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STRUCTURAL COMPARISON OF THE CHORION SURFACE OF FIVE *PHILONTHUS* SPECIES (COLEOPTERA: STAPHYLINIDAE)

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Abstract.—Surface structures of eggs of five species of *Philonthus* are illustrated with the aid of scanning electron micrographs. Eggs studied are of the following cow-dung-inhabiting species in northcentral Florida: *Philonthus longicornis* Stephens, *P. flavolimbatus* Erichson, *P. ventralis* (Gravenhorst), *P. sericans* (Gravenhorst) and *P. hepaticus* Erichson. The eggs of *P. longicornis, P. flavolimbatus* and *P. ventralis* have high, longitudinal ridges, which are 4–5 for *P. ventralis,* but 7–8 for the other two species. The ridges of *P. longicornis* eggs are continuous and wider, and the egg surface between the ridges is covered by tubercles, whereas the ridges of *P. flavolimbatus* eggs are discontinuous (with weak connections between the tubercles) and narrower, and the egg surface between the ridges is covered by sharp teeth. Eggs of *P. sericans* have low, parallel and connected ridges, which consist of minute granules and scattered enlarged granules over the entire surface. Eggs of *P. hepaticus* are covered by compact, minute granules and cone-shaped structures resembling volcanoes. Aeropyles occur on the egg ridges in *P. longicornis* and *P. flavolimbatus,* but on the surface between the ridges in *P. ventralis* and *P. sericans.* A key is given to the five species of *Philonthus* eggs.

Key Words: *Philonthus,* eggs, surface structure

Staphylinids are considered to be the most important predators in cow dung, due to the diversity of species and high population levels. Members of the genus *Philonthus* are predacious on eggs and larvae of Diptera (Harris and Blume 1986, Laurence 1956, Sanders and Dobson 1966). They are considered to be efficient predators of the horn fly, *Haematobia irritans* (Fincher and Summerlin 1994, Harris and Oliver 1979, Hunter et al. 1989, Macqueen and Beirne 1975, Roth 1982, Thomas and Morgan 1972), and the face fly, *Musca autumnalis* (Valiela 1969, Wingo et al. 1974).

During a survey of the insect fauna of cow dung in two pastures in Alachua County (northcentral Florida) in 1993, we collected specimens of six species of *Philonthus,* among which one species (*P. rectangulus* Sharp) is new to Florida (only one specimen was collected). There have been no studies on the ecology of these dung-inhabiting species. Therefore we colonized the previously recorded five species (Frank 1986) for biological studies and predation tests against immature horn flies. The species included *Philonthus longicornis* Stephens, *P. flavolimbatus* Erichson, *P. ventralis* (Gravenhorst), *P. sericans* (Gravenhorst) and *P. hepaticus* Erichson. Egg specimens of each species were prepared for this study.

The surface structure of the egg chorion of some *Philonthus* species was described by Mank (1923; 1 sp.), Frank (1968; 1 sp.), Tawfik et al. (1976a, b, c; 3 spp.), and Hinton (1981b; 17 spp.) with light microscopy. Scanning electron microscopic (SEM) studies of eggs of *Philonthus* were published by Hinton (1981a) for 3 European species, and by Hunter et al. (1989) for two North American species. Among the *Philonthus* species that we collected, only the eggs of *P. longicornis* and *P. flavolimbatus* were observed under the light microscope and under SEM, respectively (Tawfik et al. 1976c, Hunter et al. 1989). These studies did not provide much detailed description. The objective of this study is to describe and compare the exochorionic surface structures of the five dung-inhabiting *Philonthus* species found in northcentral Florida.

Materials and Methods

Insect collection and culture.—Eggs were obtained from females in laboratory colonies. Female *Philonthus* were extracted from the dung using emergence boxes (Hu and Frank 1995), designed by G. T. Fincher (USDA-ARS, College Station, Texas). Fresh cow-dung pads were sampled from two pastures in Alachua County, Florida, placed individually into the emergence boxes and brought into the laboratory. Insects that left the dung in the emergence box were collected daily in a removable plastic vial and cup attached to holes on the end and bottom of the box, respectively. *Philonthus* colonies were maintained by following the method of Hunter et al. (1986). Adult females were confined in Petri dishes (5.1 cm diameter × 1.3 cm high), lined on the bottom with a moist paper towel. A water-soaked cotton ball was provided for humidity, and horn fly eggs and first-instar larvae were provided for food.

Specimen preparation.—For photography using SEM, eggs were removed from a Petri dish with a fine paintbrush and placed into 70% isopropyl alcohol. These eggs preserved in alcohol for at least 24 h, were cleaned by an ultrasonic cleaner for 3–5 min to remove pieces of paper towel or remnants of consumed immature horn flies. After cleaning, eggs were dehydrated in a graded alcohol series, then dried in a desiccator over Ca_2SO_4. Dried eggs were attached to an SEM stub using double-sided tape and sputter-coated with gold in a Denton Vacuum DESK II Gold Sputter Etch Unit. The eggs were examined with an SEM (Hitachi 570) at 20 KV and 100 mA and photographed. Five to ten eggs were used for each species.

Egg size measurements.—Less than 24 h old eggs were measured at 25× with a dissecting microscope and an ocular micrometer. *Philonthus* eggs increased in size during the incubation period (Tawfik et al. 1976a, b, c), because they became permeable to water one day after there was an appreciable secretion of serosal cuticle (Hinton 1981b). The length and width were measured for ten eggs of each species. Each sample of ten eggs was produced by 2–5 females. Mean egg length of species was compared using analysis of variance (ANOVA) and Duncan's multiple range test (SAS 1990). Measurements are presented as ranges followed by the mean and one standard deviation.

Terminology.—The terms for morphological description used in this paper follow those of Hinton (1981b), including ridges, processes, projections, tubercles, and aeropyles. Terms that were not provided by Hinton were adopted from Endris et al. (1987) for connected ridges, and Woodruff (1973) for granules.

Results

General.—Most eggs were laid under the cotton ball or at the edge of Petri dishes under the paper towel. Female *P. longicornis* often tried to bury their eggs under macerated paper towel. Cannibalism of eggs by adult females was observed for all five species, especially for *P. longicornis.*

The eggs of all species are oval, with sculptures on the surface of the exochorion (Figs. 1A–E). The sculpture includes high

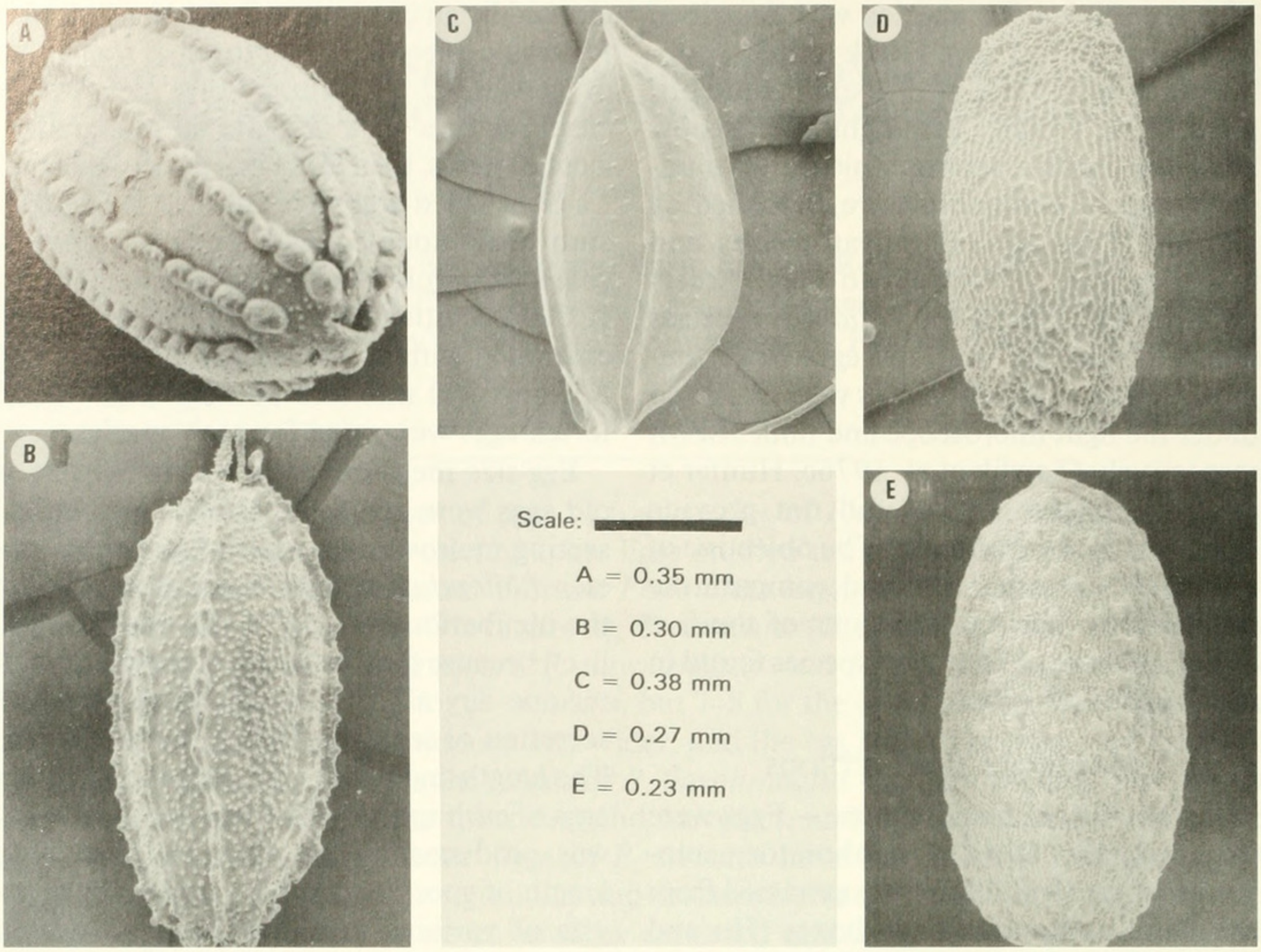

Fig. 1. Scanning electron micrographs of lateral view of the whole egg. (A) *P. longicornis*; (B) *P. flavolimbatus*; (C) *P. ventralis*; (D) *P. sericans*; (E) *P. hepaticus*.

longitudinal ridges, low connecting ridges, tubercles, granules and cone-shaped structures resembling volcanoes. When newly deposited, the eggs are pale yellow in color; twenty-four h later, the color becomes darker, the size increases, and the sculpture becomes more distinct. The incubation period lasts 2–4 days. From the second day, the embryo can be seen easily through the transparent chorion, and the eye spots attain a pale brown color. Descriptions of eggs of each species follow.

P. longicornis Stephens.—Size: length, 1.07–1.20 (1.13 ± 0.03) mm; width, 0.52–0.57 (0.55 ± 0.02) mm. The egg is extended anteriorly into a club-shaped tube-like process of ca. 0.4–0.6 mm long and posteriorly with a small projection. The exochorion has 7–8 high longitudinal ridges. The ridges are well developed (continuous; Fig. 1A) and consist of 19–35 tubercles on each ridge. The distance between the ridges across the middle line of the egg is 0.24–0.41 mm. The surface of the exochorion between the ridges is covered by scattered blunt tubercles that are 6–9 μm long (Fig. 2A). Aeropyles occur on the tubercles of the ridges.

P. flavolimbatus Erichson.—Size: length, 0.79–0.88 (0.83 ± 0.03) mm; width, 0.39–0.50 (0.44 ± 0.03) mm. The egg is extended anteriorly into a club-shaped, tubular process and posteriorly with a small projection. The exochorion has 7–8 high longitudinal ridges. Compared to *P. longicornis,* the ridges are not so well developed. They are narrower and discontinuous between some tubercles (Fig. 1B). Each ridge has 16–21 tubercles. The distance between the ridges across the middle line of the egg is 0.14–0.21 mm. The surface of the exochorion

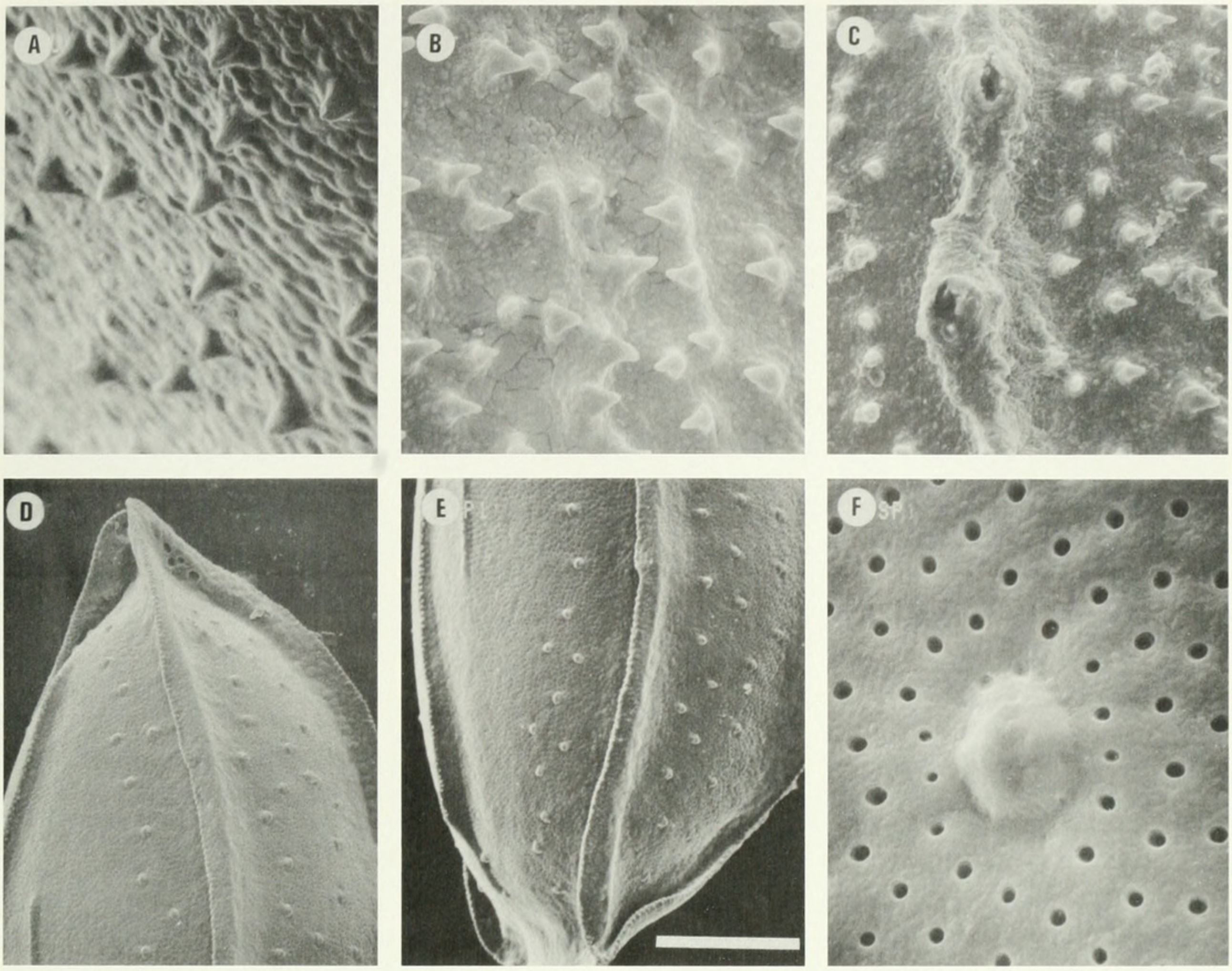

Fig. 2. Scanning electron micrographs of lateral view of egg structures. (A) *P. longicornis,* showing blunt tubercles; (B) *P. flavolimbatus,* showing sharp teeth; (C) *P. flavolimbatus,* showing aeropyles on ridges; (D–F) *P. ventralis:* (D) anterior end; (E) posterior end; (F) Showing a tubercle and aeropyles. Magnifications are based on scale bar in E: A = 23 μm, B = 30 μm, C = 42 μm, D = 0.18 mm, E = 0.18 mm, F = 15 μm.

between the ridges is covered by densely distributed sharp teeth, which are 6–10 μm long (Fig. 2B). The aeropyles occur on the tubercles of the ridges (Fig. 2C). They are elongated with a size of approximately 18 × 15 μm.

P. ventralis (Gravenhorst).—Size: length, 1.04–1.21 (1.13 ± 0.06) mm; width, 0.48–0.59 (0.54 ± 0.03) mm. The egg is extended anteriorly into a pointed process and posteriorly with a projection (Figs. 1C, 2D, E). The egg is asymmetrical dorso-ventrally, dorsally domed, and ventrally somewhat flat. The exochorion has 4–5 high longitudinal ridges, with two on the dorsal surface, one on each side, and one or none on the ventral surface. The ridges are well developed (Fig. 1C) and have many small cross-ridges (Figs. 2D, E). The distance between the ridges across the middle line of the egg is 0.21–0.32 mm. The surface of the exochorion between the ridges is covered by scattered tubercles (Figs. 2D, E) and compact aeropyles (Fig. 2F). Each tubercle is 14–17 μm in diameter, and each aeropyle is 1.5–2.5 μm in diameter.

P. sericans (Gravenhorst).—Size: length, 0.79–0.91 (0.83 ± 0.04) mm; width, 0.38–0.50 (0.46 ± 0.04) mm. The egg is extended anteriorly into a club-shaped, tubular process, and lacks a process toward the posterior end (Fig. 1D). The exochorion has low and thin ridges which consist of minute granules. The ridges are flat in the middle

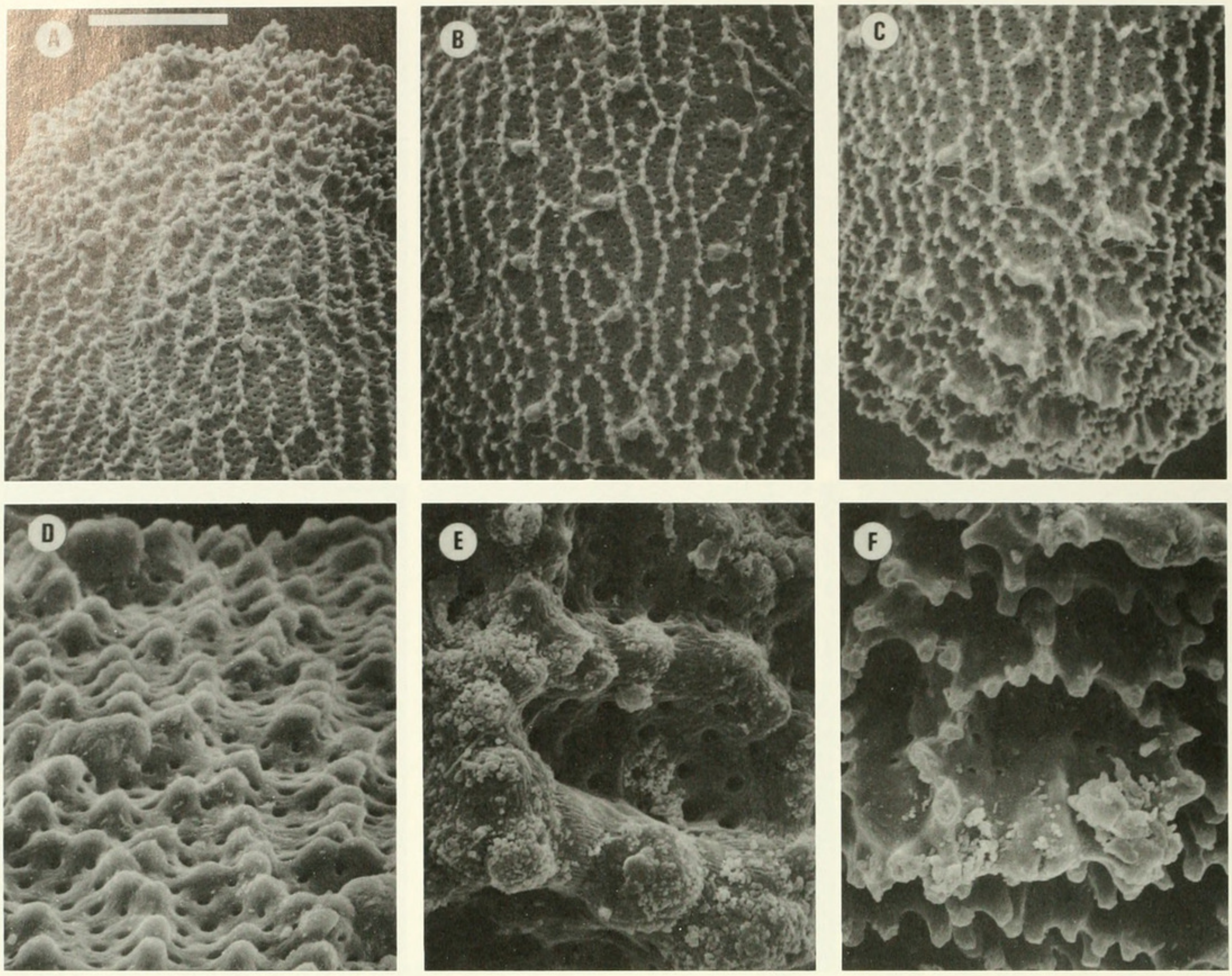

Fig. 3. Scanning electron micrographs of *P. sericans* eggs. (A) lateral view of anterior end, showing compact granular ridges; (B) lateral view of middle portion, showing flat connected ridges; (C) lateral view of posterior end, showing strengthened and compact ridges; (D) anterolateral view, magnification of aligned minute granules on front end; (E) lateral view, showing toe-like granules of circular structure on the middle portion; (F) posterolateral view, showing tooth-like granules on a circular structure. Magnifications are based on scale bar in A: A = 0.1 mm, B = 0.1 mm, C = 0.1 mm, D = 25 μm, E = 23 μm, F = 15 μm.

portion of the egg, and are parallel and connected by cross-ridges (Fig. 3B). High magnification shows that the granules of the flat ridges are toe-like (Fig. 3E). At the anterior end they become higher and compact (Figs. 3A, D). At the posterior end they are highest, and form a circular structure (7–30 μm in diameter; Fig. 3C). The granules on the thin ridges of the posterior end are tooth-like (Fig. 3F). The surface of the exochorion between the thin ridges is covered by scattered enlarged granules (Figs. 3A–C) and compact aeropyles (Figs. 3A–F). The enlarged granules are 10–15 μm in diameter and the aeropyles are 1.5–2 μm in diameter.

P. hepaticus Erichson.—Size: length, 0.79–0.88 (0.83 ± 0.03) mm; width, 0.39–0.50 (0.44 ± 0.03) mm. The egg is extended anteriorly into a small projection (Figs. 1E, 4A) and lacks a process toward the posterior end (Figs. 1E, 4B). The exochorion is covered by compact minute granules and scattered irregular enlarged granules. These enlarged granules are alternated with pits, forming cone-shaped structures resembling volcanoes (Figs. 1E, 4A, B) which are evenly distributed over the entire surface (Fig. 1E). The minute granules on the enlarged ones are short and scattered (Fig. 4C), but tooth-like and aligned into lines between the cone-shaped structures (Fig. 4D). The granules form no precise patterns.

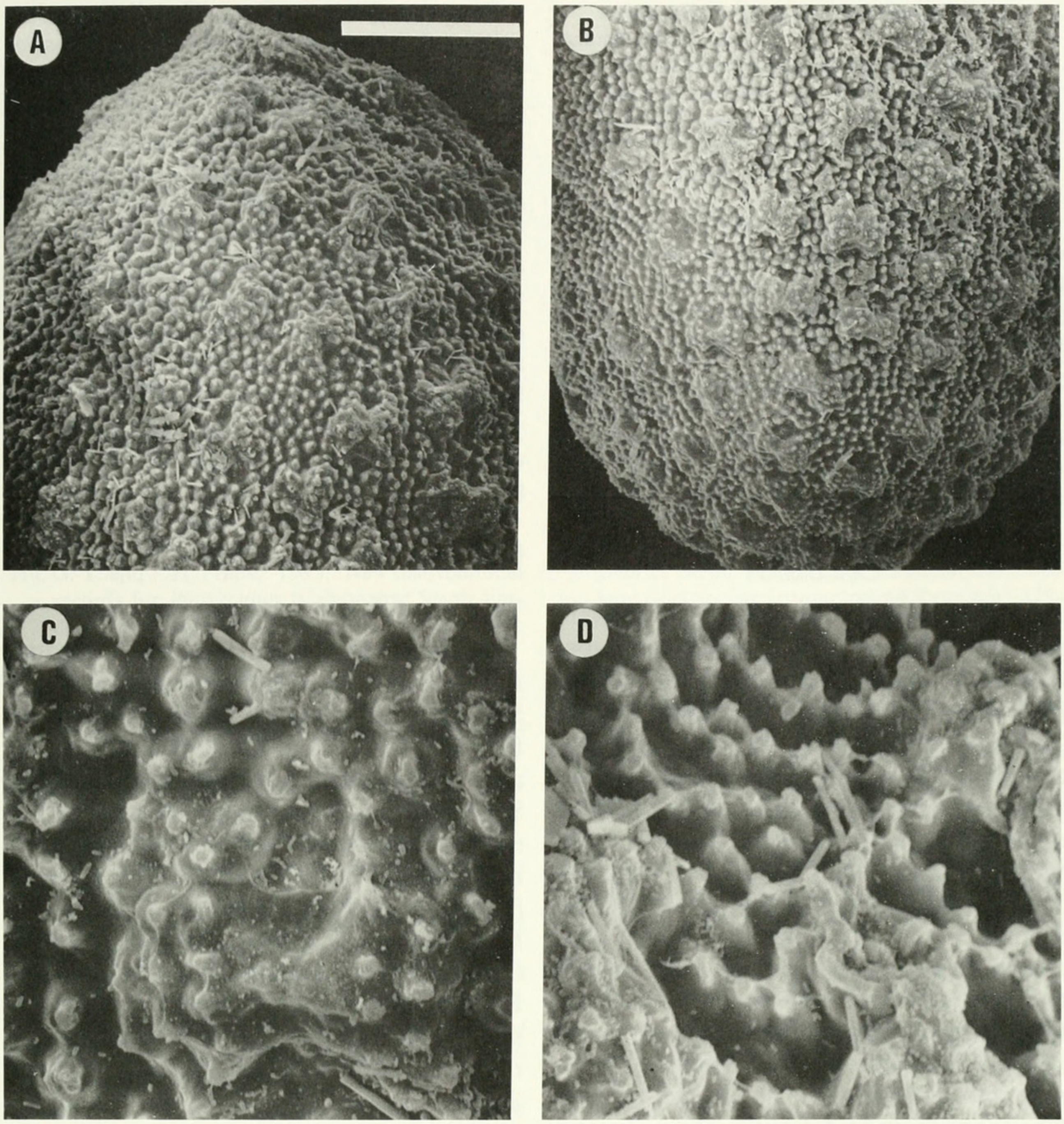

Fig. 4. Scanning electron micrographs of *P. hepaticus* eggs. (A) anterolateral view; (b) posterolateral view, showing volcano-like structure; (C) lateral view, showing minute granules on the enlarged ones; (D) lateral view, showing minute granules on the egg surface between the enlarged ones. Magnifications are based on scale bar in A: A = 0.10 mm, B = 0.10 mm, C = 20 μm, D = 20 μm.

Key to the Eggs of Dung-Inhabiting *Philonthus* in Northcentral Florida

1. Exochorion with strong, longitudinal ridges (Figs. 1A–C) 2
– Exochorion without strong longitudinal ridges (Figs. 1D, E) 4
2. With 4–5 ridges; exochorion between ridges covered with aeropyles and scattered tubercles (Fig. 2F) .... *ventralis* (Gravenhorst)
– With 7–8 ridges; exochorion between ridges covered by tubercles or granular teeth 3
3. All ridges well-developed (continuous); tubercles present on the exochorion between the ridges (Fig. 2A) .... *longicornis* Stephens
– Not all ridges well developed (discontinuous); granular teeth present on the exochorion between the ridges (Fig. 2B) *flavolimbatus* Erichson
4. Exochorion with weak and thin granular ridges

and enlarged granules over the entire surface (Figs. 3A–C). . . . . *sericans* (Gravenhorst)
– Exochorion with compact minute granules and scattered enlarged granules which are alternated with pits, forming cone-shaped structures over the entire surface (Figs. 1E, 4A, B) . . . . *hepaticus* Erichson

Egg size.—Mean egg length was found to vary significantly between species ($F = 167.08$; df = 4,45; $P < 0.01$). Duncan's multiple range test determined that the eggs of *P. longicornis* and *P. ventralis* were statistically similar to each other yet significantly larger (longer) than those of *P. flavolimbatus, P. sericans* and *P. hepaticus.* Egg of *P. flavolimbatus, P. sericans* and *P. hepaticus* did not differ significantly in size.

Discussion

Alcohol fixation and desiccation were tried using staphylinid eggs of several species from three subfamilies. Most eggs of *Philonthus* and those of *Neohypnus pusillus* (Sachse) (Staphylininae) did not collapse, but almost all the eggs of *Aleochara notula* and *Atheta* sp. (Aleocharinae) and *Platystethus americanus* Erichson and *Anotylus insignitus* (Gravenhorst) (Oxytelinae) collapsed. This may be because the egg chorion of *Philonthus* spp. and *N. pusillus* is thicker than that of the other species and is sculptured over the entire outer surface. The sculpture forms supportive structure for the egg shells and may help the eggs resist external pressure and substances from decaying dung. The egg chorion of *A. notula, Atheta* sp. and *P. americanus,* however, is thin and smooth, without any surface sculpture.

Egg size is not a reliable character for separating eggs of these five species of *Philonthus,* because the size is similar for *P. longicornis* and *P. ventralis,* and for *P. flavolimbatus, P. sericans* and *P. hepaticus.* Microsculpture is the only egg character that can be used for species determination of *Philonthus* studied here. These include patterns and presence of ridges, tubercles, processes and projections, granular teeth, granules, cone-shaped structures and aeropyles.

The egg of *P. cruentatus* Gmelin was described by Hunter et al. (1989). Its shape and ridge characteristics are similar to those of *P. longicornis.* Further detailed study is needed for separating these two species because they have overlapping distribution (Blume 1985).

Tiny holes evenly cover the egg surface of *P. ventralis* and *P. sericans,* and open on the tubercles of the egg ridges of *P. longicornis* and *P. flavolimbatus.* These holes were named aeropyles by Hinton (1970, 1981a, b), because they are used for absorbing oxygen from the ambient air. Aeropyles are common on the eggs of terrestrial insects (Hinton 1970).

Philonthus eggs were most abundant in field-collected dung of about 7 d old.

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Literature Cited

Blume, R. R. 1985. A checklist, distributional record, and annotated bibliography of the insects associated with bovine droppings on pastures in America north of Mexico. Southwestern Entomologist 9: 1–55.

Endris, R. G., D. G. Young, and P. V. Perkins. 1987. Ultrastructural comparison of egg surface morphology of five *Lutzomyia* species (Diptera: Psychodidae). Journal of Medical Entomology 24: 412–415.

Fincher, G. T. and J. W. Summerlin. 1994. Predation on the horn fly by three exotic species of *Philonthus.* Journal of Agricultural Entomology 11: 45–48.

Frank, J. H. 1968. Notes on the biology of *Philonthus decorus* (Grav.) (Col., Staphylinidae). Entomologist's Monthly Magazine 103: 273–277.

———. 1986. A preliminary checklist of the Staphylinidae (Coleoptera) of Florida. Florida Entomologist 363–382.

Harris, R. L. and L. M. Oliver. 1979. Predation of *Philonthus flavolimbatus* on the horn fly. Environmental Entomology 8: 259–260.

Harris, R. L. and R. R. Blume. 1986. Beneficial insects inhabiting bovine droppings in the United Stages, pp. 10–15. *In* R. S. Patterson & D. A. Rutz, eds., Biological Control of Muscoid Flies. Entomological Society of America Miscellaneous Publications No. 61.

Hinton, H. E. 1970. Insect eggshells. Scientific American 223: 84–91.

———. 1981a. Biology of Insect Eggs, Vol 1. Pergamon Press, Oxford.

———. 1981b. Biology of Insect Eggs. Vol 2. Pergamon Press, Oxford.

Hu, G. Y. and J. H. Frank. 1995. New distributional records for *Platystethus* (Coleoptera: Staphylinidae: Oxytelinae) with notes on the biology of *P. americanus*. Florida Entomologist 78: 137–144.

Hunter, J. S. III, G. T. Fincher, and D. E. Bay. 1986. Methods for rearing *Philonthus* spp. associated with cattle droppings. Journal of Entomological Science 21: 83–86.

Hunter, J. S. III, D. E. Bay, and G. T. Fincher. 1989. Laboratory and field observations on the life history and habits of *Philonthus cruentatus* and *Philonthus flavolimbatus*. Southwestern Entomologist 14: 41–47.

Laurence, B. R. 1956. The larval inhabitants of cow pats. Journal of Animal Ecology 23: 234–260.

Macqueen, A. and B. P. Beirne. 1975. Influence of other insects on production of horn fly, *Haematobia irritans* (Diptera: Muscidae), from cattle dung in South Central British Columbia. Canadian Entomologist 107: 1255–1264.

Mank, H. G. 1923. The biology of the Staphylinidae. Annals of the Entomological Society of America 16: 220–37.

Roth, J. P. 1982. Predation on the horn fly, *Haematobia irritans* (L.). Southwestern Entomologist 7: 26–30.

SAS Institute. 1990. User's Guide: Statistics. Ver. 6, 4th ed. SAS Institute Inc., Cary, North Carolina.

Sanders, D. P. and R. C. Dobson. 1966. The insect complex associated with bovine manure in Indiana. Annals of the Entomological Society of America 59: 955–59.

Tawfik, M. F. S., K. T. Awadallah, E. D. Ammar, and S. M. Abul-Ela. 1976a. The life history of *Philonthus misor* Tott. (Coleoptera: Staphylinidae). Bulletin de la Societe Entomologique d'Egypte 60: 345–355.

———. 1976b. The life history of the staphylinid *Philonthus turbidus* Er. (Coleoptera: Staphylinidae). Bulletin de la Societe d'Entomologique Egypte 60: 357–366.

———. 1976c. On the bionomics of *Philonthus longicornis* Steph. (Coleoptera: Staphylinidae). Bulletin de la Societe Entomologique Egypte 60: 379–387.

Thomas, G. D. and C. E. Morgan. 1972. Field-mortality studies of the immature stages of the horn fly in Missouri. Environmental Entomology 1: 453–459.

Valiela, I. 1969. An experimental study of the mortality factors of larval *Musca autumnalis* DeGeer. Ecological Monographs 39: 119–225.

Wingo, C. W., G. D. Thomas, G. N. Clark and C. E. Morgan. 1974. Succession and abundance of insects in pasture manure: relationship to face fly survival. Annals of the Entomological Society of America 67: 386–390.

Woodruff, R. E. 1973. The scarab beetles of Florida. Pt. 1. Arthropods of Florida and Neighboring Land Areas. Vol. 8. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville.