (129.9–302.1,  $\pm 51.11$ , 30).

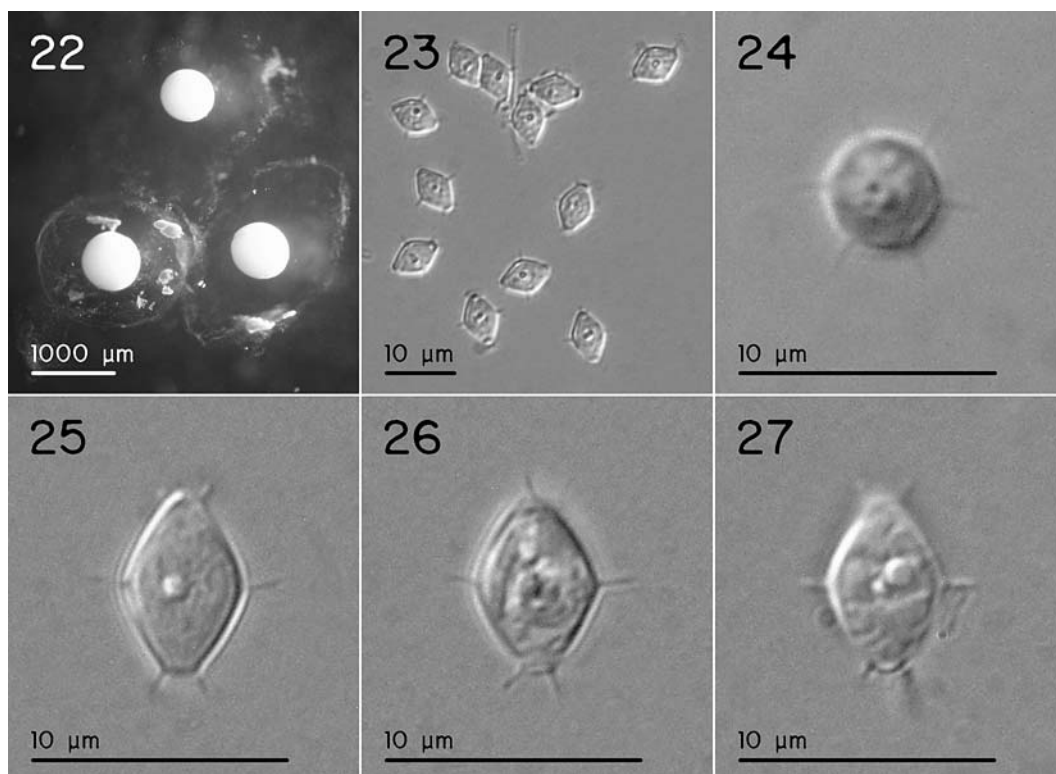
Deutomerite narrowly obovoid to very narrowly obdeltoid, anterior margin truncate at junction with protomerite, length (DL) 1,087.1 (657.0–1,826.4,  $\pm 299.9$ , 30), width at equatorial transect (DWE) 196.5 (124.8–267.8,  $\pm 42.61$ , 30), maximum width (DWM) 271.4 (167.0–416.3,  $\pm 65.92$ , 30), distance from protomerite–deutomerite septum to DWM (DLA) 89.8 (15.9–165.7,  $\pm 32.11$ , 30), distance from DWM to posterior end of deutomerite (DLP) 999 (587.0–1697.8,  $\pm 288.02$ , 30). Total length (TL) 1,255 (793.4–2,047.9,  $\pm 318.44$ , 30). Indices: HL/EpL 1.3 (1.1–1.6,  $\pm 0.12$ , 30), EpL/EpW 0.5 (0.3–0.7,  $\pm 0.09$ , 30), HL/DiL 4.6 (2.9–8.6,  $\pm 1.19$ , 30), DiL/DiW 0.3 (0.2–0.7,  $\pm 0.11$ , 30), EpW/DiL 7.5 (4.6–13.4,  $\pm 2.17$ , 30), EpL/DiL 3.5 (1.8–7.6,  $\pm 1.17$ , 30), PL/PWE 0.6 (0.4–0.8,  $\pm 0.12$ , 30), PL/PWM 0.5 (0.3–0.7,  $\pm 0.11$ , 30), PL/PDSW 0.5 (0.3–0.8,  $\pm 0.12$ , 30), PLA/PL 0.7 (0.5–1.0,  $\pm 0.11$ , 30), PLA/PLP 3.5 (1–10.6,  $\pm 2.79$ , 30), PWM/PWE 1.1 (1–1.2,  $\pm 0.05$ , 30), DiL/PL 0.1 (0.1–0.2,  $\pm 0.04$ , 30), DL/DWE 5.6 (3.7–10.6,  $\pm 1.45$ , 30), DL/DWM 4.1 (2.9–6.2,  $\pm 0.8$ , 30), DLA/DL 0.1 (0.1–0.1,  $\pm 0.03$ , 30), DLA/DLP 0.1 (0.1–0.2,  $\pm 0.03$ , 30), DWM/DWE 1.4 (1.2–1.8,  $\pm 0.17$ , 30), TL/PL 11.6 (8.3–17.7,  $\pm 2.23$ , 30), DL/PL 10 (6.9–16,  $\pm 2.2$ , 30), DWM/PWM 1.3 (1.1–1.5,  $\pm 0.12$ , 30), TL/DL 1.2 (1.1–1.2,  $\pm 0.03$ , 30). Nucleus (Figs. 16, 17, 20, 21) broadly elliptoid, placement axial, anterior length (NL) 52.7 (42.2–69.2,  $\pm 6.4$ , 30), width (NW) 47.8 (36.6–63.7,  $\pm 7.32$ , 30); with numerous distinct, circular karyosomes (Figs. 20, 21), nucleus contained within a distinct membranous sack or nuclear suspensorium apparently derived from protomerite–deutomerite septum (Figs. 16, 17, 20, 21), distance from protomerite–deutomerite septum to nucleus (NDS) 15.0 (7.0–30.9,  $\pm 6.34$ , 30), NL/NW 1.1 (0.8–1.4,  $\pm 0.16$ , 30), NSD/NL 0.3 (0.1–0.6,  $\pm 0.11$ , 30), DL/NSD 20.6 (14.3–32.8,  $\pm 4.94$ , 30).

*Gamonts and association* (Fig. 18): Solitary gamonts rare, isogamontic, similar in morphology and size to mature trophozoites but lacking holdfast structures; gamonts enter cephalolateral association immediately before syzygy.

*Gametocysts* (Fig. 22): White in color; roughly spherical, diameter (GD) 702.6 (658.7–734.1,  $\pm 39.23$ , 3), hyaline coat present but of irregular thickness, minimum thickness (HC) 555.2 (444.9–689.2,  $\pm 123.86$ , 3), overall diameter (GDT) 1,795.5 (1,597.6–1,915.0,  $\pm 172.67$ , 3), HC/GD 0.8 (0.6–1,  $\pm 0.23$ , 3), HC/GDT 0.3 (0.3–0.4,  $\pm 0.05$ , 3), GD/GDT 0.4 (0.4–0.5,  $\pm 0.06$ , 3). Gametocysts stored



**Figures 13–21.** Trophozoites of *Calyxocephalus karyopera*, g. nov., sp. nov. **13–15.** Young trophozoites with incompletely developed epimerite complexes. **16, 17.** Mature trophozoites with fully developed epimerite complexes. **16.** A photograph of the holotype. **18.** Gamont separated from partner at onset of lateral association. **19.** Mature epimerite complex, en face aspect demonstrating apopetalous bifid nature of marginal petaloid lobes. **20, 21.** Nucleus of mature trophozoite demonstrating nuclear suspensorium and distinct polykaryotic nature of nucleus.



**Figures 22–27.** Gametocysts and oocysts of *Calyxocephalus karyopera*, g. nov., sp. nov. **22.** Gametocysts with gelatinous hyaline sheaths, bright field. **23.** Oocysts en masse, differential interference contrast. **24.** Oocyst, optical section through equator in polar aspect, differential interference contrast. **25–27.** Oocysts, optical sections in lateral view demonstrating placement and nature of spines and oocyst residua, differential interference contrast.

under distilled water mature within 40 hr and dehisce by simple rupture to release individual oocysts en masse; conspicuous sporoducts absent; epispore packet absent, no gametocyst residuum observed.

*Oocysts* (Figs. 1, 2, 23–27): Axially symmetric, very uniform in size and shape, dipyramidic in shape with slight polar truncations, length (OL) 7.2 (6.9–7.7,  $\pm 0.16$ , 36), width at equator (OW) 4.8 (4.5–5.1,  $\pm 0.14$ , 36), width of oocyst at polar truncation (OWP) 0.9 (0.6–1.1,  $\pm 0.14$ , 36), distance from oocyst equator to oocyst pole (OLA) 3.6 (3.3–3.8,  $\pm 0.1$ , 36), bearing 6 equatorial spines, 1 at each equatorial vertex, length of equatorial spine (ESL) 1.6 (1.4–1.9,  $\pm 0.15$ , 36), 6 terminal spines obliquely inserted at each pole, 1 at each vertex created by polar truncation, length of dorsal or polar spine (DSL) 1.1 (0.7–1.3,  $\pm 0.14$ , 36), oocyst residuum present, smooth, diameter (OrD) 1.0 (0.6–1.3,  $\pm 0.14$ , 36), distance from oocyst residuum to base of closest equatorial spine (OrESD) 1.7 (1.2–2.0,  $\pm 0.18$ , 36),

oocyst residuum eccentricity or distance from oocyst residuum to oocyst pole (OrPD) 3.0 (2.4–3.2,  $\pm 0.18$ , 36), accessory oocyst residuum present, smooth, diameter (OSrD) 0.5 (0.3–0.7,  $\pm 0.07$ , 36); released from gametocyst by simple rupture as individual oocysts en masse. Indices: OL/OW 1.5 (1.4–1.6,  $\pm 0.05$ , 36), OL/OWP 8.2 (6.3–12.5,  $\pm 1.5$ , 36), OL/OLA 2 (1.9–2.1,  $\pm 0.05$ , 36), OL/OrPD 2.5 (2.2–3,  $\pm 0.15$ , 36), OL/DSL 7 (5.5–9.8,  $\pm 0.99$ , 36), OW/OWP 5.4 (4.0–8.1,  $\pm 0.94$ , 36), OW/ESL 3.0 (2.4–3.6,  $\pm 0.28$ , 36), ESL/DSL 1.6 (1.2–2.4,  $\pm 0.24$ , 36), OW/OrESD 2.9 (2.5–4.1,  $\pm 0.34$ , 36), OrD/OSrD 2.0 (1.6–2.6,  $\pm 0.23$ , 36).

### Taxonomic summary

*Host:* *Calopteryx maculata* (Beauvois, 1805) (Odonata: Zygoptera: Calopterygidae), Ebony Jewelwing damselfly.

*Symbiotype:* Two symbiotype specimens (authors' specimens REC030263, REC030255) are deposited

in the Sam Houston State University Insect Collection (SHSUIIC), Department of Biology, Sam Houston State University, Huntsville, Texas, U.S.A. Both symbiotype specimens are stored in 95% ethanol and include a 2-line collection label: "Turkey Creek, Johnson Co., Nebraska, USA; August 19, 2003, R E Clopton, leg." The reverse is labeled with the author's specimen number and the word "SYMBIOTYPE" (SHSUIIC does not assign individual accession numbers).

*Host records:* *Calopteryx maculata*; adults.

*Localities:* Turkey Creek tributary, Johnson Co., Nebraska, U.S.A. (40°15'75.3"N, 96°21'40.4"W); Turkey Creek, Johnson Co., Nebraska, U.S.A. (40°15'49.5"N, 96°21'03.3"W) (type locality).

*Infection site:* Trophozoites and gamonts were collected from the ventriculus. Gametocysts were collected from the rectum or passed feces.

*Prevalence:* Overall sample prevalence 51.9% (40 of 77 damselflies examined postmortem); males, 27.3% (12/44); females, 84.8% (28/33). Prevalence was significantly higher in female damselflies than in males ( $P [\chi^2] < 0.00001$ ). July sample prevalence: overall, 53.8% (14/26); males, 35.7% (5/14); females 75% (9/12). Prevalence was significantly higher in female damselflies than in males ( $P [\chi^2] = 0.045$ ). August sample prevalence: overall, 51.0% (26/51); males, 23.3% (7/30); females, 90.5% (19/21). Prevalence was significantly higher in female damselflies than in males ( $P [\chi^2] < 0.00001$ ).

*Specimens deposited:* The holotype and paratype slides are deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. The holotype slide HWML 45474 (author's slide REC030244C) contains multiple trophozoites. The holotype specimen is marked by an etched circle; the remaining individuals are paratypes. The paratype series includes 47 slides containing trophozoites and gamonts: HWML 45475–45502 (REC030108A-B, REC030110, REC030111A-E, REC030115A-B, REC030122, REC030123, REC030125, REC030127A-B, REC030129, REC030130, REC030131, REC030132A-B, REC030230, REC030241, REC030244A-B, REC030250A-C, REC030251A, REC030252, REC030253, REC030254A-D, REC030256B-C, REC030257, REC030258A-C, REC030261A-B, REC030261D-F, REC030262, REC030268). No paratype specimen is retained by the authors.

*Etymology:* The specific epithet *karyopera*, transliterally "nut sack," is derived from Greek and is given to mark the unique nuclear suspensorium characteristic of the species.

## Remarks

This work establishes *Calyxocephalus* within the Acanthosporinae with the description of *C. karyopera*. The morphometric character set established for the genus includes both nuclear and oocyst characters worthy of comment.

In their ultrastructural study of the nucleus of *Hoshideia polyhamatus* (Hoshide, 1977) Sarkar, 1995, (= *Hoplorhynchus polyhamatus* Hoshide, 1977, = *Odonaticola polyhamatus* (Hoshide, 1977) Amoji and Kori, 1992) Hoshide and Janovy (2002) reported a "thread-like structure that connected the nucleus to the center of the septum" and a fine fibrous network encasing the nucleus. This nuclear suspensorium effectively fixed the position of the nucleus within the deutomerite. A distinct nuclear suspensorium is also reported for *C. karyopera*. As in *H. polyhamatus*, the nuclear suspensorium of *C. karyopera* fixes the axial and equatorial position of the nucleus, but rather than a thin thread-like suspensor, the suspensorium in this case is a broad sack that is readily observed in stained specimens under light microscopy (Figs. 16, 17, 20, 21). Hoshide and Janovy (2002) demonstrate that nuclear position can have a structural basis among the gregarines and suggest that nuclear position may be an important character for gregarine taxonomy. The data presented herein confirm their assertion and suggest that the form and structure of the nuclear suspensorium may also be systematically informative.

It is difficult to establish clear homologies between the gametocysts and oocysts of gregarines and similar ontogenetic structures among other apicomplexan taxa. (As the initial work of zygotes reflecting the immediate products of fertilization, oocysts appear to be uniformly homologous across apicomplexan groups, but gregarine gametocysts and coccidian sporocysts, for example, have no obvious mutual homologs.) However, analogous structures across Apicomplexa can indicate potentially informative taxonomic characters. The extended morphometric set delineated herein establishes the oocyst residuum and the accessory oocyst residuum as taxonomic characters. Whereas their eventual utility cannot be determined until additional data are collected across a range of gregarine taxa, they are analogs of the sporocyst residuum and accessory sporocyst residuum in



**Table 1.** Sample size, prevalence, and  $P$  ( $\chi^2$ ) of a significant difference in prevalence between host sexes for adults of 7 odonate species infected with gregarines.

| Host taxon                     | Sample size |     |     | Prevalence (%) |      |       | $P$ ( $\chi^2$ ) |
|--------------------------------|-------------|-----|-----|----------------|------|-------|------------------|
|                                | ♂ + ♀       | ♂   | ♀   | ♂ + ♀          | ♂    | ♀     |                  |
| <i>Calopteryx maculata</i>     | 77          | 44  | 33  | 51.9           | 27.3 | 84.8  | <0.00001         |
| <i>Calopteryx virgo</i> *      | 187         | 104 | 83  | 49.2           | 8.7  | 100.0 | <0.00001         |
| <i>Pyrrhosoma nymphula</i> †   | 138         | 87  | 51  | 73.9           | 78.2 | 66.7  | 0.137737         |
| <i>Enallagma cyathigerum</i> † | 265         | 156 | 109 | 80.8           | 78.2 | 84.4  | 0.207852         |
| <i>Coenagrion hastulatum</i> † | 38          | 26  | 12  | 84.2           | 84.6 | 83.3  | 0.919753         |
| <i>Lestes sponsa</i> †         | 427         | 297 | 130 | 27.2           | 24.6 | 33.1  | 0.069277         |
| <i>Sympetrum danae</i> †       | 437         | 297 | 140 | 79.6           | 78.8 | 81.4  | 0.522425         |

\* Data from Åbro (1996).

† Data from Åbro (1974).

coccidians, 2 characters of distinct taxonomic utility within *Eimeria*.

## DISCUSSION

Comprehensive understanding of gregarine evolution depends on a conceptual framework encompassing probable selective pressures. Previous studies suggest that selective pressures on gametocysts and oocysts play a pivotal role gregarine in evolution (Clopton and Janovy, 1993; Clopton and Gold, 1995, 1996). Adaptations of gametocyst and oocyst stages to exogenous and host selective pressures directly influence transmission patterns, mechanisms, and host encounter rates, providing a parsimonious route to exogenous niche segregation, reproductive isolation, and eventual speciation. Detailed knowledge of host behavior, diet, and habitat use is requisite to conceptualize parasite transmission and consequently parasite adaptation and primary selective pressures. The *C. karyopera*–*C. maculata* host–parasite system is particularly well suited to studies of gregarine transmission and species isolating mechanisms because *Calopteryx* includes charismatic, well-known insect species often used in studies of parasite ecology, insect behavior, and evolutionary ecology (Johnson, 1962; Waage, 1972; Forsyth and Montgomerie, 1987; Marden and Wagge, 1990; Marden and Rollins, 1994; Åbro, 1996; Taylor and Merriam, 1996; Siva-Jothy and Plaistow, 1999; Fitzstephens and Getty, 2000; Jonsen and Taylor, 2000; Misof et al., 2000; Siva-Jothy, 2000; Kirkton and Schultz, 2001; Beukema, 2002; Burcher and Smock, 2002; see also studies referenced by Dunkle, 1990; Westfall and May, 1996), and thus their natural history is well documented.

When combined with known *Calopteryx* natural history, infection patterns of *C. karyopera* within

*C. maculata* populations suggest the broad mechanism of transmission, potential gregarine species isolating mechanisms, and limiting factors for parasite distribution in this host–parasite system. Prevalence of *C. karyopera* is significantly higher in adult females than in adult males of *C. maculata* (Table 1). Extraction and reanalysis of gregarine infection data for 6 other odonate species (Åbro, 1974, 1996) reveal no sex-biased difference within samples of *Sympetrum danae* (Sulzer, 1776) (Odonata: Anisoptera: Libellulidae), *Pyrrhosoma nymphula* (Sulzer, 1776) (Odonata: Zygoptera: Coenagrionidae); *Enallagma cyathigerum* (Charpentier, 1840) (Odonata: Zygoptera: Coenagrionidae); *Coenagrion hastulatum* (Charpentier, 1825) (Odonata: Zygoptera: Coenagrionidae); or, *Lestes sponsa* (Hansemann, 1823) (Odonata: Zygoptera: Lestidae) (Table 1; Åbro, 1974, 1996). However, gregarine infections in *Calopteryx virgo* (Linnaeus, 1758) (Odonata: Zygoptera: Calopterygidae) display the same pattern of significantly higher prevalence in females than in males found in *C. maculata* (Table 1; Åbro, 1996). Sex-biased differences in gregarine prevalence indicate a difference in oocyst encounter rates between adult male and female damselflies. That prevalence differences appear unique to Calopterygidae suggests that these differential encounter rates are the product of host natural history. Differences in relative prevalence by sex across host taxa (Table 1) indicate that differences in the behavior of male and female *Calopteryx* decrease the oocyst encounter rate of males, whereas females encounter oocysts at a rate equivalent to or greater than that of other odonates.

The life cycle of *C. maculata* revolves around small, shaded, forest streams where adults mate and oviposit and naiads develop in association with organic debris dams (Johnson, 1962; Smock, 1988;

Dunkle, 1990; Burcher and Smock, 2002). Adult males are extraordinarily territorial (Johnson, 1962; Dunkle, 1990; Waage, 1972), defending with extreme fidelity a relatively small (2–3 m) permanent territory from other *C. maculata* males for up to 20 d of their 35 d adult lifespan (Dunkle, 1990; Westfall and May, 1996; Kirkton and Schultz, 2001). These mating territories tend to overlook or overhang the stream and serve as both perches for mate acquisition and overstream feeding flights (Johnson, 1962; Kirkton and Schultz, 2001). Teneral males pass through a brief (4 d) preterritorial stage in which their behavior is markedly different and emulates the nonmating behavior of the adult female (Waage, 1972; Kirkton and Schultz, 2001). In contrast with the strict territoriality of adult males, adult females and preterritorial males of *C. maculata* tend to wander from the stream proper, foraging for flying insects in sunny forest glades and roosting in forest vegetation far from the stream itself (Forsyth and Montgomerie, 1987; Taylor and Merriam, 1996; Westfall and May, 1996).

Sexual differences in the foraging habit of territorial and nonterritorial adult *C. maculata* result in differential prey use between sexes as well as between territorial and preterritorial males. Åbro (1996) examined prey availability and use by *P. nymphula* and *E. cyathigerum*, 2 coenagrionid damselflies with a woodland–riparian hunting and roosting strategy similar to that of female *C. maculata*. Four dipteran families constituted 88% of available prey (Chironomidae: Orthoclaadiinae, 64%; Ceratopogonidae, 11%; Empididae, 8%; and Cecidomyiidae, 5%) and 88% of prey items recovered in a damselfly gut analysis study (Chironomidae: Orthoclaadiinae, 59%; Ceratopogonidae, 15%; Empididae, 6%; and Cecidomyiidae, 8%) (Åbro, 1996). These insects are associated with aquatic systems but their larvae are primarily terrestrial, developing in moist soils and lichens (Chironomidae: Orthoclaadiinae) (McAlpine, 1990; Teskey, 1990; Hilsenhoff, 1991; Chu and Cutkomp, 1992; Delettre, 2000); leaf axils, tree holes and moist soil (Ceratopogonidae) (McAlpine, 1990; Teskey, 1990; Hilsenhoff, 1991; Chu and Cutkomp, 1992; Ronderos and Diaz, 2002); decaying vegetation, dead wood, soil, and mosses (Empididae) (McCafferty, 1983; McAlpine, 1990; Teskey, 1990; Hilsenhoff, 1991; Chu and Cutkomp, 1992; Delettre et al., 1998); and, moist humus, soil, and under the bark of fallen trees (Cecidomyiidae) (McAlpine, 1990; Teskey, 1990; Chu and Cutkomp, 1992). Thus, they form a woodland–riparian fauna that is associated with, but distinct from, the adjacent stream fauna (Teskey,

1990; Delettre et al., 1992, 1998; Delettre, 2000; Delettre and Morvan, 2000). In contrast to the woodland–riparian hunting and roosting habitat of females and preterritorial males of *C. maculata*, nonorthoclinid chironomid midges are the dominant component of the aerial community in most stream systems, followed by ceratopogonids and trichopterans (e.g., Delettre et al., 1992; Lynch et al., 2002). Thus, sexual differences in foraging habit (stream vs. woodland–riparian) result in differential prey use with territorial males concentrating primarily on aquatic chironomids and ceratopogonids emerging from the stream itself, whereas preterritorial males and females hunt ceratopogonid and orthoclinid chironomid midges that complete their life cycle in the moist humus and duff of the woodland floor. If the prey act as mechanical vector for gregarine oocysts, sexual differences in prey use would be reflected by sexual differences in gregarine prevalence and would also suggest how mechanisms for parasite isolation can arise from the interaction of vector-based oocyst transmission and host behavior.

Åbro (1996) recovered wild ceratopogonid and orthoclinid chironomid midges with acanthosporine gregarine oocysts entangled in the bristles of their tarsal segments and postulated that these insects acted as mechanical vectors of gregarines infecting *P. nymphula* and *E. cyathigerum*, 2 coenagrionid damselflies with a forest hunting and roosting strategy similar to that of *C. maculata*. It seems likely that transmission of *C. karyopera* involves a similar mechanical vector strategy that results in a higher oocyst encounter rate for preterritorial male and female *C. maculata* than for territorial males. High-fidelity male territoriality forces the use of a different insect resource base that does not include species that act as mechanical vectors for *C. karyopera*. Thus, the parasite population is probably supported largely by female hosts: all male infections could easily be the result of preterritorial foraging behavior. The lack of naiad infections in areas where gregarine prevalence in adults approaches 85% supports the hypothesis of a forest-based exogenous cycle for *C. karyopera*. Environmental conditions and resulting selective pressures on gametocyst and oocyst development, in addition to the specialized oocyst morphology required to use a mechanical prey–vector provide mechanisms for isolation and speciation of gregarines parasitizing *Calopteryx* from sympatric gregarines parasitizing other Zygoptera. Clopton et al. (2004) suggested that strict host habitat fidelity contributes to isolation and subsequent speciation of gregarine populations,

producing higher parasite diversity than might otherwise be expected. Territoriality and male aggression in this damselfly system apparently functions in a similar manner with subtle but significant implications for the evolution of gregarine diversity. The system provides isolation from other sympatric zygopteran-gregarine systems but also includes selective pressures that stabilize nonaquatic exogenous gregarine development dependent on a mechanical prey-vector for stable transmission.

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