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TWO NEW SPECIES OF *XIPHOCEPHALUS* IN *ELEODES TRICOSTATA* AND *ELEODES FUSIFORMIS* (COLEOPTERA: TENEBRIONIDAE: ELEODINI) FROM THE SANDHILLS OF WESTERN NEBRASKA

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ABSTRACT: *Xiphocephalus triplogemmatus* n. sp. and *Xiphocephalus quadratogemmatus* n. sp. (Apicomplexa: Eugregarinida) are described from *Eleodes tricostata* and *Eleodes fusiformis* (Coleoptera: Tenebrionidae), respectively, collected from Keith County in the sandhills of western Nebraska. Gamonts can be diagnosed with some confidence based on relative size and shape of the deutomerite, but these taxa are distinguished by differences in oocyst size, shape, and residuua number. Together with *Xiphocephalus ellisi* from *Eleodes opacus* in the same region, *X. triplogemmatus* and *X. quadratogemmatus* form a distinct Nearctic xiphocephalid group that is morphologically distinct from groups that occur in the Palearctic, Ethiopian, and Oriental regions.

During an ongoing biotic survey of the gregarine parasites of North American insects, 2 heretofore unknown gregarine species were discovered in populations of *Eleodes tricostata* (Say, 1824) and *Eleodes fusiformis* (Say, 1824) (Coleoptera: Tenebrionidae: Eleodini) collected in the sandhills of western Nebraska. The gregarine populations recovered are taxonomically distinct from known gregarine species and represent new species of *Xiphocephalus* Théodoridès, 1963 sensu Clopton (1999). The work presented herein extends the gregarine morphometric set and non-metric epimerite descriptive methods introduced by Clopton (1999, 2000), uses the standard nomenclature for plane shapes introduced by Clopton (2004b), describes 2 new species within *Xiphocephalus*, and recognizes biogeographical trends in xiphocephalid morphology.

MATERIALS AND METHODS

Eleodes tricostata adults (n = 233) were collected by hand from beneath cattle dung pats in the following localities: Beckius Ranch (east windmill), Keith County, Nebraska (41°11′17.2″N, 101°35′77.2″W), 3 August 2003 (n = 73); Beckius Ranch (north windmill), Keith County, Nebraska (41°12′25.8″N, 101°36′56.2″W), 15 July 1997 (n = 50), and 21 July 1999 (n = 2); Cedar Point Biological Station (CPBS), Keith County, Nebraska (41°12′25.8″N, 101°36′56.8″W), 21 July 1998 (n = 64), 21 July 1999 (n = 1), and 9 August 2004 (n = 5); Nevens Ranch, Keith County, Nebraska (41°12′40.7″N, 101°25′09.4″W) on 22 July 1998 (n = 4), 13 July 1999 (n = 1), 3 August 2003 (n = 25); and the Carl Fischer Ranch, Keith County, Nebraska (41°14′37.7″N, 101°32′84.3″W) on 3 August 2003 (n = 8). *Eleodes fusiformis* adults (n = 56) were collected by hand from beneath cattle dung pats in the following localities: CPBS, 20 July–4 August 1999 (n = 31), 9 August 2004 (n = 1); Nevens Ranch, 3–4 August, 2003 (n = 23), 8 August 2004 (n = 1).

Beetles were transported to the laboratory at CPBS, University of Nebraska, Keith County, Nebraska, divided by species into lots of 10–12 individuals each, and held in 250-ml glass culture dishes (Carolina Culture Dishes, Carolina Biological Supply Company, Burlington, North Carolina) with damp filter paper. Beetles were held for at least 6 hr for gametocyst shedding and then either preserved as permanent specimens or examined for gregarine infection within 48 hr of collection. Beetles 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 (Clopton 1996, 1999, 2000, 2002, 2004a; Clopton and Nolte, 2002; Clopton et al., 2004). Subsamples of gregarines infecting 5 beetles of each host species were preserved in 100% ethanol for future genetic analysis. Gametocysts were isolated from feces collected in culture dishes, triple-rinsed in insect muscle saline, photographed for morphometric analysis, triple-rinsed in 17-MΩ deionized water, dried briefly on filter paper, and transferred to 4-mm black cardstock disks previously sterilized in 1:1,000 formalin and placed in individual 4 × 12-mm glass microvials (BioQuip Products, Gardena, California). Vials were sealed with white silicon stoppers and gametocysts were held for maturation and dehiscence. Gametocysts were observed daily and any changes in structure, maturation, or dehiscence noted. Oocyst structure and dimensions were taken from fresh preparations of oocysts in wet mounts and agar monolayer mounts (Clopton, 1999, 2000, 2002, 2004a; Clopton and Nolte, 2002; Clopton et al., 2004) prepared as follows. Molten agar (1.5% solution) was pipetted onto a clean glass slide, allowed to drain, and the slide was quickly chilled on a cold aluminum block to produce a thin, uniform layer of agar. Oocysts were placed in a small (ca. 5 μl) drop of water on a 12-mm round cover glass (#0 thickness), and the agar slide was inverted to pick up the cover glass. The resulting preparation produces a monolayer of oocysts trapped between the agar layer and the cover glass. The monolayer technique provides a uniform object plane well suited for light microscopy using dry or oil-immersion objectives.

Observations were made using an Olympus B-Max 50 compound microscope with ×4, ×20, ×40, and ×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). 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).

An extended xiphocephalid morphometric character set is delineated in Figures 1–2. The morphometric set used here 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. 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 μm as mean values followed by range values, standard deviations, and sample sizes in parentheses. The shape and relative proportion of structures in mature trophozoites, particularly the epimerite, comprise an important diagnostic character suite among the Stylocephalidae. Although shape and relative proportion are useful, significant developmental variation within taxa precludes the use of absolute metrics taken from trophozoites (Watwood et al., 1997; Clopton, 1999). Terminology for parasite ontogenetic stages and anatomy largely follows that proposed by Levine (1971). Terminology for shapes of planes and solids follows Clopton (2004b). Additional descriptive terminology is derived from Harris and Harris (1994).

The following abbreviations for morphometric characters are used herein: length of diamerite, DiaL; width of diamerite, DiaW; length of deutomerite, DL; distance from anterior end of deutomerite to widest point, DLAM; distance from posterior end of deutomerite to widest point, DLPM; equatorial width of deutomerite, DWE; maximum width

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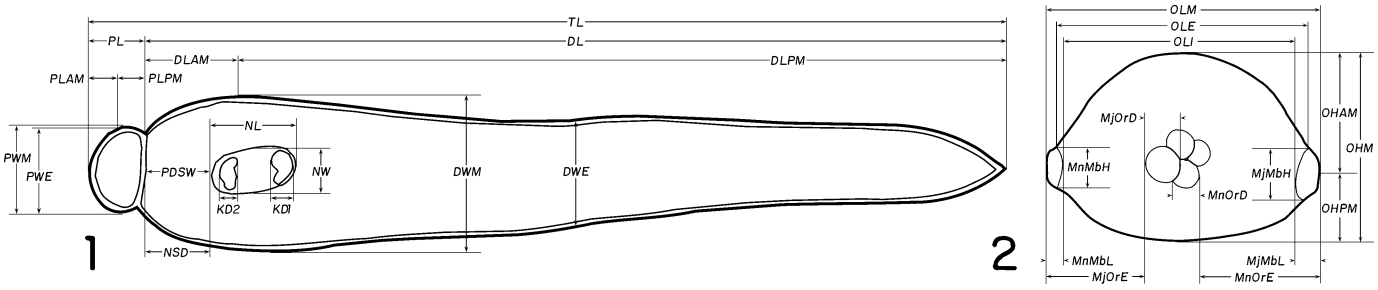


FIGURES 1–2. Morphometric character set for gamonts and oocysts of *Xiphocephalus*. (**1**) Gamont. (DL, length of deutomerite; DLAM, distance from anterior end of deutomerite to widest point; DLPM, distance from posterior end of deutomerite to widest point; DWE, equatorial width of deutomerite; DWM, maximum width of deutomerite; KD1, diameter of primary nuclear karyosome; KD2, diameter of secondary nuclear karyosome; NL, length of nucleus; NSD, distance from nucleus to protomerite-deutomerite septum; NW, width of nucleus; PDSW, width at protomerite-deutomerite septum; PL, length of protomerite; PLAM, distance from anterior end of protomerite to widest point; PLPM, distance from posterior end of protomerite to widest point; PWE, equatorial width of protomerite; PWM, maximum width of protomerite; TL, total length.) (**2**) Oocyst. (MjMbH, height of major oocyst micropyle body; MjMbL, length of major oocyst micropyle body; MjOrD, diameter of major oocyst residuum; MjOrE, eccentricity of major oocyst residuum; MnMbH, height of minor oocyst micropyle body; MnMbL, length of minor oocyst micropyle body; MnOrD, diameter of minor oocyst residuum; MnOrE, eccentricity of minor oocyst residuum; OHAM, dorsal height of oocyst from meridiofrontal plane of micropyle bodies; OHM, maximum oocyst height; OHPM, ventral height of oocyst to meridiofrontal plane of micropyle bodies; OLE, external length of oocyst [excluding micropyles]; OLI, interior length of oocyst [shortest distance between micropyle bodies]; OLM, maximum oocyst length.)

of deutomerite, DWM; maximum width of deutomerite, DWM; length of epimerite, EpiL; width of epimerite, EpiW; diameter of anterior polar (primary) nuclear endosome, KD1; diameter of posterior polar (secondary) nuclear endosome, KD2; height of major oocyst micropyle body, MjMbH; length of major oocyst micropyle body, MjMbL; diameter of major oocyst residuum, MjOrD; eccentricity of major oocyst residuum, MjOrE; height of minor oocyst micropyle body, MnMbH; length of minor oocyst micropyle body, MnMbL; diameter of minor oocyst residuum, MnOrD; eccentricity of minor oocyst residuum, MnOrE; length of nucleus, NL; distance from nucleus to protomerite-deutomerite septum, NSD; width of nucleus, NW; dorsal height of oocyst from meridiofrontal plane of micropyle bodies, OHAM; maximum height of oocyst, OHM; ventral height of oocyst to meridiofrontal plane of micropyle bodies, OHPM; external length of oocyst excluding micropyles, OLE; interior length of oocyst or the shortest distance between oocyst micropyle bodies, OLI; maximum length of oocyst, OLM; width at protomerite–deutomerite septum, PDSW; length of protomerite, PL; distance from anterior end of protomerite to widest point, PLAM; distance from posterior end of protomerite to widest point, PLPM; equatorial width of protomerite, PWE; maximum width of protomerite, PWM; total length, TL.

Discriminant analyses of gregarine gamont and oocyst data sets were conducted separately to evaluate the utility of morphometric characters for taxon identification. Unweighted variables were added stepwise and evaluated using Wilks' lambda (maximum significance of F to enter = 0.05; minimum significance of F to remove = 0.10). The effectiveness of the resulting canonical discriminant functions was evaluated by reclassification of the original data set. All analyses were conducted using SPSS Base 10.0 (SAS Institute, Carey, North Carolina).

DESCRIPTION

Xiphocephalus triplogemmatus n. sp.

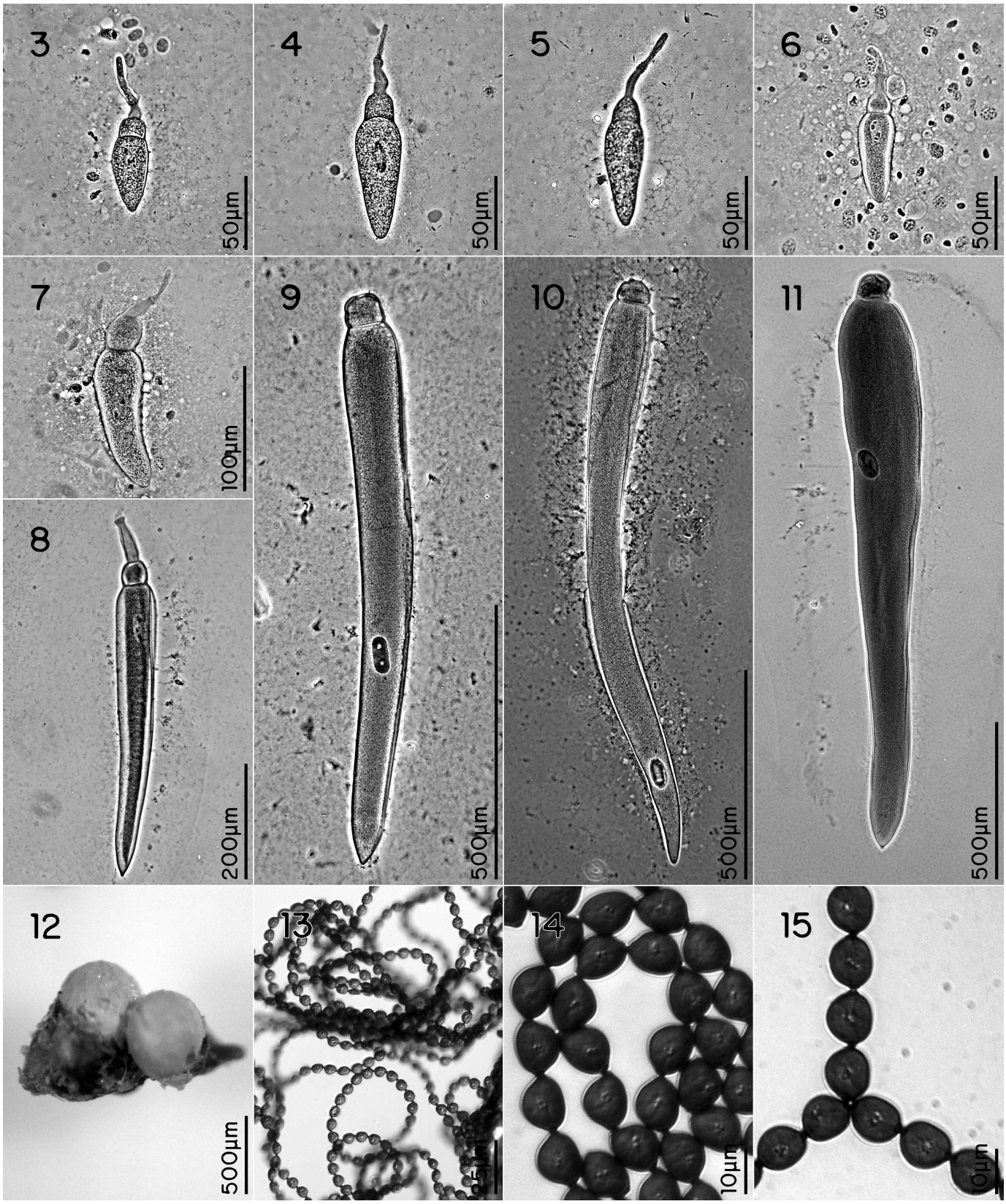
(Figs. 3–15)

Diagnosis: Eugregarinida Léger, 1892 sensu stricto Levine et al. (1980); *Septatina* Lankester, 1885, sensu stricto Levine et al. (1980); *Stenophoricae* Levine, 1984 sensu Chakravarty, 1960; *Stylocephalidae* Ellis, 1912, with the characters of *Xiphocephalus* Théodoridès, 1963 sensu Clopton (1999): Epimerite complex elongated into cylindrical, often filiform diamerite, expanding terminally to form epimerite proper; epimerite elongated into xiphoid process (including deltoid, ensiform, lanceolate, and gladiate forms), terminating in sharp or rounded point; gametocysts papillate, with internal pseudocyst residuum; oocysts axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect (including hat-, purse-, stone-, and seed-shaped of previous authors), emerging in chains.

Young trophozoite (Figs. 3–7): Developing trophozoites solitary, attached to host ventricular epithelium. Holdfast an epimerite complex comprising terminal epimerite proper joined to protomerite by intercalating diamerite (Filipponi, 1949; Clopton, 1999, 2000). Epimerite elongate and xiphoid, very narrowly dolioform to gladiate, terminally obtuse, with toroid basal tumidus at junction with diamerite; diamerite roughly cylindrical, very narrowly oblong to very narrowly lomentiform, flattening anteriad to form basal tumidus of epimerite, no longitudinal fold observed; without visible septum at junction with protomerite but clearly differentiated by decreased density of cytoplasm. Protomerite finely to deeply deltoid. Protomerite–deutomerite septum clearly marked and constricted. Deutomerite spatulate to narrowly obpyriform. Nucleus narrowly ellipsoid, with 2–3 distinct polysomal endosomes. Relative morphometrics: EpiL $\geq$ DiaL, EpiW $\leq$ DiaW, EpiL $\geq$ PL, DiaL $\geq$ PL, DL/PL $\approx$ 4, PWM $<$ DWM, PL $\approx$ PW, DWM/DL $\approx$ 4.

Mature trophozoite (Fig. 8): Infection site and complex nature of epimerite similar to young trophozoites. Epimerite very narrowly trullate; diamerite narrowly dolioform, expanding to very shallowly dolioform tumidus at junction with epimerite, with fine longitudinal folds in epicuticle; junction with protomerite clearly marked by constriction, pseudoseptum, and differential cytoplasmic density. Protomerite broadly to very broadly dolioform. Protomerite–deutomerite septum clearly marked and constricted. Deutomerite very narrowly obdeltiod, spatulate. Nucleus narrowly ellipsoid; with 2–3 distinct polysomal endosomes. Relative morphometrics: EpiL $<$ DiaL, DiaL/EpiL $\approx$ 3, EpiW $<$ DiaW, DiaW/EpiW $\approx$ 3, EpiL $<$ PL, PL/EpiL $\approx$ 2, DiaL $>$ PL, PL/DiaL $\approx$ 2, DL/PL $\approx$ 10–12, PWM $<$ DWM, DWM/PWM $\approx$ 2, PL $\approx$ PW, DWM/DL $\approx$ 7.

Gamont (Figs. 9–11): Solitary, isogamontic, epimerite–diamerite complex absent. Protomerite very shallowly ovoid to ovoid; PL 63.24 (46.13–87.2, $\pm$11.91, 30), PWE 72.83 (50.54–111.06, $\pm$17.06, 30), PWM 81.44 (47.61–122.39, $\pm$19.54, 30), PLAM 41.46 (19.92–71.16, $\pm$10.09, 30), PLPM 21.51 (10.2–36.44, $\pm$7.16, 30), PL/PWE 0.89 (0.7–1.19, $\pm$0.13, 30), PL/PWM 0.8 (0.57–1.06, $\pm$0.13, 30), PLAM/PL 0.65 (0.43–0.87, $\pm$0.1, 30), PLAM/PLPM 2.17 (0.74–5.65, $\pm$1.04, 30), PWM/PWE 1.12 (0.94–1.34, $\pm$0.08, 30). Protomerite–deutomerite septum clearly marked and constricted; PDSW 77.04 (52.16–118.45, $\pm$17.37, 30), PL/PDSW 0.83 (0.65–1.05, $\pm$0.11, 30). Deutomerite spatulate to linearly obdeltoid; DL 1118.1 (807.19–1748.21, $\pm$274.62, 30), DWM 139.17 (80.3–246.94, $\pm$39.88, 30), DLAM 165.74 (75.13–289.86, $\pm$52.98, 30), DLPM 954.73 (640.24–1569.17, $\pm$265.65, 30), DWE 97.5 (50.18–173.76, $\pm$27, 30), DL/DWE 12.07 (6.22–19.55, $\pm$3.66, 30), DL/DWM 8.31 (4.56–10.78, $\pm$1.7, 30), DLAM/DL 0.15 (0.08–0.31, $\pm$0.06, 30), DLAM/DLPM 0.19 (0.09–0.45, $\pm$0.09, 30), DWM/DWE 1.45 (0.92–2.11, $\pm$0.27, 30). Total length (TL) (1,176.09



FIGURES 3–15. *Xiphocephalus triplogemmatus* n. sp. **(3–7)** Young trophozoites. **(8)** Mature trophozoite. **(9–11)** Gamonts. **(12)** Immature gametocysts embedded in host frass. **(13–15)** Oocysts, with up to 3 oocyst residuua.

(862.7–1,833.92, ±278.16, 30). Morphometric indices: TL/PL 18.89 (11.73–27.6, ±4.29, 30), DL/PL 17.97 (10.66–26.58, ±4.26, 30), DWM/PWM 1.71 (1.44–2.47, ±0.25, 30), TL/DL 1.05 (1.03–1.1, ±0.01, 30). Nucleus elliptoid to narrowly elliptoid; NL 72.88 (51.02–96.18, ±10.91, 30), NW 32.34 (15.89–59.41, ±9.91, 30), NSD 340.17 (38.35–1,116.77, ±315.71, 30), NL/NW 2.44 (1.35–4.06, ±0.76, 30), NSD/NL 4.79 (0.6–15.21, ±4.51, 30), DL/NSD 6.93 (1.21–24.45, ±5.87, 30); typically with 3 polysomal endosomes (2 polar, 1 meriodontal), but up to 5 polysomal endosomes (2 polar, 3 meriodontal) have been observed, KD1 12.61 (8–19.82, ±2.97, 30), KD2 10.96 (6.27–19.14, ±2.83, 30).

Association: Frontal; isogamontic; late and ephemeral; leading directly to syzygy, associated pairs fusing laterally during syzygy; associations, syzygial pairs and gametocysts located between host ventricular peritrophic membrane and posterior ventricular epithelium. Gamonts in association morphometrically similar to solitary gamonts; epimerite absent.

Gametocyst (Fig. 12): White to opalescent in color, becoming tan to light brown with maturity; roughly spherical; no hyaline coat apparent, gametocyst wall desiccating to become paper-like, papillated. Gametocysts mature within 48–72 hr and dehisce by simple rupture of the gametocyst wall. Oocysts extruded in a coiled chain to form single, tangled, sticky mass; epispore packet absent, gametocyst residuum present.

Oocyst (Figs. 13–15): Axially asymmetric, shallowly ovoid in lateral aspect, uniform in size and shape; OLM 13.4 (12.9–13.8, ±0.22, 30), OLE 11.8 (11.2–12.6, ±0.38, 30), OLI 11.4 (10.8–11.9, ±0.31, 30), OHM 10.1 (9.4–10.7, ±0.28, 30), OHAM 6.3 (5.8–6.8, ±0.23, 30), OHPM 3.9 (3.6–4.1, ±0.16, 30); with asymmetric terminal micropyle bodies; major micropyle body oblong, MjMbL 0.9 (0.6–1.5, ±0.18, 30), MjMbH 1.5 (1.1–2.1, ±0.24, 30); minor micropyle body narrowly oblong MnMbL 0.9 (0.6–1.3, ±0.16, 30), MnMbH 1.3 (0.9–1.7, ±0.18, 30); with 3 central, anisomorphic, smooth, spherical oocyst residuua (immature oocysts often with 2 oocyst residuua), MjOrD 1.1 (0.9–1.4, ±0.14, 30), MjOrE 5.4 (4.5–6.1, ±0.4, 30), MnOrD 1.4 (1–1.9, ±0.2, 30), MnOrE 5.6 (4.5–6.4, ±0.44, 30); octozooic nature confirmed by optical sectioning. Extruded in chains. Oocysts dark brown under transmitted light, black under reflected light. Morphometric indices: OLM/OHM 1.3 (1.2–1.5, ±0.05, 30), OLE/OHM 0.9 (0.8–0.9, ±0.02, 30), OHAM/OHM 0.6 (0.6–0.7, ±0.01, 30), OHAM/OLM 0.5 (0.4–0.5, ±0.02, 30), MjMbH/MjMbL 1.7 (1.1–3.1, ±0.42, 30), MnMbH/MnMbL 1.4 (0.8–2.3, ±0.32, 30), MjMbH/MnMbH 1.2 (1–1.9, ±0.19, 30), MjMbL/MnMbL 1 (0.6–1.7, ±0.25, 30).

Taxonomic summary

Host: Eleodes tricostata Say, 1824, adults (Coleoptera: Tenebrionidae: Eleodini).

Symbiotype: Twenty symbiotype specimens are deposited in the Sam Houston State University Insect Collection (SHSUIC), Department of Biology, Sam Houston State University, Huntsville, Texas (SHSUIC does not assign individual accession numbers).

Host records: Eleodes tricostata, adults.

Type locality: Beckius Ranch (east windmill), Keith County, Nebraska (41°11′17.2″N, 101°35′77.2″W).

Other locality records: Beckius Ranch (north windmill), Keith County, Nebraska (41°12′25.8″N, 101°36′56.2″W); CPBS, Keith County, Nebraska (41°12′25.8″N, 101°36′56.8″W;); Nevens Ranch, Keith County, Nebraska (41°12′40.7″N, 101°25′09.4″W); and, the Carl Fischer Ranch, Keith County, Nebraska (41°14′37.7″N, 101°32′84.3″W).

Infection site: Trophozoites and gamonts were collected from the ventriculus. Associations primarily located in the ileum. Gametocysts were collected from the host hindgut, rectum, and frass.

Prevalence: Overall sample prevalence 59% (137 of 233 beetles examined postmortem). Overall site sample prevalence was as follows: Beckius Ranch (east windmill), 70% (51 of 73); Beckius Ranch (north windmill), 83% (43 of 52); CPBS, 33% (23 of 70); Nevens Ranch, 40% (12 of 30); and Carl Fischer Ranch 100% (8 of 8).

Specimens deposited: The holotype slide is deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. The holotype slide HWML48116 (author's slide REC030281 b) is a hapantotype. The paratype series includes 167 slides containing trophozoites, gamonts, and associations deposited in 11 lots as follows: HWML48109 (REC030188 a–c; REC030189; REC030281 a, c; REC030282; REC030298 a–b; REC030299 a–g, REC030301; REC030303 a, c–d; REC030304 a–d; REC030305 a–f; REC030306 a–e; REC030310 a–b; REC030314 a–f; REC030337 a–d; REC030339; REC030340 a–b; REC030341 a–c; REC030342 a–b; REC030343; REC030344; REC030374 a–e; REC030377 a–b; REC030378 a–c; REC04109; REC04110 a–c; REC04111 a–e; REC04112); HWML48110 (REC99219 a–b); HWML48111 (MCN980068, MCN980072, MCN980076, MCN980078, MCN980079, MCN980081, MCN980082, MCN980263 a–c, MCN980265 a–b, MCN980345, MCN980349, MCN980375, MCN980380, REC980502, REC980503); HWML48112 (REC99216 a–c); HWML48113 (REC04120); HWML48114 (REC030329 a–k, REC030330 a–c, REC030331 a–d, REC030332, REC030333 a–e, REC030334 a–f, REC030335 a–c, REC030336 a–b); HWML48115 (REC030184 a–g, REC030186 a–d, REC030187 a–e, REC030274 a–d, REC030277, REC030347 a–c, REC030350 a–g). No paratype specimen is retained by the authors.

Etymology: The specific epithet *triplogemmatus* is taken from the Latin (*triplex* ''3-fold'' + *gemma* ''a bud or precious stone'') to mark the 3 spherical residuua characteristic of the mature oocyst.

Xiphocephalus quadratogemmatus n. sp.

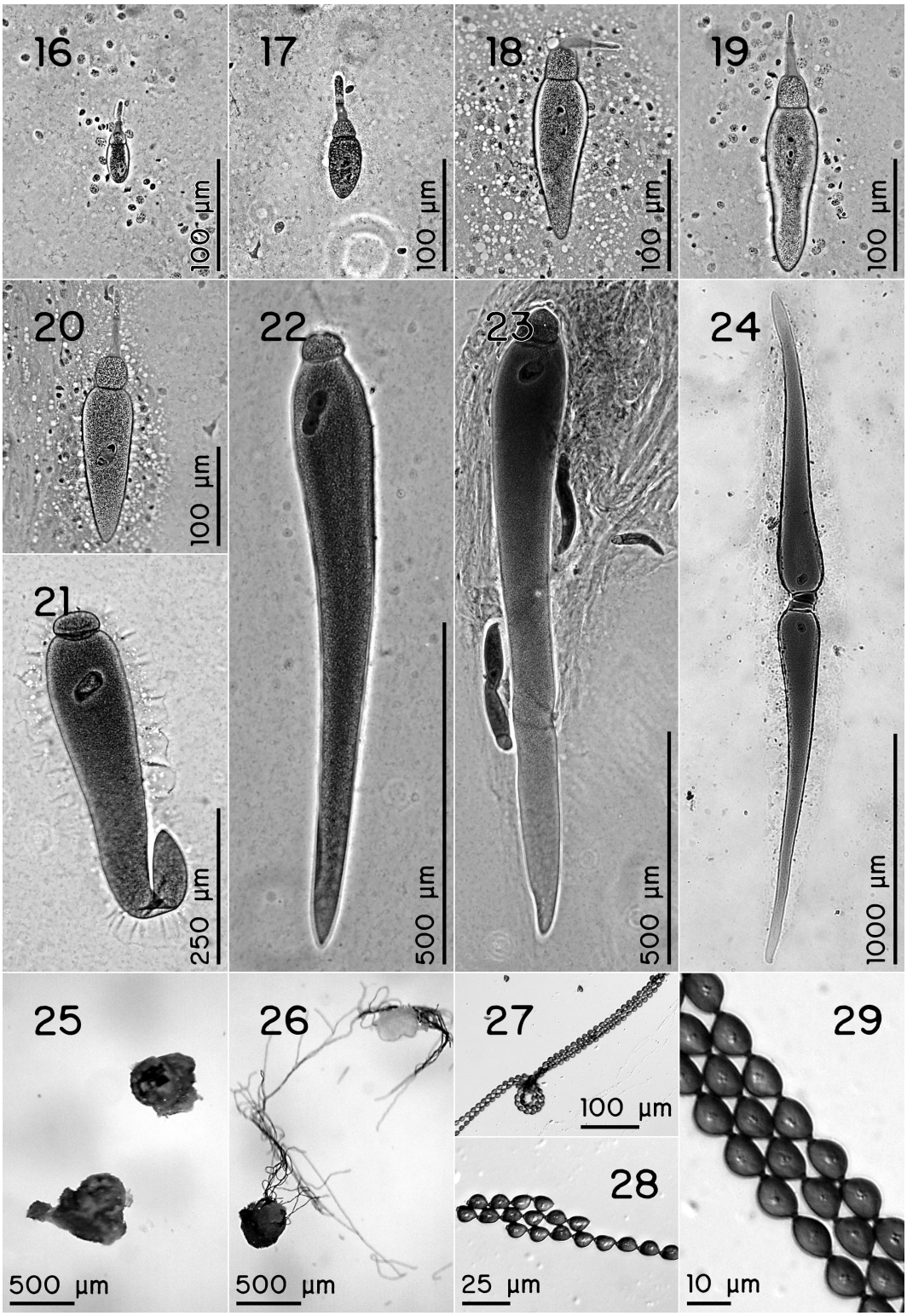
(Figs. 16–29)

Diagnosis: Eugregarinida Léger, 1892 sensu stricto Levine et al. (1980); *Septatina* Lankester, 1885, sensu stricto Levine et al. (1980); *Stenophoricae* Levine, 1984 sensu Chakravarty, 1960; *Stylocephalidae* Ellis, 1912, with the characters of *Xiphocephalus* Théodoridès, 1963 sensu Clopton (1999): Epimerite complex elongated into a cylindrical, often filiform diamerite, expanding terminally to form epimerite proper; epimerite elongated into xiphoid process (including deltoid, ensiform, lanceolate, and gladiate forms), terminating in sharp or rounded point; gametocysts papillate, with internal pseudocyst residuum; oocysts axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect (including hat-, purse-, stone-, and seed-shaped of previous authors), emerging in chains.

Young trophozoite (Figs. 16–17): Developing trophozoites solitary, attached to host ventricular epithelium. Holdfast an epimerite complex comprising terminal epimerite proper joined to protomerite by intercalating diamerite (Filipponi, 1949; Clopton, 1999, 2000). Epimerite elongate and xiphoid, narrowly dolioform to very narrowly lomentiform, terminally obtuse, with basal tumidus at junction with diamerite; diamerite very narrowly trullate with tumidus at junction with epimerite, no longitudinal fold observed; without visible septum at junction with protomerite but clearly differentiated by decreased density of cytoplasm. Protomerite broadly to very broadly deltoid. Protomerite–deutomerite septum clearly marked and constricted. Deutomerite narrowly obdeltoid to very deeply obovoid. Nucleus narrowly ellipsoid; with 1–2 distinct polysomal endosomes. Relative morphometrics: EpiL ≤ DiaL, EpiW ≤ DiaW, EpiL ≈ PL, DiaL ≥ PL, DL/PL ≈ 4.5, PWM < DWM, PL ≈ PW, DWM/DL ≈ 4.5.

Mature trophozoite (Fig. 18–20): Infection site and complex nature of epimerite similar to young trophozoites. Epimerite very narrowly trullate; diamerite linearly trullate, expanding to very shallowly dolioform tumidus at junction with epimerite, with fine longitudinal folds in epicuticle; junction with protomerite clearly marked by constriction, pseudoseptum, and differential cytoplasmic density. Protomerite broadly dolioform to broadly ovoid. Protomerite–deutomerite septum clearly marked and constricted. Deutomerite very narrowly obdeltiod, spatulate. Nucleus narrowly ellipsoid; with 2 distinct polysomal endosomes. Relative morphometrics: EpiL < DiaL, DiaL/EpiL ≈ 3.5, EpiW < DiaW, DiaW/EpiW ≈ 1.5, EpiL < PL, PL/EpiL ≈ 2, DiaL > PL, PL/DiaL ≈ 2, DL/PL ≈ 8–10, PWM < DWM, DWM/PWM ≈ 1.5, PL ≈ PW, DWM/DL ≈ 5.

Gamont (Figs. 21–23): Solitary, isogamontic, epimerite–diamerite complex absent. Protomerite very shallowly ovoid to very broadly ovoid; PL 50.89 (38.49–91.18, ±12.18, 30), PWE 61.01 (38.67–105.06, ±13.28, 30), PWM 66.96 (43.71–107.05, ±12.71, 30), PLAM 33.99 (21.76–50.58, ±7.88, 30), PLPM 16.99 (5.78–39.66, ±7.11, 30), PL/PWE 0.84 (0.54–1.16, ±0.15, 30), PL/PWM 0.77 (0.5–1.03, ±0.13, 30), PLAM/PL 0.67 (0.5–0.86, ±0.1, 30), PLAM/PLPM 2.42 (1.04–6.7, ±1.47, 30), PWM/PWE 1.1 (1–1.33, ±0.07, 30). Protomerite–deutomerite septum clearly marked and constricted, PDSW 61.76 (38.65–



FIGURES 16–29. *Xiphocephalus quadratogemmatus* n. sp. (**16–17**) Young trophozoites. (**18–20**) Mature trophozoites. (**21–23**) Gamonts. (**24**) Frontal association. (**25**) Immature gametocysts embedded in host frass. (**26**) Mature gametocyst undergoing exsporulation of oocyst chains. (**27–29**) Oocysts, with up to 4 oocyst residuua.

92.36, ±11.79, 30), PL/PDSW 0.83 (0.56–1.16, ±0.15, 30). Deutomerite very narrowly to linearly obdeltoid; DL 884.16 (597.98–1443.39, ±277.83, 30), DWM 107.53 (62.78–161.34, ±25.68, 30), DLAM 105.57 (31.12–248.73, ±53.42, 30), DLPM 773.25 (385.35–1351.43, ±255.03, 30), DWE 71.73 (34.23–136.83, ±21.39, 30), DL/DWE 12.93 (6.52–22.31, ±4.14, 30), DL/DWM 8.44 (4.87–17.06, ±2.69, 30), DLAM/DL 0.12 (0.04–0.2, ±0.04, 30), DLAM/DLPM 0.14 (0.04–0.26, ±0.05, 30), DWM/DWE 1.54 (1.1–2.03, ±0.24, 30). Total length 928.24 (644.97–1,492.67, ±284.31, 30). Morphometric indices: TL/PL 18.45 (10.85–32.17, ±4.77, 30), DL/PL 17.58 (10.12–31.1, ±4.71, 30), DWM/PWM 1.6 (1.24–2.09, ±0.19, 30), TL/DL 1.05 (1.02–1.08, ±0.01, 30). Nucleus ellipsoid, typically abaxial; NL 61.98 (52.09–78.35, ±6.77, 30), NW 29.52 (22.16–42.26, ±5.31, 30), NSD 108.53 (11.3–1074.11, ±190.06, 30), NL/NW 2.15 (1.3–2.81, ±0.36, 30), NSD/NL 1.66 (0.19–14.16, ±2.51, 30), DL/NSD 21.16 (1.18–107.44, ±25, 30); typically with 2 polysomal endosomes (2 polar), but up to 3 polysomal endosomes (2 polar, 1 meriodontal) have been observed, KD1 12.62 (9.4–17.05, ±2.05, 30), KD2 10.12 (7.15–14.02, ±1.61, 30).

Association (Fig. 24): Frontal; isogamontic; late and ephemeral; leading directly to syzygy, associated pairs fusing laterally during syzygy; associations, syzygial pairs, and gametocysts located between host ventricular peritrophic membrane and posterior ventricular epithelium. Gamonts in association morphometrically similar to solitary gamonts; epimerite absent.

Gametocyst (Figs. 25–26): White to opalescent in color, becoming tan to light brown with maturity; roughly spherical; no hyaline coat apparent, gametocyst wall desiccating to become paper-like, papillated. Gametocysts mature within 48–72 hr and dehisce by simple rupture of gametocyst wall. Oocysts extruded in coiled chain to form single, tangled, sticky mass (Fig. 26); epispore packet absent, gametocyst residuum present.

Oocyst (Figs. 27–29): Axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect, very uniform in size and shape; OLM 12 (11.5–12.4, ±0.18, 30), OLE 10.3 (9.7–10.7, ±0.26, 30), OLI 10.1 (9.5–10.7, ±0.29, 30), OHM 8.5 (8.1–8.8, ±0.18, 30), OHAM 5.3 (4.9–5.7, ±0.17, 30), OHPM 3.2 (2.8–3.4, ±0.15, 30); with asymmetric terminal micropyle bodies; major micropyle body oblong, MjMbL 1.1 (0.8–1.5, ±0.13, 30), MjMbH 2.4 (1.6–2.8, ±0.29, 30); minor micropyle body narrowly oblong MnMbL 0.9 (0.6–1.2, ±0.16, 30), MnMbH 1.4 (1–1.9, ±0.21, 30); with 4 central, anisomorphic, smooth, spherical oocyst residuua (immature oocysts often with 2–3 oocyst residuua), MjOrD 1.1 (0.8–1.3, ±0.15, 30), MjOrE 4.8 (4–5.6, ±0.49, 30), MnOrD 1.4 (1.1–1.8, ±0.18, 30), MnOrE 4.9 (3.6–5.5, ±0.48, 30); octozooic nature confirmed by optical sectioning. Extruded in chains (Fig. 26–29). Oocysts dark brown under transmitted light, black under reflected light. Morphometric indices: OLM/OHM 1.4 (1.4–1.5, ±0.03, 30), OLE/OHM 0.9 (0.8–0.9, ±0.02, 30), OHAM/OHM 0.6 (0.6–0.7, ±0.02, 30), OHAM/OLM 0.4 (0.4–0.5, ±0.02, 30), MjMbH/MjMbL 2.2 (1.5–2.7, ±0.29, 30), MnMbH/MnMbL 1.6 (1.2–2.2, ±0.28, 30), MjMbH/MnMbH 1.8 (1–2.4, ±0.35, 30), MjMbL/MnMbL 1.3 (0.9–2, ±0.25, 30).

Taxonomic summary

Host: Eleodes fusiformis Say, 1824, adults (Coleoptera: Tenebrionidae: Eleodini).

Symbiotype: Twenty symbiotype specimens are deposited in the Sam Houston State University Insect Collection, Department of Biology, Sam Houston State University, Huntsville, Texas (SHSUIC does not assign individual accession numbers).

Host records: Eleodes fusiformis, adults.

Type locality: Cedar Point Biological Station, Keith County, Nebraska (41°12′25.8″N, 101°36′56.8″W).

Other locality records: Nevens Ranch, Keith County, Nebraska (41°12′40.7″N, 101°25′09.4″W).

Infection site: Trophozoites and gamonts were collected from the ventriculus. Associations primarily located in the ileum. Gametocysts were collected from the host hindgut, rectum, and frass.

Prevalence: Overall sample prevalence 80% (45 of 56 beetles examined postmortem). Overall site sample prevalence was as follows: CPBS, 91% (29 of 32); and Nevens Ranch, 67% (16 of 24).

Specimens deposited: The holotype slide is deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. The holotype slide HWML48104 (author's slide REC04122 d) is a hapantotype. The paratype series includes 87 slides containing trophozoites, gamonts, and associations deposited in 6 lots as follows: HWML48101 (DDH990422, LJK990163, LJK990164 a–d, LJK990165, LJK990170, LJK990171 a–b, LJK990172 a–b, LJK990173, LJK990174, LJK990175, REC990211 a–c, REC990212, REC990213, REC990214, REC990220 a–b, REC990316 a–c, REC990317, REC990318 a–d, REC990320, REC990321 a–f, REC990322 a–c, REC990325 a–c): HWML48102 (REC04122 a–c); HWML48103 (REC030201 a–c, REC030202, REC030203, REC030204, REC030210 a–c, REC030211, REC030213 a–b, REC030214, REC030215, REC030319 a–d, REC030322 a–e); HWML48117 (REC030323 a–d); HWML48118 (REC030324 a–c); HWML48119 (LJK990168). No paratype specimen is retained by the authors.

Etymology: The specific epithet *quadratogemmatus* is taken from the Latin (*quadratos* ''4-fold'' + *gemma* ''a bud or precious stone'') to mark the 4 spherical residuua characteristic of the mature oocyst.

DISCUSSION

The eugregarine *Xiphocephalus* includes 9 previously described species: *Xiphocephalus africanus* (Théodoridès, Desportes, and Jolivet, 1965) Corbel, 1971; *Xiphocephalus ellisi* Clopton, 1999; *Xiphocephalus gladiator* (Blanchard, 1905) Corbel, 1971; *Xiphocephalus gonocephali* Patil and Amoji, 1985; *Xiphocephalus karnatakaensis* (Devdhar and Amoji, 1977) Levine, 1984; *Xiphocephalus latipes* Patil and Amoji, 1985; *Xiphocephalus phaleriae* (Tuzet and Ormières, 1955) Corbel, 1971; *Xiphocephalus reitterae* Patil and Amoji, 1985; and *Xiphocephalus serpentula* (Devdhar and Amoji, 1977) Levine, 1984. All are described from tenebrionid beetles, but only *X. ellisi* is reported from the Nearctic. *Xiphocephalus quadratogemmatus* and *X. triplogemmatus* are the second and third species representing the genus in the Nearctic, and their morphology indicates a generalized Nearctic morphotype distinct from that observed in the Palearctic, Ethiopian, and Oriental zoogeographical regions as defined by Darlington (1957) (Fig. 30).

Regional groups within *Xiphocephalus* are united by epimerite shape in trophozoites and deutomerite shape in gamonts. Within the Nearctic group (*X. ellisi*, *X. triplogemmatus*, and *X. quadratogemmatus*), gamonts possess a very-narrowly obdeltoid deutomerite and trophozoite epimerites are very narrowly trullate with a ornamented basal tumidus marking the junction with the diamerite (Fig. 30). In contrast, xiphocephalid gregarines of the Palearctic group (*X. gladiator* and *X. phaleriae*) possess gamonts with very narrowly elliptoid deutomerite (the gamonts of *X. phaleriae* are unknown), and their epimerites are narrowly trullate but lack a basal tumidus at the junction with the diamerite (Fig. 30). Xiphocephalid gregarines of the Oriental group (*X. gonocephali*, *X. karnatakaensis*, *X. latipes*, *X. reitterae*, and *X. serpentula*) include species with gamont deutomerites ranging from very narrowly obdeltoid (*X. latipes*, *X. reitterae*, *X. karnatakaensis*) to serpentine and linearly obdeltoid (*X. gonocephali*, *X. serpentula*), but their epimerites are all cordate in shape (the epimerite of *X. gonocephali* is cordate in young trophozoites and becomes ensiform in mature trophozoites) (Fig. 30). *Xiphocephalus africanus* is the sole known representative of the Ethiopian group, doubtless a reflection of incomplete survey rather than true faunistic poverty within the region. The gamontic deutomerite of *X. africanus* is narrowly obovoid in shape and possesses a unique and distinctive wrinkled ectosarc, whereas the epimerite of trophozoites is very

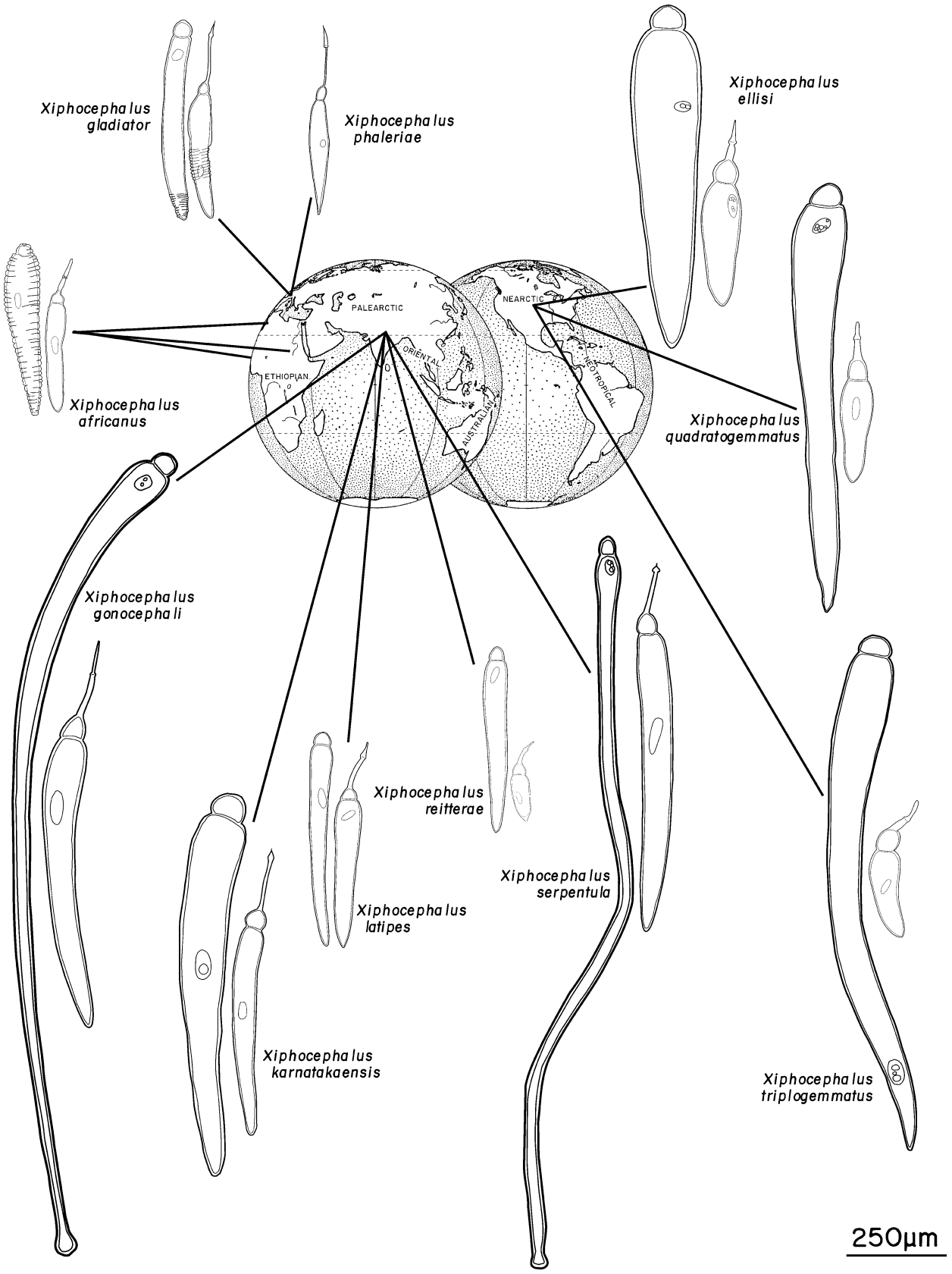


FIGURE 30. Biogeographical morphotypes of *Xiphocephalus*: Nearctic, *Xiphocephalus ellisi*, *Xiphocephalus triplogemmatus*, and *Xiphocephalus quadratogemmatus*; Palearctic, *Xiphocephalus gladiator* and *Xiphocephalus phaleriae*; Oriental, *Xiphocephalus gonocephali*, *Xiphocephalus karnatakaensis*, *Xiphocephalus latipes*, *Xiphocephalus reitterae*, and *Xiphocephalus serpentula*; and Ethiopian, *Xiphocephalus africanus*.

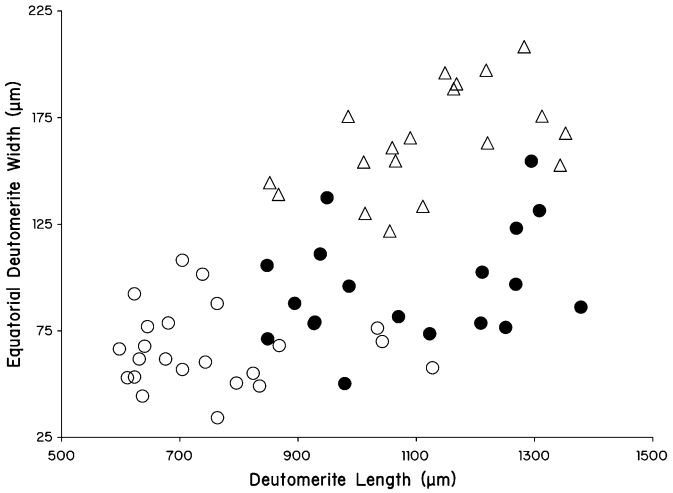


FIGURE 31. Morphotype centroid separation of *Xiphocephalus ellisi* (Δ), *Xiphocephalus triplogemmatus* (●), and *Xiphocephalus quadratogemmatus* (○) based on deutomerite length and width. Misclassification occurs primarily when large gamonts of *X. quadratogemmatus* are diagnosed as small gamonts of *X. triplogemmatus*.

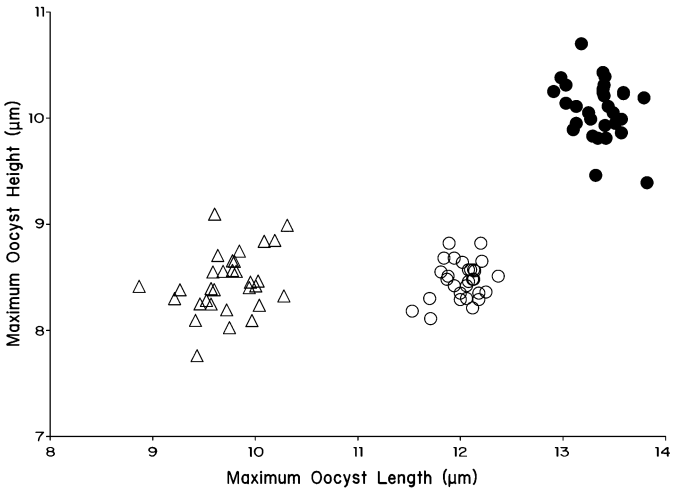


FIGURE 32. Morphotype centroid separation of *Xiphocephalus ellisi* (Δ), *Xiphocephalus triplogemmatus* (●), and *Xiphocephalus quadratogemmatus* (○) based on oocyst height and length. Taxa are clearly separated with little opportunity for misclassification.

narrowly ovoid (Fig. 30). Although there are distinct morphological differences among these regional groups, sufficient data do not exist to resolve nomenclatural or phylogenetic relationships among them. Until such data become available, these regional groups should remain the constituents of a single genus in the interest of nomenclatural stability.

Species comprising the Nearctic *Xiphocephalus* group (*X. ellisi*, *X. triplogemmatus*, and *X. quadratogemmatus*) are difficult to distinguish using gamonts alone. Given data from populations, 97%, 87%, and 70% of *X. ellisi*, *X. triplogemmatus*, and *X. quadratogemmatus* gamonts, respectively, are correctly classified using the following canonical discriminate functions: F1 = 1.129 DWE − 0.471 DLAM − 0.337 NL; F2 = 0.108 DWE + 0.595 DLAM + 0.725 NL. These functions categorize gamonts primarily based on the relative size and shape of the deutomerite, and most cases of misclassification occur when large specimens *X. quadratogemmatus* are classified as *X. triplogemmatus* (Fig. 31). These taxa are readily distinguished based on their oocysts, either using traditional oocyst height and length ratios (Fig. 32), or based solely on the number of oocyst residuua in mature oocysts. *Xiphocephalus triplogemmatus* and *X. quadratogemmatus* possess 3 and 4 spherical oocyst residuua each, whereas mature oocysts of *X. ellisi* typically contain 2 spherical residuua. Clopton (1999) reported a single oocyst residuum for oocysts of *X. ellisi*, but recollection and evaluation of oocysts reveal that single residuum oocysts are immature and develop a second residuum on maturity. All 3 Nearctic species of *Xiphocephalus* appear to be restricted to single tenebrionid host taxa: *X. ellisi* in *Eleodes opacus*, *X. triplogemmatus* in *E. tricostata*, and *X. quadratogemmatus* in *E. fusiformis*. Although all 3 host species are often collected in the same general area, they specialize on the seeds of different range grasses, providing some segregation of the microhabitat. Experimental cross-infections or nucleic acid analyses will provide additional insights into their phylogenetic relationship.

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