DOMADRACUNCULUS JANOVYI N. GEN., N. SP. (APICOMPLEXA: ACTINOCEPHALIDAE) FROM ADULTS OF ISCHNURA VERTICALIS (ODONATA: ZYGOPTERA) IN TEXAS*

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ABSTRACT: Domadracunculus janovyi n gen., n. sp. (Apicomplexa: Actinocephalidae) is described from trophozoites, gamonts, and oocysts collected from adult Ischnura verticalis (Odonata: Zygoptera) in Brazos County, Texas. Oocysts of the new species are axially asymmetric, smooth, and crescentic, features that unite the taxon with the Menosporinae. The new genus is distinguished from existing menosporid genera by an epimerite in the form of a distinctly pleated cup or sucker, without the anterior digitations of Hoplorhynchus or the crown of hooks diagnostic of Menospora.

Léger (1892) established the taxon Menosporidae to include gregarine forms possessing irregular, i.e., axially asymmetric, crescentic oocysts. Later, Grassé (1953) reduced Menosporidae to a subfamily, Menosporinae, within the family Actinocephalidae.

Three genera currently constitute the Menosporinae. Menospora comprises 2 species and is reported from Europe (Léger, 1892; Geus, 1969) and India (Devdhar and Deshpande, 1971; Sarkar and Haldar, 1980[1982]). Hoplorhynchus comprises 10 species and is reported from Europe (Carus, 1863; Geus, 1969; Théodoridès et al., 1976), India (Sarkar and Haldar, 1980; Sarkar and Mazumder, 1983; Shailaja and Amoji, 1992), and Japan (Hukui, 1952; H. Hoshide, 1953, 1954, 1959; Obata, 1953; K. Hoshide, 1977). Odonaticola Sarkar and Haldar, 1981 comprises 13 species, all described from India (Sarkar, 1981; Sarkar and Haldar, 1981; Kori and Amoji, 1984, 1986; Levine, 1988; Amoji and Kori, 1992). No genus of Menosporinae has been described or reported from the New World. The general paucity of the known North American gregarine fauna has been noted (Clopton et al., 1993). This pattern is more likely a result of insufficient survey work rather than true faunistic poverty.

A gregarine parasite was collected from *Ischnura verticalis* (Common Forktail Damselfly [Zygoptera: Coenagrionidae]) populations in Brazos County, Texas during an ongoing survey of the gregarine fauna of New World damselflies. The smooth, crescentic oocysts are diagnostic of the Menosporinae sensu Léger (1892); however, the fundamental structure of the epimerite is clearly distinct among existing Menosporid genera. The unique form of the epimerite prompted the present proposal of a new genus and species of eugregarine. This is the first report of a member of Menosporinae in the New World.

MATERIALS AND METHODS

The methods used in this study generally follow those of Clopton et al. (1993). Adult damselflies were isolated in 5-ml test tubes with 1 ml of water for 24 hr. The collected feces were examined for gregarine gametocysts. Gametocysts were freed from feces, pipetted with $10~\mu l$ of water into individual wells of a tissue culture plate, measured, and held for maturation and dehiscence. Oocyst structure and dimensions were taken from fresh preparations of oocysts suspended in water. Oocysts rotated freely in these preparations and the full 3-dimensional structure was observed.

After gametocyst collection, adult damselflies were dissected in insect saline without sucrose (Belton and Grundfest, 1962). Thirty-eight of 40 damselflies examined in April 1994 were infected. Permanent preparations of oocysts and trophic gregarines were made. Fresh material was fixed for 3 min in Schaudinn's fixative, stained with either Heidenhain's hematoxylin according to Cable's (1961) method or Semichon's acid carmine, dehydrated, cleared in xylene, and mounted in Canada balsam. Measurements were taken on no more than 5 individuals from each host. Widths of protomerites and deutomerites were taken at the widest points. Measurements in μ m were taken on fixed specimens and are presented as range values followed by means, standard deviations, and sample sizes in parentheses.

Terminology for parasite ontogenetic stages used here is consistent with Levine's (1971) uniform terminology for the Apicomplexa. Terminology for shapes of planes is consistent with that suggested by the Systematics Association Committee for Descriptive Biological Terminology (Anonymous, 1962a, 1962b) and is used to derive terminology for shapes of solids used in this description.

Drawings were made with the aid of a camera lucida. All observations were made using a Wild binocular compound microscope with $10 \times$ wide-field eye-pieces and $10 \times, 20 \times$, and $40 \times$ objectives. Measurements were made over a blue filter. Color observations were made using a Nikon Alphaphot binocular compound microscope with $10 \times$ wide-field eyepieces and a daylight filter.

DESCRIPTION

Domadracunculus n. gen.

Diagnosis: Eugregarinorida Léger, 1900 sensu Levine, Corliss, Cox, Deroux, Grain, Honigberg, Leedale, Loeblich, Lom, Lynn, Merinfeld, Page, Poljansky, Sprague, Vavra, and Wallace, 1980; Septatorina Lankester, 1885 sensu Levine, 1988; Stenophoricae Levine, 1984; Actinocephalidae Léger, 1892; Menosporinae Léger, 1892 sensu strictu. Gametocysts spherical; hyaline coat erratic (not present on all gametocysts); sporulation by simple dehiscence; no spore tubes or packet membranes were observed. Oocysts crescentic. Development solitary; association lateral, late. Epimerite borne on a narrow basal stalk; a pleated cup or sucker, very broadly ovoid in lateral view, circular en face; with distinct pleats rising from the interior of the cup to form a rosette or crown.

Taxonomic summary

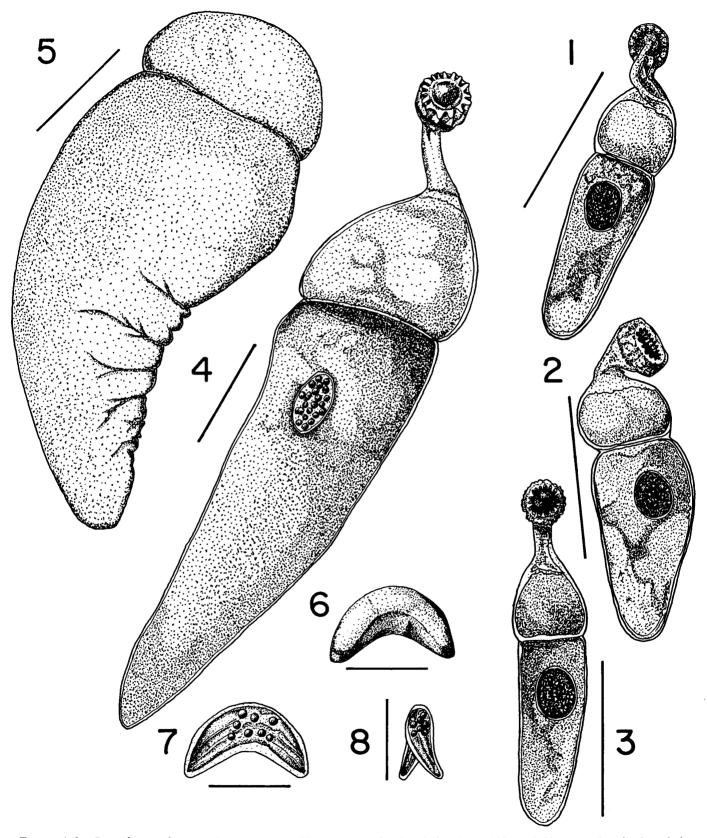
Etymology: The generic name is derived from the latin doma + dracon (gift of the little dragon).

Remarks

Léger (1892) established the taxon Menosporidae to include gregarine forms possessing irregular, i.e., axially asymmetric, crescentic oocysts. Menosporidae was 1 of 3 families Léger (1892) erected to encompass what is now Actinocephalidae, but he indicated that as many as 7 distinct macrotaxa might exist within the group. (Many of these families were later erected by Geus [1969], but his systematic arrangement of the group has not been generally accepted.) Labbé (1899) included Léger's general description of member genera (Menospora Léger, 1892 and Ho-

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FIGURES 1–8. Domadracunculus janovyi n. gen., n. sp. 1. Young trophozoite, dorsal view; bar = $100 \, \mu m$. 2. Young trophozoite, lateral view; bar = $100 \, \mu m$. 3. Young trophozoite, ventral view; bar = $100 \, \mu m$. 5. Gamont with disassociated nucleus, lateral view; bar = $100 \, \mu m$. 6. Oocyst, unstained surface topography, oblique aspect; bar = $10 \, \mu m$. 7. Oocyst with internal structure as revealed by iron hematoxylin stain, lateral aspect; bar = $10 \, \mu m$. 8. Oocyst with internal structure as revealed by iron hematoxylin stain, terminal aspect; bar = $10 \, \mu m$.

plorhynchus Carus, 1863) in his diagnosis of Menosporidae. It is not clear from Labbé's work that he intended to emend the family, but his diagnosis was retained by later authors of major works concerning the group (Watson, 1916; Grassé, 1953; Chakravarty, 1959[1960]; Levine, 1985, 1988). Later, Grassé (1953) reduced Menosporidae to a subfamily Menosporinae within the family Actinocephalidae. The current rank of the Menosporinae within Actinocephalidae reflects the arrangement of Grassé (1953). Domadracunculus is placed with the Menosporinae sensu strictu Léger (1892) because oocysts of the new genus are irregular, i.e., axially asymmetric, and crescentic. The structure of the epimerite accounts for characters diagnostic at the generic level rather than at the subfamily or family level as suggested by Labbé (1899) or Grassé (1953).

Three subfamilies, Actinocephalinae, Acanthosporinae, and Menosporinae, currently constitute Actinocephalidae. These subfamilies are separated by general oocyst morphology. The Menosporinae are united by the presence of a smooth, crescentic oocyst without spines or polar thickenings. The new genus shares this general oocyst morphology. The 3 named genera of Menosporinae, Menospora, Hoplorhynchus, and Odonaticola are separated by differences in the structure of the epimerite. Specimens of Menospora possess a cupule bordered with recurved hooks (Léger, 1892). Specimens of Hoplorhynchus possess a flattened bulb bordered anteriorly with digitations (Carus, 1863). Specimens of Odonaticola possess a hat-shaped or umbrelliform disk with a margin of petaloid spines (Sarkar and Haldar, 1981). The new genus does not possess an epimerite consistent with any menosporid genus, although it most closely resembles the epimerite of the type genus, Menospora. The new genus is distinguished from Menospora by the occurrence of distinct pleats inside the epimerite cup and the lack of a corona of recurved hooks.

Domadracunculus janovyi n. sp.

(Figs. 1-8)

Trophozoite (Figs. 1-4): Attached to host ventricular epithelium, solitary. Epimerite borne on a narrow basal stalk; length 12.0-200.0 $(75.1, \pm 42.5, 47)$, width 4.0-24.0 $(10.7, \pm 5.0, 47)$; increasing proportionally with maturity. Epimerite a pleated cup or sucker, in lateral view very broadly ovoid, length 24.0-48.0 (30.0, \pm 5.7, 47); circular en face. diameter 24.0–48.0 (29.5, \pm 5.2, 47); with 16 distinct pleats, length 8.0; rising from the interior of the cup to form a rosette or crown. (A few specimens show only 14 or 15 pleats; however, it is likely that these pleat counts represent artifacts of dissection technique or abnormal growth rather than variation in pleat number among members of the population.) Protomerite broadly ovoid to very broadly ovoid; protomerite length (LP) 44.0-144.0 (77.3, ± 19.5 , 47), protomerite width (WP) 36.0-120.0 (82.4, ± 19.5 , 47), LP/WP 0.67-1.42 (0.96, ± 0.20 , 47); tapering anteriad to junction with epimerite stalk, with strong posteriad constriction at protomerite-deutomerite septum. Deutomerite narrowly obvoid to obvoid, becoming distinctly obvoid in older trophozoites; deutomerite length (LD) 120.0-384.0 (235.2, ± 73.2 , 47), deutomerite width (WD) 40.0-120.0 (83.8, ± 20.3 , 47), LD/WD 2.00-3.90 (2.81, ± 0.52 , 47); broadly rounded posteriad, especially in young trophozoites. Total length without epimerite (TL) 160.0-528.0 (309.9, ± 87.38 , 47); LP/TL 0.18-0.33 (0.25, ± 0.04 , 47); LD/TL 0.66-0.98 $(0.75, \pm 0.06, 47)$; LP/LD 0.22-0.50 $(0.34, \pm 0.06, 47)$; WP/WD 0.67-1.50 (0.99, ± 0.12 , 47). Nucleus spheroid to elliptoid; nucleus length (LK) 32.0-96.0 (41.7, ±9.9, 47), nucleus width (WK) 24.0-64.0 (33.2, ± 6.1 , 47), LK/WK 1.00-1.63 (1.26, ± 0.14 , 47); placement roughly axial, equatorial; nuclear endosomes variable, usually a single, large, central endosome filling most of the nucleus. Fresh trophozoites with endocyte clear to slightly granular when young, becoming opaque with maturity, epicyte clear. Color white under reflected light, brownishorange under transmitted light. Hematoxylin staining yields specimens with protomerite white to light blue, deutomerite light blue to purple. Structural details of the epimerite are somewhat ambiguous with hematoxylin stain but are clearly distinguished by acid carmine stain.

Gamont (Fig. 5): Partially enrobed in host ventricular peritrophic membrane, solitary or in association. Epimerite absent, shed with maturity. Protomerite very broadly ovoid to depressed ovoid; LP 64.0–112.0 (90.1, ± 13.3 , 15), WP 104.0–176.0 (132.0, ± 25.7 , 15), LP/WP 0.4–0.9 (0.7, ± 0.2 , 15); rounded anteriad, with strong posteriad constriction at protomerite–deutomerite septum. Protomerite usually offset at junction with deutomerite. Deutomerite narrowly obvoid to obvoid;

LD 272.0–480.0 (354.7, \pm 60.0, 15), WD 120.0–232.0 (162.1, \pm 36.34, 15), LD/WD 1.6–2.9 (2.3, \pm 0.4, 15). TL 360.0–568.0 (433.6, \pm 63.01, 15); LP/TL 0.15–0.29 (0.21, \pm 0.03, 15); LD/TL 0.72–0.88 (0.82, \pm 0.03, 15); LP/LD 0.18–0.36 (0.26 \pm 0.04, 15); WP/WD 0.72–0.88 (0.82, \pm 0.04, 15). Nucleus broadly ellipsoid to ovoid; LK 32.0–48.0 (39.6, \pm 4.5, 11), WK 32.0–48.0 (35.6, \pm 5.5, 11); placement roughly axial, equatorial, opaque. Nucleus undifferentiated in late gamonts. Fresh gamonts granular to opaque, epicyte clear. Color white under reflected light, brownishorange under transmitted light. Hematoxylin staining yields specimens with protomerite white to light blue, deutomerite dark blue to opaque black.

Association: Syzygy late and ephemeral, latero-associative while caudally enrobed in peritrophic membrane. The progression of syzygy and gametocyst formation reported for *Nubenocephalus nebraskensis* Clopton, Percival, and Janovy, 1993 is very similar to that observed for the present species (Clopton et al., 1993).

Gametocyst: Opalescent and spheroid; diameter 224.0-248.0 (239.1, ±9.3, 18); hyaline coat erratic, not present on all gametocysts. Gametocysts collected and stored under (ca. 22 C) water mature and rupture by simple dehiscence within 40-48 hr. No spore tubes or packet membranes were observed.

Oocyst (Figs. 6–8): Crescentic ("biconical, bent"), axially symmetric in lateral view; length (tip-to-tip) 13.5-16.7 (15.1, ± 0.8 , 30), axial depth of bend 2.3-4.5 (3.5, ± 0.6 , 30); circular in axial cross section, breadth 4.0-6.3 (4.9, ± 0.5 , 30).

Taxonomic summary

Type host: Ischnura verticalis (Odonata: Zygoptera: Coenagrionidae). Host records: Ischnura verticalis adults.

Type locality: Country Club Lake (30°38'23.3"N, 96°21'36.2"W [United States Geological Survey: Bryan East Quadrangle]), City of Bryan, Brazos County, Texas.

Specimens deposited: Two (1 with multiple trophozoites, 1 with multiple oocysts) type slides were deposited in the United States National Museum Helminthological Collection (USNMHC), Biosystematic Parasitology Laboratory, Beltsville, Maryland. The holotype is a trophozoite on slide USNMHC no. 84015 and is marked by an etched circle. The remaining trophozoites (USNMHC no. 84015) and oocysts (USNMHC no. 84016) are paratypes. Trophozoites and oocysts on slides REC-94-0045-0068 are paratypes and are retained in the author's personal collection. One specimen of *I. verticalis* was deposited as voucher specimen TAMU 611 in the Entomology Museum, Department of Entomology, Texas A&M University, College Station, Texas.

Infection site: Trophozoites and gamonts were observed along the entire length of the host ventriculus. Trophozoites were attached to the ventricular epithelium. Gamonts were free in the ventricular lumen or caudally enrobed in the ventricular peritrophic membrane.

Etymology: The specific name janovyi is given in honor of my mentor and friend, Dr. John Janovy, Jr., whose interest and work with the gregarine parasites of odonates, orthopterans, and coleopterans initiated a renewed interest in the gregarine fauna of North America and inspired my own work within the group.

Remarks

Domadracunculus janovyi is the type species of Domadracunculus.

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