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Gregarina niphandrodes (Eugregarinorida: Septatorina): Oocyst Surface Architecture

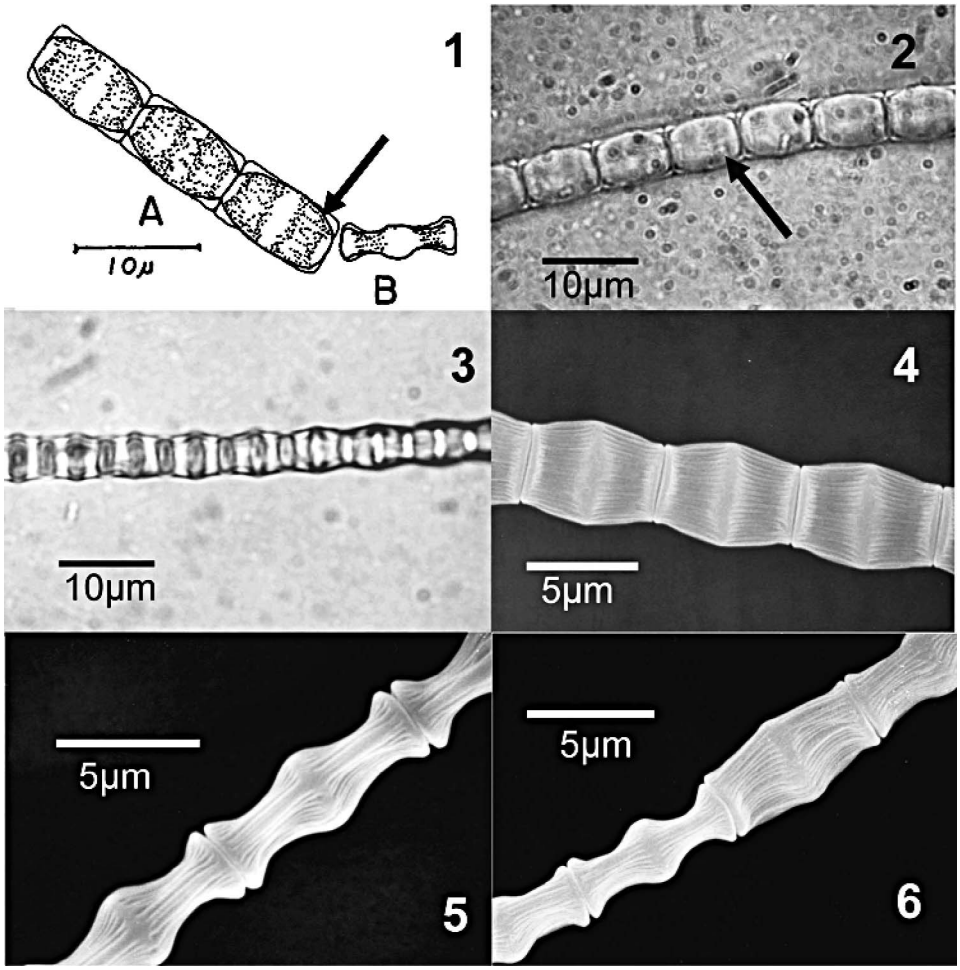
J. Janovy, Jr., M. G. Bolek, J. Detwiler, S. Schwank, A. Knipes, and G. J. Langford, School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588-0118. *e-mail: jjanovy1@unl.edu*

ABSTRACT: The surface architecture of oocysts produced by *Gregarina niphandrodes* (Eugregarinorida) from *Tenebrio molitor* adults (Coleoptera: Tenebrionidae) as revealed by scanning electron microscopy is reported. Gametocysts were allowed to dehisce on 15-mm, round cover glasses; the cover glasses with their oocysts chains were then mounted on stubs without further processing, and sputter-coated with 20-nm gold–palladium. Scanning electron microscopy was performed at 10–15 kV with a Hitachi 3000N SEM. Oocysts retained their characteristic shapes as reported in the original species description but showed longitudinal ridges of relatively uniform height, width, and spacing, in separate fields on either side of a central equatorial bulge in the oocysts. There was no ultrastructural evidence of an enclosing external sheath holding the oocysts in a chain. Oocyst ends were flared slightly, and the chain itself was twisted, with adjacent oocysts offset slightly from one another. This article now provides an additional set of structural characters potentially useful in gregarine systematics.

The class Gregarinasina (Apicomplexa) is perhaps the most diverse eukaryotic taxon because its members parasitize invertebrates, especially annelids and arthropods, although gregarine species have been described from most invertebrate phyla. Descriptive work, however, is somewhat hindered by a number of factors, such as gregarines' lack of medical importance, their seeming paucity of structural features, and the fact that so many potential hosts in interesting parts of the world are relatively inaccessible to workers because of logistical constraints, political turmoil, and lack of funds. Nevertheless, in recent years, Clopton and various coworkers have established criteria by which new taxa should be described and have standardized terminology for shapes and proportions (Clopton et al., 1991, 1992; Clopton, 2004; Clopton et al., 2004). This body of published work shows clearly that oocyst shape and size are highly stable characters of major taxonomic importance.

The present study was intended to increase the number of characters available for gregarine systematics by examining surface architecture of oocysts using scanning electron microscopy. *Gregarina niphandrodes* Clopton, Percival, and Janovy, 1991, from adult *Tenebrio molitor* (Coleoptera: Tenebrionidae) was chosen as the material because both host and parasite are common, well-studied species, and adult beetles are generally infected with only this single gregarine species (Clopton et al., 1992). In addition, *G. niphandrodes* gametocysts are relatively large and easily handled, and oocyst production (''sporogeny'') is easily achieved in moist chambers (Clopton and Janovy, 1993).

Insects used in this study were from cultures maintained for many years at the University of Nebraska–Lincoln using wheat bran and potatoes as food. These are the same cultures from which the type hosts



FIGURES 1–6. (**1**) Drawing of *Gregarina niphandrodes* oocysts from the original species description (Clopton et al., 1991) showing characteristic morphology as seen in 1:1 glycerin:water suspensions. Arrow points to patterns that can now be interpreted as longitudinal ridges. (**2**) Bright-field light micrograph of a *G. niphandrodes* oocyst chain suspended in 1:1 glycerin:water. Arrow points to an area that can now be interpreted as showing longitudinal ridges. (**3**) Bright-field light micrograph of the same *G. niphandrodes* oocyst chain shown in Figure 2, but taken before submersion in glycerin:water. (**4–6**) Scanning electron micrographs of *G. niphandrodes* oocysts, showing surface ridges and the nature of joints between oocysts.

and parasite type specimens were taken (Clopton et al., 1991). Gametocysts were harvested by isolating adult beetles overnight in a plastic shoebox with a slightly moistened paper towel. Shed gametocysts were picked up with a fine camel hair brush and transferred to 1% neutral buffered formalin to be washed for a few minutes before isolation for dehiscence. Gametocysts were placed on 15-mm-diameter, circular cover glasses coated with poly-L-lysine (Sigma, St. Louis, Missouri) according to package instructions and placed in the center well of covered Falcon plastic organ-culture dishes with water in the moat; dehiscence occurred within 3 days. Oocysts were prepared for scanning electron microscopy by simply attaching the cover glass, with its oocyst chains, to a stub then sputter-coating the specimen with 200-nm gold–palladium using a Technics Hummer II sputterer. Scanning electron microscopy was performed at 10–15 kV with a Hitachi 3000N SEM.

Oocysts also were prepared for light microscopy by placing oocyst chains dry on a slide and covering them with a 22 × 22 mm No. 1 cover glass, tacked down at the corners with Elmer's glue. This kind of preparation allowed photographs to be taken of oocysts under oil immersion but as they occur ''naturally,'' i.e., in air. The same oocysts chain could then be photographed after flooding with 1:1 glycerin:water as was done in the original description (Clopton et al., 1991). Digital photographs were taken with a Nikon Coolpix 995 camera fitted with an Optem 25-70-14 adapter (www.optemintl.com). Photographs were converted to grayscale, and brightness and contrast were adjusted slightly using Adobe Photoshop 6.0. The plate of figures was assembled using Adobe Illustrator 10.

Figure 1 is from the original species description and is a drawing of oocysts suspended in 1:1 glycerin:water. In this kind of preparation, oocysts can move freely, so they can be seen in various profiles. Under these conditions, *G. niphandrodes* oocysts are remarkably uniform in size and shape, with a raised center portion and flared ends as seen from the side, and an oblong, almost rectangular shape but with rounded corners and an apparent sheath holding the chain together (Fig. 2). At the same magnification, but without flooding (Fig. 3), the raised equatorial portion is evident, and the oocysts have a truncated diamond shape when viewed ''from above,'' that is, so that maximum length and width were apparent. There is no evidence that sputter-coating and photographing the oocysts without prior use of standard SEM preparation techniques (glutaraldehyde and osmium fixation, dehydration, critical point drying) altered overall shape. The profile as seen from the side is well preserved in the SEM specimens (Figs. 5, 6); additional features not obvious at the light level are the regularly spaced longitudinal ridges of almost uniform height, width, and spacing on either side of the central raised area. However, the original description drawings hint at these ridges (Fig. 1, labeled 4A in the original description), and they can be seen, although not clearly, in the glycerin preparations (Figs. 1, 2, arrows). Immersion in glycerin:water evidently makes a sheath of some kind visible, perhaps by swelling it, thus giving the oocysts a more

rectangular appearance then when dry (cf. Figs. 1, 2 vs. Fig. 3). The broad, truncated diamond shape seen in dry oocysts is also clearly seen in the SEMs (Figs. 3, 4). There was no ultrastructural evidence of an enclosing external sheath holding the oocysts in a chain. Oocyst ends were flared slightly, and the chain itself was twisted with adjacent oocysts offset slightly from one another.

There are 2 main contributions of this study. First, the demonstration that oocyst structure as seen under the light microscope is preserved through sputter-coating and scanning electron microscopy, even though standard fixation, dehydration, and critical-point drying are not performed on the specimens. Second, there are distinct, fine folds or ridges, arranged in a distinctive pattern, on the oocysts. Although these structural features are potentially useful in future gregarine systematic work, such use requires comparative information on oocyst surface architecture from a variety of gregarine taxa.

The differences between oocyst structure as seen in glycerin:water suspensions versus in air at oil immersion magnifications could be the result of refraction of the light beam, although there is also a possibility that the cysts are contained within a sheath of some kind that is expanded in glycerin:water. These differences also emphasize the importance of reporting oocyst preparation methods in detail, for example, as exemplified by Clopton et al. (2004), when using such measurements in taxonomic studies.

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Helminths of Hudsonian Godwits, *Limosa haemastica*, From Alaska and Manitoba

John M. Kinsella, Andy S. Didyk*, and Albert G. Canaris†, HelmWest Laboratory, 2108 Hilda Avenue, Missoula, Montana 59801; *University of New Brunswick, Moncton, New Brunswick E1C 4B7, Canada, †P.O. Box 717, Hamilton, Montana 59480. *e-mail: wormdwb@aol.com*

ABSTRACT: In total, 21 Hudsonian godwits, *Limosa haemastica* (Charadriiformes: Scolopacidae), were examined for helminths, 10 from Bristol Bay, Alaska, and 11 from Churchill, Manitoba. Seventeen species of helminths (9 trematodes, 6 cestodes, 2 nematodes) were collected, but only 1 trematode species, *Plagiorchis elegans*, was found in common between the 2 sample sites. All 17 species are new records for this host and 2 cestodes, *Capsulata edenensis* and *Malika limosa*, are new records for North America. In general, both prevalence and intensities were low, and species richness ranged from 1 to 6 (mean = 2.4). Most of the differences in the helminth faunas between the 2 sites were attributed to difference in habitats, freshwater in Manitoba versus saltwater in Alaska.

The Hudsonian godwit, *Limosa haemastica* (Linnaeus, 1758) (Charadriiformes: Scolopacidae), is a large shorebird that breeds in 5 disjunct areas in North America, including 2 distinct sites along Hudson Bay (1 in Ontario, the other in Manitoba), the Arctic Coast of the Northwest Territories, the southern coast of Alaska, and the west coast of Alaska, and winters in southern South America, especially in Argentina (Elphick and Klima, 2002). This species is found in the boreal forest–tundra transition zone and breeds in areas where open sedge meadows intermix with forests. During staging and migration, godwits feed primarily on invertebrates in coastal tidal flats.

Although the helminths of its Palearctic congeners, the black-tailed godwit, *Limosa limosa* (Linnaeus, 1758) and the bar-tailed godwit, *L. lapponica* (Linnaeus, 1758), have been comparatively well studied (Sergienko, 1972; Schmidt and Allison, 1989; Kornyushin and Greben, 2000; Piersma et al., 2001), almost nothing is known about the helminths of *L. haemastica*. The description of *Wardium villocirrosus* (= *Hymenolepis villocirrosus*) from the Hudsonian godwit in Alaska by Deblock and Rausch (1967) appears to be the only published record of a helminth from this host.

Ten Hudsonian godwits were collected between 13 and 24 June 1991, and a single godwit on 19 June 1992 by ASD under scientific permit numbers WS-M54 and WS-M26 issued by the Canadian Wildlife Service. All birds were collected in the vicinity of the Twin Lakes (58°39′24″N, 93°51′05″W) in the Cape Churchill Wildlife Management Area in Manitoba, Canada. The area is freshwater habitat consisting of scattered clumps of black spruce and tamarack forest, sedge meadows, and lichen heaths. Between 23 and 29 July 2001, 10 Hudsonian godwits were collected by A.G.C. and J.M.K. from a 6.0-km-long section of littoral zone just north of the mouth of the Egegik River, Bristol Bay, Alaska, between Bishop Creek (58°14′31″N, 157°29′43″W), and Big Creek (58°17′01″N, 157°32′5″W).

All hosts were killed with a shotgun, and each was examined within 6 hr of collection. In the Manitoba birds, only the lower intestinal tract (small intestine, ceca, large intestine, cloaca) was examined. In the Alaska birds, all internal organs were examined. The koilin of the ventriculus was removed, and both the ventriculus and proventriculus tissues were teased apart. Skin and blood were not examined in either sample. Nematodes were studied in temporary mounts of lactophenol, and trematodes and cestodes were stained with either Meyer's carmine or Ehrlich's hematoxylin, cleared in methyl salicylate, and mounted in Canada balsam. Voucher specimens were deposited in the National Parasite Collection, Beltsville, Maryland under accession numbers 99447–99463.

In total, 17 species of helminths (9 trematodes, 6 cestodes, 2 nematodes) were recovered from 21 godwits (Table I). All are new host records for *L. haemastica*. Godwits from Alaska were infected with 9 species (5 trematodes, 2 cestodes, 2 nematodes), and those from Manitoba were also infected with 9 species (5 trematodes, 4 cestodes), but only 1 trematode species, *Plagiorchis elegans*, was present in birds at both sites. Four godwits from Alaska and 3 from Manitoba (7 of 21) were negative for helminths. In general, prevalence and intensities were low (Table I), and species richness ranged from 1 to 6 species per host (mean = 2.4 ± 1.2 SD).

Two cestode species, *Capsulata edenensis* and *Malika limosa*, are