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Host Utilization and Distribution of Nubenocephalid Gregarines (Eugregarinorida: Actinocephalidae) Parasitizing *Argia* spp. (Odonata: Zygoptera) in the Central United States

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ABSTRACT: Gregarine host specificity has been the cornerstone of gregarine taxonomy for nearly a century. Several laboratory experiments have accepted strict host specificity by failure to cross-infect distantly related hosts with unrelated gregarine species. These empirical studies are not feasible for all gregarine hosts, especially nondomesticated groups. Additionally, studies of gregarine distributions have always focused on insect hosts of disparate groups, rather than targeting potential hosts species within a single genus and their congeneric gregarines. This study addresses host utilization of nubenocephalid gregarines parasitizing the odonate genus *Argia* (Odonata: Zygoptera: Coenagrionidae). Populations of 9 species of adult *Argia* were collected, dissected, and observed for gregarine infection during the April–September flight seasons in 2007 from 17 localities in the central United States. On average, 2.5 species of *Argia* were collected at each locality. A species of *Nubenocephalus*—*Nubenocephalus nebraskensis, Nubenocephalus secundus,* or *Nubenocephalus* spp.—was collected from every infected population of *Argia* except for the *Argia vivida* population at Prairie Dog Town Fork-Red River, Randall County (Co.), Texas, U.S.A. *Nubenocephalus secundus* utilizes at least 7 of the 9 argid hosts sampled whereas *N. nebraskensis* was collected from only 2 argid species. Only *Argia translata* was observed to host both *N. secundus* and *N. nebraskensis*. These patterns of host utilization by nubenocephalid gregarines represent an ecotypic gregarine assemblage rather than a vicariant assemblage, demonstrating that nubenocephalid gregarines do not differentiate between species of *Argia* as hosts.

KEY WORDS: Actinocephalidae, Apicomplexa, *Argia apicalis*, *Argia fumipennis*, *Argia immunda*, *Argia moesta*, *Argia nahuana*, *Argia sedula*, *Argia tibialis*, *Argia translata*, *Argia vivida*, Coenagrionidae, ecotypic assemblage, Eugregarinorida, damselfly, gregarine, host specificity, Kansas, Nebraska, *Nubenocephalus secundus*, *Nubenocephalus nebraskensis*, Odonata, Oklahoma, Texas, Zygoptera.

Host specificity has been a paradigmatic cornerstone of gregarine taxonomy for almost a century. "Host specificity" is a vague term that reflects a critical evolutionary phenomenon: parasites have evolved in concert with their hosts and these evolutionary changes tend to increase compatibility, transmissibility, parasite survival, and ultimately gene-pool divergence and speciation. Studies of gregarine host specificity exist (Corbel, 1968, 1971; Clopton et al., 1992; Clopton and Gold, 1996; Watwood et al., 1997; Wise et al., 2000; Detwiler and Janovy, 2008; Smith and Cook, 2008), but most lack a phylogenetic context: they attempt to cross-infect distantly related hosts with distantly related gregarines and accept the failure to cross-infect as general evidence of host specificity among gregarines (Corbel, 1968, 1971; Wise et al., 2000; Smith and Cook, 2008). Host specificity is usually fairly rigid and can be constricted to developmental stages of a single host species (Clopton et al., 1991, 1992) or rarely expand to include several related host species (Corbel, 1968, 1971; Levine, 1988; Clopton and Gold, 1996), but there is little evidence to suggest that the host specificity of gregarines justifies a maxim of "different host = different gregarine" (Levine, 1979, 1988).

Empirical host-specificity studies are appropriate for insects and gregarines in laboratory colonies, but are infeasible for most insect–gregarine systems, which are not readily domesticated. In these cases, only intensive field survey can provide insight into host specificity. Existing large-scale gregarine surveys focus on disparate host groups, often including several insect orders in a single geographically based survey (e.g., the Nebraska Sandhills [Clopton, 1995a; 1999; 2000; 2006; Clopton and Nolte, 2002; Hays et al., 2004; Clopton and Hays, 2006], the Texas Big Thicket [Clopton et al., 2004; 2007; 2008a, b; Hays et al., 2007], the eastern United States [Watson, 1915, 1916a, b, c, 1918; Watson Kamm, 1922], Poland

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[Lipa, 1967]; France [Desportes, 1963]; Japan [Hoshide, 1951a, b, 1952a, b, 1953a–c, 1954], and Indochina [Théodoridès et al., 1965; 1975; 1976; Théodoridès and Desportes, 1966, 1967]). The host use and distributional data from these surveys can shed little light on gregarine host specificity. No large-scale gregarine survey has targeted potential host species within a single genus.

Damselflies of the genus *Argia* (Odonata: Zygoptera) are an ideal target for a large-scale survey of gregarines. The genus is specious (ca. 100 species) and restricted to the Western Hemisphere (Westfall and May, 1996). Gregarines are known from only 3 argid species. *Nubenocephalus nebraskensis* is described from *Argia bipunctulata* in Nebraska (Clopton et al., 1993), *Nubenocephalus secundus* is described from *Argia sedula* in Texas (Hays et al., 2007) and *Nubenocephalus xunantunichensis* is described from *Argia chelata* in Belize, Central America (Clopton et al., 2010). No other New World species of *Nubenocephalus* is known. The current study was designed to sample gregarines parasitizing an array of *Argia* species across the central United States to elucidate nubenocephalid host specificity based on patterns of argid host use.

MATERIALS AND METHODS

Populations of *Argia* spp. were collected from the following 17 sites constituting a longitudinal transect spanning Nebraska through Texas as well as sites representing 6 of the largest 10 biogeographical provinces of Texas as recognized by Abbott (2005) (Fig. 1): USA: Kansas: Atchison Co.: Clear Creek, 39°39′01″N; 95°24′53″W; Brown Co.: Delaware River, 39°40′03″N; 95°39′35″W; Sedgwick Co.: Cowskin Creek, 37°38′41″N; 97°26′54″W; Nebraska: Nemaha Co.: Deroin Creek, 40°15′38″N; 95°38′07″W; Richardson Co.: Easly Creek, 40°03′30″N; 95°52′37″W; Sardine Creek, 40°13′58″N; 95°41′41″W; Oklahoma: Rogers Co.: Coal Creek, 36°12′26″N; 95°54′50″W; Texas: Collin Co.: Russell Creek, 33°05′47″N; 96°45′04″W; Kerr Co.: Guadalupe River, 30°00′45″N; 99°07′11″W; Kleberg Co.: Tranquitas Creek, 27°31′18″N; 97°51′22″W; Lee Co.: Yegua Creek, 30°19′10″N; 96°44′28″W; Parker Co.: Lake Mineral Wells, 32°48′59″N; 98°02′31″W; Polk Co.: Big Sandy Creek, 30°40′16″N; 94°41′21″W; P-Creek, 30°39′26″N; 94°40′06″W; Randall Co.: Prairie Dog Town Fork-Red River, 34°57′08″N; 101°40′02″W; Reeves Co.: San Solomon Springs, 30°56′41″N; 103°47′01″W; Walker Co.: Harmon Creek, 30°44′42″N; 95°28′17″W. Table 1 summarizes *Argia* sample size, nubenocephalid prevalence, and gregarine species identified by collecting site, which corresponds with Figure 1. All sites are lotic systems ranging from small first-order streams to large fifth-order rivers except Lake Mineral Wells, which is lentic. Substrates were typically variable by site, ranging from rock, clay, sand, or sandy/pebble substrates in low-order streams to mud/silt substrates in high-order rivers and lakes.

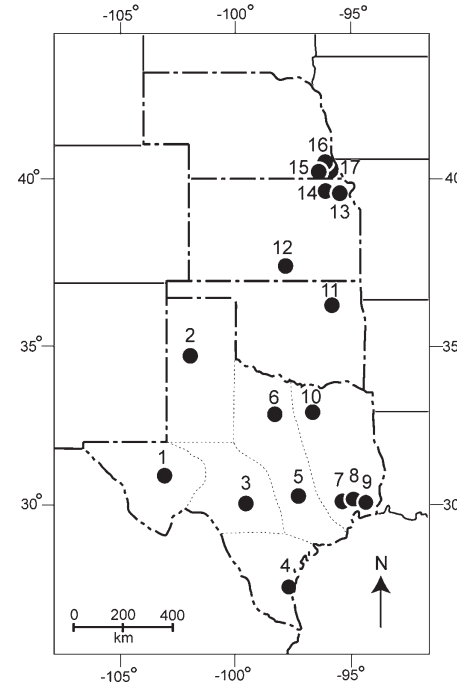


Figure 1. Map of Nebraska, Kansas, Oklahoma, and Texas, U.S.A., indicating 17 collecting sites (1, San Solomon Springs; 2, Prairie Dog Town Fork-Red River; 3, Guadalupe River; 4, Tranquitas Creek; 5, Yegua Creek; 6, Lake Mineral Wells; 7, Harmon Creek; 8, Big Sandy Creek; 9, P-Creek; 10, Russell Creek; 11, Coal Creek; 12, Cowskin Creek; 13, Clear Creek; 14, Delaware River; 15, Easly Creek; 16, Deroin Creek; 17, Sardine Creek). Collecting localities sample the largest 6 biogeographical provinces within Texas (Chihuahuan [site 1]; Kansan [site 2]; Balconian [site 3]; Tamaulipan [site 4]; Texan [sites 5 and 6], and Austroriparian [sites 7–10]). Biogeographical provinces within Texas are bounded by dotted lines. States are bounded by telegraph lines.

Adult damselflies were collected with aerial nets at each locality during the 2007 flight season from April to September. Damselflies were placed in 1-liter plastic containers and stored on ice until processing (<1 hr). For identified hosts at least 1 individual damselfly representing each sex (if applicable), from each locality was preserved in 70% EtOH in a glass vial and identified to species using Westfall and May (1996). Individual damselflies were isolated abdomen-down in 5-ml plastic test tubes with ca. 1 ml distilled water and held for 6 hr to 12 hr to collect feces and embedded gregarine gametocysts. Shed gametocysts were freed using a fine paintbrush, triple-rinsed in insect muscle saline (Belton and Grundfest, 1962) and distilled water, and transferred to individual 4 × 12–mm glass microvials (BioQuip Products, Gardena, California, U.S.A.) with ca. 50 µl distilled water. Vials were sealed with white silicon stoppers and gametocysts held for 3–5 d to observe maturation and dehiscence. Fresh preparations of oocysts were examined as wet mounts sealed with paraffin oil.

Table 1. Collecting sites indicating the species of *Argia* collected, sample size, and nubenocephalid gregarine prevalence.

Site*	Location/coordinates (U.S.A.)	*Argia* spp.	Infected/ sample Size	Prevalence	*Nubenocephalus* spp.
1	Texas, Reeves Co., Solomon Springs 30°56′41″N; 103°47′01″W	*A. immunda*	8/36	22.2	*N. secundus*
		A. moesta	4/5	80	*Nubenocephalus* sp.
		A. sedula	15/60	25	*N. secundus*
2	Texas, Randall Co., Prairie Dog Town Fork-Red River 34°57′08″N; 101°40′02″W	*A. apicalis*	26/53	49.1	*N. secundus*
		A. moesta	9/36	25	*N. secundus*
		A. sedula	14/38	36.8	*N. secundus*
		A. vivida	0/13†	0	
3	Texas, Kerr Co., Guadalupe River 30°00′45″N; 99°07′11″W	*A. moesta*	2/16	12.5	*N. secundus*
		A. sedula	34/58	58.6	*N. secundus*
		A. translate	41/60	68.3	*N. secundus*
4	Texas, Kleberg Co., Tranquitas Creek 27°31′18″N; 97°51′22″W	*A. immunda*	0/4	0	
		A. sedula	17/65	26.2	*N. secundus*
5	Texas, Lee Co., Yegua Creek 30°19′10″N; 96°44′28″W	*A. apicalis*	10/26	38.4	*N. secundus*
		A. tibialis	56/61	91.8	*N. nebraskensis*
6	Texas, Parker Co., Lake Mineral Wells 32°48′59″N; 98°02′31″W	*A. apicalis*	97/101	96	*N. secundus*
		A. moesta	2/2	100	*Nubenocephalus* sp.
		A. sedula	18/23	78.3	*N. secundus*
7	Texas, Walker Co., Harmon Creek 30°44′42″N; 95°28′17″W	*A. fumipennis*	1/2	50	*Nubenocephalus* sp.
		A. sedula	16/35	45.7	*N. secundus*
		A. tibialis	25/26	96.2	*N. nebraskensis*
		A. translata	10/16	62.5	*N. nebraskensis*
8	Texas, Polk Co., Big Sandy Creek 0°40′16″N; 94°41′21″W	*A. moesta*	5/5	100	*N. secundus*
9	Texas, Polk Co., P-Creek 30°39′26″N; 94°40′06″W	*A. moesta*	0/5	0	
		A. tibialis	16/20	80	*N. nebraskensis*
10	Texas, Collin Co., Russell Creek 33°05′47″N; 96°45′04″W	*A. immunda*	0/15	0	
		A. sedula	3/31	9.7	*Nubenocephalus* sp.
		A. vivida	0/6	0	
11	Oklahoma, Rogers Co., Coal Creek 36°12′26″N; 95°54′50″W	*A. apicalis*	4/4	100	*N. secundus*
		A. sedula	34/48	70.8	*N. secundus*
		A. translate	6/10	60	*Nubenocephalus* sp.
		A. vivida	7/27	25.9	*Nubenocephalus* sp.
12	Kansas, Sedgwick Co., Cowskin Creek 37°38′41″N; 97°26′54″W	*A. apicalis*	53/72	73.6	*N. secundus*
13	Kansas, Atchison Co., Clear Creek 39°39′01″N; 95°24′53″W	*A. fumipennis*	6/13	46.2	*Nubenocephalus* sp.
14	Kansas, Brown Co., Delaware River 39°40′03″N; 95°39′35″W	*A. apicalis*	21/31	67.7	*N. secundus*
		A. moesta	2/2	50	*Nubenocephalus* sp.
15	Nebraska, Richardson Co., Easly Creek 40°03′30″N; 95°52′37″W	*A. apicalis*	2/2	100	*Nubenocephalus* sp.
		A. fumipennis	16/33	48.5	*N. secundus*
		A. moesta	0/2	0	
		A. nahuana	4/19	31.6	*Nubenocephalus* sp.
		A. vivida	3/7	42.9	*Nubenocephalus* sp.
16	Nebraska, Nemaha Co., Deroin Creek 40°15′38″N; 95°38′07″W	*A. fumipennis*	0/15	0	
		A. moesta	1/25	4	*Nubenocephalus* sp.
		A. vivida	0/13	0	
17	Nebraska, Richardson Co., Sardine Creek 40°13′58″N; 95°41′41″W	*A. vivida*	39/74	52.7	*N. secundus*
Total			627/1,217	51.5	

* Sites correspond to those indicated in Figure 1.

† 5/13 *A. vivida* were infected with an unidentified, nonnubenocephalid gregarine.

Observations were made using an Olympus B-Max 50 compound microscope with ×10, ×20, ×40, and ×60 universal planapochromatic objectives with differential interference contrast prisms and an infinity-optics turret image doubler. Digital images were captured using an Olympus DP-70 camera through the aforementioned microscope. Oocyst morphometrics were taken from digitized images of oocysts using Image-Pro Discovery® v. 4.0 image analysis software (Media Cybernetics, L.P., Silver Spring, Maryland, U.S.A.). Gregarines were identi-

fied through comparison with original descriptions of gregarines in the genus *Nubenocephalus* (Clopton et al., 1993; Hays et al., 2007) and other New World damselfly gregarines (Ellis, 1914; Richardson and Janovy, 1990; Percival et al., 1995; Clopton, 1995b; 2004; Clopton et al., 2007; 2010).

Voucher specimens of *Nubenocephalus* spp. from each host species from each locality were deposited in the Harold W. Manter Laboratory for Parasitology, Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (accession numbers HWML 49347–49498). Voucher specimens of *Argia* species collected and identified in this study are deposited in the Sam Houston State University Insect Collection, Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, U.S.A. (accession numbers SHSUE000161–SHSUE000231).

RESULTS

Nine *Argia* species were collected: *Argia apicalis, Argia fumipennis, Argia immunda*, *Argia moesta, Argia nahuana*, *Argia sedula, Argia tibialis, Argia translata,* and *Argia vivida*, collectively hosting 2 nubenocephalid species: *Nubenocephalus secundus* Hays, Clopton, and T. J. Cook, 2007, and *Nubenocephalus nebraskensis* Clopton, Percival, and Janovy, 1993. In some cases, gregarine samples sizes were too small to permit positive species identification; such populations are reported simply as *Nubenocephalus* spp. Host use, geographical distribution of parasites, and uninfected host populations are reported by host species, below. Number of infected hosts and sample size followed by prevalence are indicated for each locality when a gregarine species was collected and identified from multiple localities.

Argia apicalis

Nubenocephalus secundus

Prevalence: 211 of 287 hosts sampled (73.5%).

Other reported hosts: A. fumipennis, A. immunda, A. moesta, A. sedula, A. translata, A. vivida.

Locality records: Kansas: Brown Co.: Delaware River (21/31; 67.7%), Sedgwick Co.: Cowskin Creek (53/72; 73.6%); Oklahoma: Rogers Co.: Coal Creek (4/4; 100%); Texas: Lee Co.: Yegua Creek (10/26; 38.4%), Parker Co.: Lake Mineral Wells (97/101; 96%), Randall Co.: Prairie Dog Town Fork-Red River (26/53; 49.1%).

Nubenocephalus spp.

Prevalence: 2 of 2 hosts sampled (100%).

Other reported hosts: A. fumipennis, A. moesta, A. nahuana, A. sedula, A. translata, A. vivida.

Locality records: Nebraska: Richardson Co.: Easly Creek.

Argia fumipennis

Nubenocephalus secundus

Prevalence: 16 of 33 hosts sampled (48.5%).

Other reported hosts: A. apicalis, A. immunda, A. moesta, A. sedula, A. translata, A. vivida.

Locality records: Nebraska: Richardson Co.: Easly Creek.

Nubenocephalus spp.

Prevalence: 7 of 15 hosts sampled (46.7%).

Other reported hosts: A. apicalis, A. moesta, A. nahuana, A. sedula, A. translata, A. vivida.

Locality records: Kansas: Atchison Co.: Clear Creek (6/13; 46.2%); Texas: Walker Co.: Harmon Creek (1/2; 50.0%).

Uninfected

Prevalence: 0 of 15 hosts sampled.

Locality records: Nebraska: Nemaha Co.: Deroin Creek.

Argia immunda

Nubenocephalus secundus

Prevalence: 8 of 36 hosts sampled (22.2%).

Other reported hosts: A. apicalis, A. fumipennis, A. moesta, A. sedula, A. translata, A. vivida.

Locality records: Texas: Reeves Co.: San Solomon Springs.

Uninfected

Prevalence: 0 of 19 hosts sampled.

Locality records: Texas: Collin Co.: Russell Creek (0/15; 0%), Kleberg Co.: Tranquitas Creek (0/4; 0%).

Argia moesta

Nubenocephalus secundus

Prevalence: 16 of 57 hosts sampled (28.1%).

Other reported hosts: A. apicalis, A. fumipennis, A. immunda, A. sedula, A. translata, A. vivida.

Locality records: Texas: Kerr Co.: Guadalupe River (2/16; 12.5%), Polk Co.: Big Sandy Creek (5/5; 100%), Randall Co.: Prairie Dog Town Fork-Red River (9/36; 25.0%).

Nubenocephalus spp.

Prevalence: 7 of 36 hosts sampled (19.4%).

Other reported hosts: A. apicalis, A. fumipennis, A. nahuana, A. sedula, A. translata, A. vivida.

Locality records: Kansas: Brown Co.: Delaware River (2/4; 50.0%); Nebraska: Nemaha Co.: Deroin Creek (1/25; 4.0%); Texas: Parker Co.: Lake Mineral Wells (2/2; 100%), Reeves Co.: San Solomon Springs (4/5%; 80.0%).

Uninfected

Prevalence: 0 of 7 hosts sampled.

Locality records: Nebraska: Richardson Co.: Easly Creek (0/2; 0%); Texas: Polk Co.: P-Creek (0/5; 0%).

Argia nahuana

Nubenocephalus spp.

Prevalence: 4 of 19 hosts sampled (21.1%).

Other reported hosts: A. apicalis, A. fumipennis, A. moesta, A. sedula, A. translata, A. vivida.

Locality records: Nebraska: Richardson Co.: Easly Creek.

Argia sedula

Nubenocephalus secundus

Prevalence: 148 of 327 hosts sampled (45.3%).

Other reported hosts: A. apicalis, A. fumipennis, A. immunda, A. moesta, A. translata, A. vivida.

Locality records: Oklahoma: Rogers Co.: Coal Creek (34/48; 70.8%); Texas: Kerr Co.: Guadalupe River (34/58; 58.6%), Kleberg Co.: Tranquitas Creek (17/65; 26.2%), Parker Co.: Lake Mineral Wells (18/23; 78.3%), Randall Co.: Prairie Dog Town Fork-Red River (14/38; 36.8%), Reeves Co.: San Solomon Springs (15/60; 25.0%), Walker Co.: Harmon Creek (16/35; 45.7%).

Nubenocephalus spp.

Prevalence: 3 of 31 hosts sampled (9.7%).

Other reported hosts: A. apicalis, A. fumipennis, A. moesta, A. nahuana, A. translata, A. vivida.

Locality records: Texas: Collin Co.: Russell Creek.

Argia tibialis

Nubenocephalus nebraskensis

Prevalence: 97 of 107 hosts sampled (90.7%).

Other reported hosts: A. translata.

Locality records: Texas: Lee Co.: Yegua Creek (56/61; 91.8%), Polk Co.: P-Creek (16/20; 80.0%), Walker Co.: Harmon Creek (25/26; 96.2%).

Argia translata

Nubenocephalus nebraskensis

Prevalence: 10 of 16 hosts sampled (62.5%).

Other reported hosts: A. tibialis.

Locality records: Texas: Walker Co.: Harmon Creek.

Nubenocephalus secundus

Prevalence: 41 of 60 hosts sampled (68.3%).

Other reported hosts: A. apicalis, A. fumipennis, A. immunda, A. moesta, A. sedula, A. vivida.

Locality records: Texas: Kerr Co.: Guadalupe River.

Nubenocephalus spp.

Prevalence: 6 of 10 hosts sampled (60%).

Other reported hosts: A. apicalis, A. fumipennis, A. moesta, A. nahuana, A. sedula, A. vivida.

Locality records: Oklahoma: Rogers Co.: Coal Creek.

Argia vivida

Nubenocephalus secundus

Prevalence: 39 of 74 hosts sampled (52.7%).

Other reported hosts: A. apicalis, A. fumipennis, A. immunda, A. moesta, A. sedula, A. translata.

Locality records: Nebraska: Richardson Co.: Sardine Creek.

Nubenocephalus spp.

Prevalence: 10 of 34 hosts sampled (29.4%).

Other reported hosts: A. apicalis, A. fumipennis, A. moesta, A. nahuana, A. sedula, A. translata.

Locality records: Nebraska: Richardson Co.: Easly Creek (3/7; 14.3%); Oklahoma: Rogers Co.: Coal Creek (7/27; 25.9%).

Uninfected

Prevalence: 0 of 32 hosts sampled.

Locality records: Nebraska: Nemaha Co.: Deroin Creek (0/13; 0.0%); Texas: Collin Co.: Russell Creek (0/6; 0.0%), Randall Co.: Prairie Dog Town Fork-Red River (0/13; 0.0%).

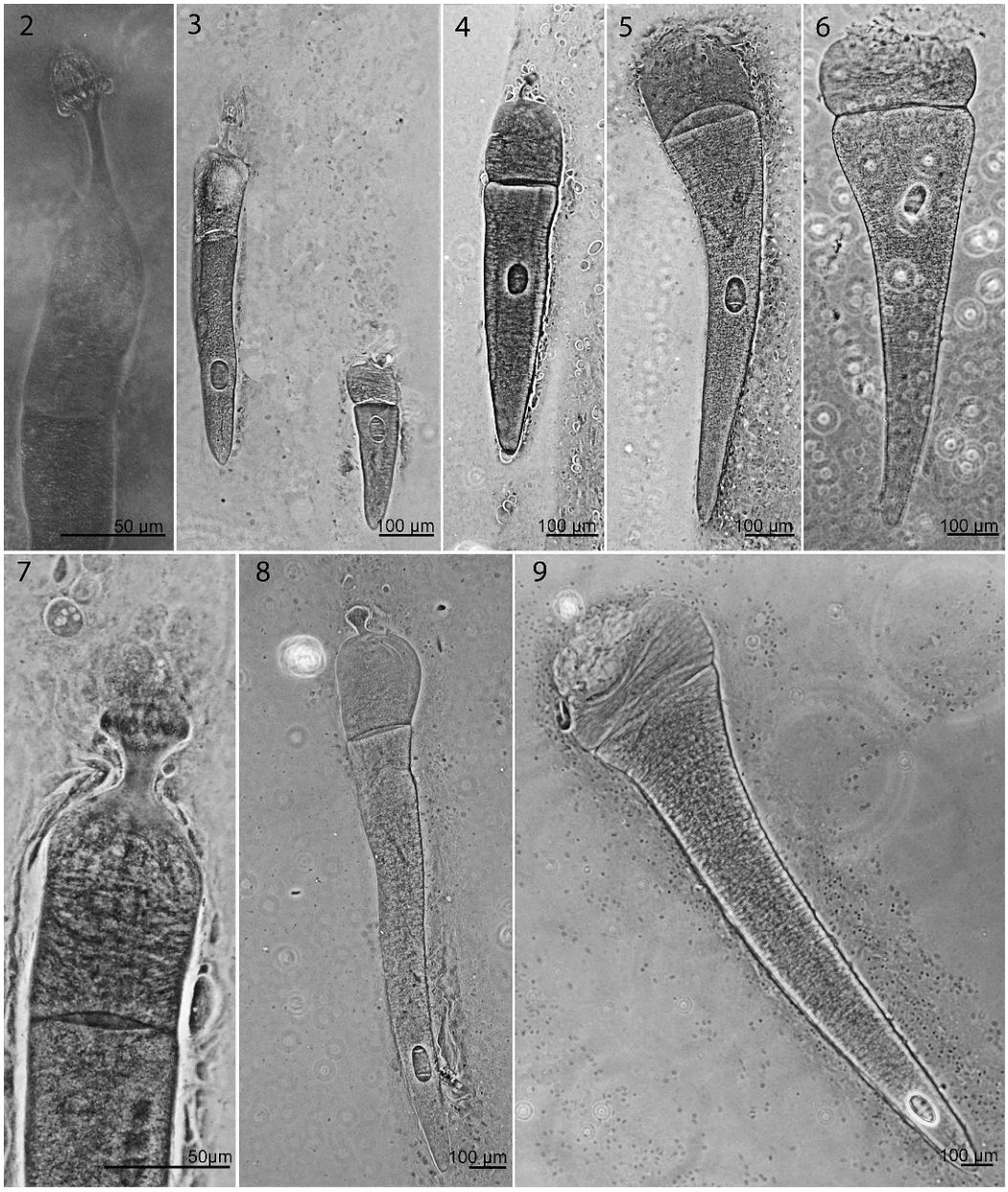
DISCUSSION

Individuals of *N. secundus* and *N. nebraskensis* were diagnosed using epimerite morphology (Figs. 2–4, 7, 8) and overall sporont size (Figs. 5, 6, 9; Hays et al. [2007] provides comparative morphometric ranges). Epimerites differ between species; *N. secundus* possesses a deeply deltoid epimerite with longitudinal pleats, whereas the epimerite of *N. nebraskensis* is deltoid with longitudinal lobes (Clopton et al., 1993; Hays et al., 2007). Sporonts of *N. secundus* are approximately 2/3 the length of *N. nebraskensis* (Hays et al., 2007).

Species of *Nubenocephalus* are broadly distributed over the central United States and rather than being host-specific, they individually utilize a range of argid species. At best, they are restricted to hosts of the genus *Argia*. At least 1 nubenocephalid species parasitized each infected host species at each sampling locality except for the *A. vivida* population ($n = 13$) at Prairie Dog Town Fork-Red River, Randall Co., Texas. *Nubenocephalus secundus* utilizes a broad range of hosts, parasitizing at least 7 species of *Argia*: *A. apicalis, A. fumipennis, A. immunda, A. moesta, A. sedula, A. translata,* and *A. vivida*. In contrast, *N. nebraskensis* was collected from only 2 argid species: *A. tibialis* and *A. translata*. Only *A. translata* serves as a suitable host for both *N. secundus* and *N. nebraskensis*. Both gregarine species co-occurred only at Harmon Creek, Walker Co., Texas, where *N. secundus* infects *A. sedula* and *N. nebraskensis* infects both *A. tibialis* and *A. translata*. Never were both species of *Nubenocephalus* observed simultaneously parasitizing the same host species at a single collecting site. Although this study sampled 9 species of *Argia* across a broad geographic scale, approximately 90 additional species of *Argia* with distributions across North and South America remain to be examined for gregarine infection. This study expands the known range of both species of *Nubenocephalus*, but further studies including collections of additional potential hosts are needed to better understand the true distribution of nubenocephalid gregarines.

It is not surprising that species of *Nubenocephalus* display relatively low host specificity. Traditional notions of host specificity assume an evolutionary vicariance model: host specificity is implicitly a function of phylogenetic history. Clopton (2009) postulated that gregarine assemblages are the products of either vicariant assemblage (phylogenetic events) or ecotypic assemblage (ecological fitting). These phenomena could theoretically be observed simultaneously within a single host–parasite assemblage. The trend revealed in this study regarding host specificity among nubenocephalids parasitizing argid damselflies appears to be an ecotypic effect. Evolutionarily, gregarine species track niche resources along lines of transmission; they do not necessarily track host species (Clopton 2009). Closely related host species share physiological and ecological characteristics that make them indistinguishable niche resources for gregarine exploitation. In this context, host specificity is an ecological phenomenon that reflects the encounter dynamics of hosts and parasite transmission stages. The question becomes "does this parasite species have sufficient spatio-temporal overlap with this host species to establish a stable infective cycle?"

Damselflies of the genus *Argia* are active fliers, moving throughout the riparian zone along streams, rivers, or ponds and tending to rest on bare, open, emergent rocks or vegetation (Westfall and May, 1996). Adults of several species commonly utilize the same habitat. In the present study, up to 5 species of *Argia* were collected from a single locality (*A. apicalis, A. fumipennis, A. moesta, A. nahuana*, and *A. vivida* at Easly Creek, Richardson Co., Nebraska) and on average, 2.5 argid species utilized each study locality. Of 17 sites sampled, only 4 (Cowskin Creek, Sedgwick Co., and Clear Creek, Atchison Co., Kansas; Sardine Creek, Richardson Co., Nebraska; Big Sandy Creek, Polk Co., Texas) were inhabited by a single argid species. Because they use the same, relatively generalized breeding habitats, argids temporally partition their habitat among species over the course of the day (Westfall and May, 1996). Nubenocephalid gregarines recognize a physiologically homogenous assemblage of hosts. These hosts are utilizing the same habitat resources and have the same spatial overlap and transmission interactions with gregarines in the environment. Although there are several damselfly species involved, they represent



Figures 2–9. Representative life cycle stages differentiating *Nubenocephalus secundus* and *Nubenocephalus nebraskensis*. **2–6.** *Nubenocephalus secundus*. **7–9.** *Nubenocephalus nebraskensis*. **2.** Epimerite of young trophozoite. **3, 4.** Trophozoites. **5, 6.** Sporonts. **7.** Epimerite of young trophozoite. **8.** Trophozoite. **9.** Sporont.

an undifferentiated line of transmission for *Nubenocephalus*. Although humans and damselflies certainly recognize species boundaries within *Argia*, there is no evidence to suggest that species of *Nubenocephalus* make the same distinction.

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