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Gregarina coronata N. Sp. (Apicomplexa: Eugregarinida) Described from Adults of the Southern Corn Rootworm, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae)

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ABSTRACT. *Gregarina coronata* n. sp. (Apicomplexa: Eugregarinida) is described from the adults of the Southern Corn Root Worm, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae). Measurements given are means, in micrometers, taken from mature gamonts in association. Primite: protomerite hemi-ellipsoidal with basal tumidus, length 47.6, width 44.0, with cytoplasmic granule, apical crown apparent; deutomerite elongate ellipsoidal, length 227.9, width 81.3; epimerite absorbed into anterior in gamont, globular in trophozoite. Satellite: protomerite hemi-ellipsoidal, truncated at association interface to appear trapezoidal, length 39.2, width 49.6, with cytoplasmic granule; deutomerite elongate ellipsoidal, length 240.6, width 80.2; epimerite absorbed into anterior in gamont. Association caudofrontal and often precocious, occurring during growth of trophozoites. Gametocysts spherical, 115.3 in diameter, 132.9 with hyaline coat; producing multiple oocyst chains under moist storage in 24–36 h. Oocysts very uniform in shape and size, dorsad: doliform, length 6.4, equatorial width 3.4, polar width 2.9; pleuron: dorso-ventrally flattened, corpus concave with bicondylic termina; corpus height 0.98, width 4.44; terminus height 1.96, width 0.98.

Key words. Eugregarinidae, parasite, Septaterina.

EUGREGARINE parasites have been described and listed for several chrysomelid beetles [9–12, 14, 30–33, 35]. They have been anecdotally reported from the gut of the Southern Corn Rootworm, *Diabrotica undecimpunctata howardi,* tangential to field surveys of the beetle's diet and pollen preference [5, 23]. Eugregarine infections in *D. undecimpunctata howardi* and related species have been discussed within the context of pathogen control in insect colonization and mass production for research programs [2, 7, 8]. Unfortunately, the taxonomic status of these infections has been of little concern to the entomological community and has remained unresolved. The eugregarines infecting the host genus *Diabrotica* may represent an excellent system to study both allopatric speciation through host divergence and the mechanisms of host-specificity in the Gregarinidae. However, the taxonomy of the parasite complex must be established to provide an initial foundation for conceptual work. The present study describes *Gregarina coronata* n. sp. from adult *D. undecimpunctata howardi.*

MATERIALS AND METHODS

Colonies of *D. undecimpunctata howardi* were established at the School of Biological Sciences, University of Nebraska-Lincoln using beetles purchased from commercial sources (French Agricultural Research, Lamberton, MN). Zucchini squash slices were presented as a *D. undecimpunctata* food item and gregarine gametocysts were subsequently collected from feces deposited during host feeding. Groups of 20 gametocysts were placed in tissue culture dishes and held in moist storage (>78% relative humidity) for maturation. Oocyst structure and dimensions were taken from fresh preparations of extruded oocyst chains. These preparations were made from oocysts suspended in water or glycerin. Oocysts rotated freely in glycerin, thus allowing elucidation of the full three-dimensional structure of the oocyst. Individual oocyst lengths, widths, and depths were measured at their widest points and are reported in micrometers. There was no evidence to suggest a change in oocyst structure as a result of osmotic tension in either water or glycerin. Seven beetles were experimentally infected with aggregate oocyst mass collected from several cysts, then dissected in insect saline without sucrose [1] 96–132 h post-infection, and examined for parasites in March, 1991. Ten free gamont associations were measured from each fresh preparation. Widths of protomerites and deutomerites were taken at the widest points. Additional observations on the morphological structure of immature trophozoites and single mature gamonts were made from beetles randomly selected from infected colonies and dissected in insect saline without sucrose. The following histological reactions and/or stains were used to study cytoplasmic granules in *G. coronata* sectioned in situ with host mesenteron: Masson's Trichrome Method (modified from Luna [24] by omission of 2% Light Green counterstain), Feulgen's reaction [13], the Periodic acid-Schiff reaction [25], and Defano's method for golgi apparatus modified by use of whole mounts rather than post-stain sectioning [6, 24]. Alcoholic Bouin's Fluid [36] was used as a standard fixative prior to embedding unless contraindicated by the reaction or staining protocol.

Measurements are presented in this paper as range values with embedded means followed by the standard deviation in parentheses. All measurements are in micrometers unless otherwise indicated. Data from fresh gamonts in association are reported.

Terminology used in this paper is consistent with that used by Levine [18]. The following abbreviations are used here: LP, length of protomerite; LD, length of deutomerite; TL, total length; WP, width of protomerite; WD, width of deutomerite.

Drawings were made with the aid of a camera lucida. All observations were made on an American-Optical Spencer binocular compound microscope with 15× wide-field eyepieces, 40× and 100× (oil immersion) objectives, and a Silge & Kuhne Orthoilluminator B. Green filters were used for measurements and daylight filters were used for observation of color in living specimens.

Description

Gregarina coronata n. sp. (Fig. 1–4)

Diagnosis. Eugregarinida Léger, 1900 [16] sensu Levine et al. [21]: Septatina Lankester, 1885 [15] sensu Levine et al. [21]: Gregarinidae Labbé, 1899 [14] sensu Levine [20] with the characters of the genus *Gregarina* Dufour, 1828 [4] sensu Levine [20]: mucron conical, button-shaped, globular or cylindrical; syzygy rather precocious: oocysts doliform, spherical or navicular; in intestine of insects.

Trophozoite. (Fig. 1, 2) Attached to host epithelium, solitary or in precocious association. Protomerite hemi-elliptical without constriction. Deutomerite of young trophozoites claviform, tumidus shifting posteriad with maturity. Deutomerite of primite and satellite in precocious association fusular; filling pos-

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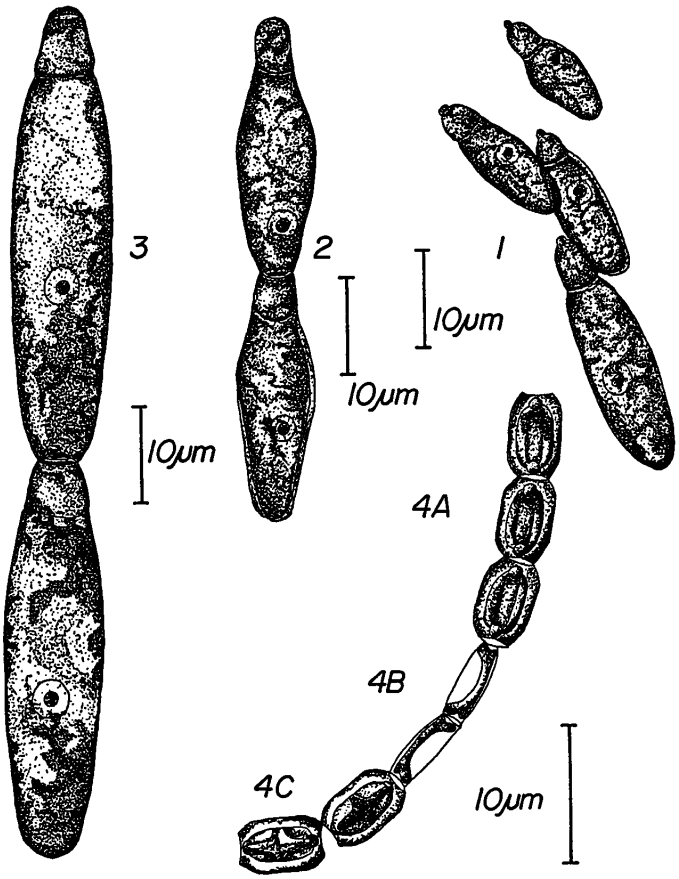

Fig. 1–4. *Gregarina coronata* n. sp. 1. Trophozoites. 2. Immature gamonts in association. 3. Mature gamonts in association. 4. Oocyst chain showing details of dorsal (A), pleural (B) and ventral (C) aspects.

teriad with maturity to reflect the elliptical structure of the gamont. Epimerite simple, globular; without visible septum; obvious in young trophozoites, apparent in some form in all trophozoite stages except satellite trophozoites in precocious association. Fresh trophozoites with endocyte clear to partially granular when very young, becoming mostly opaque with maturity, ectocyte clear to light orange in color. Color whitish under dissecting microscope with incandescent illumination, clear to orange-brown under compound microscope with daylight filter.

Gamonts in Association. See Fig. 3.

Primite. Protomerite: hemi-ellipsoidal with basal tumidus; length 40.0–**47.6**–60.0 (6.1±), width 30.0–**44.0**–70.0 (9.1±); with posterior cytoplasmic granule, apical crown visible in distal third of the protomerite. Deutomerite: elongate ellipsoidal; length 120.0–**227.9**–330.0 (52.0±), width 45.0–**81.3**–140.0 (24.0±). Total length 170.0–**275.5**–380.0 (54.8±). LP/TL ratio 0.13–**0.18**–0.29 (0.04±). LD/TL ratio 0.71–**0.82**–0.87 (0.04±). LP/LD ratio 0.15–**0.22**–0.41 (0.06±). WP/WD ratio 0.42–**0.56**–0.71 (0.07±). Epimerite absorbed into distal margin of the protomerite.

Satellite. Protomerite: hemi-ellipsoidal, truncated at association interface to appear trapezoidal; length 27.5–**39.2**–50.0 (4.2±), width 30.0–**49.6**–80.0 (13.5±); with posterior cytoplasmic granule. Deutomerite: elongate ellipsoidal; length 145.0–**240.6**–390.0 (63.3±), width 50.0–**80.2**–130.0 (22.7±). Total length 175.0–**279.9**–430.0 (65.0±). LP/TL ratio 0.09–**0.14**–0.21 (0.03±). LD/TL ratio 0.78–**0.85**–0.91 (0.03±). LP/LD ratio 0.10–**0.17**–0.28 (0.04±). WP/WD ratio 0.45–**0.63**–0.80 (0.07±). Epimerite absorbed into protomerite anterior. Satellites of fresh gamont associations with endocyte distinctly granular, non-uniform and opaque with ectocyte clear to delineate a pellicular border 2.2 wide, pellicle 2.0 thick; color whitish under dissecting microscope with incandescent light, orange-brown to black under compound microscope with daylight filter. In contrast to the primite, considerable posterior portions of the deutomerite of the satellite may be faintly granular to clear. Nuclear diameter consistent, 24.2; centered and slightly subequatorial in the deutomerite.

Association. Biassociative, caudofrontal, frequency of association nearly complete within 132 h post-infection. Association sometimes precocious; occurring during extracellular growth of trophozoite. Individuals that do not form associations apparently grow to exaggerated size.

Gametocysts. White and spherical, diameter 103.4–**115.3**–136.4 (10.7±, n = 22); hyaline coat uniform, width 8.8, increasing diameter to 121.0–**132.9**–154.0 (10.7±, n = 22), Gametocysts collected and stored under humid conditions (>78% RH) sporulate in 16–24 h, forming one to three oocyst chains in 24–36 h. Oocyst chains extruded in large, matted coils.

Oocysts. See Fig. 4. Dorsum: doliform, angles not squared by enclosing sheath; length 6.4, equatorial width 3.4, polar width 2.9. Pleuron: dorso-ventrally flattened, corpus concave with bicondylic termina; corpus, height 0.98, width 4.44; terminus, height 1.96, width 0.98. Oocysts very uniform in size and shape.

Type host. *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae), Southern Corn Root Worm; adults only.

Type locality. School of Biological Sciences research colonies, University of Nebraska–Lincoln, Lincoln, Lancaster County, NE.

Specimens deposited. Three type slides (USNM Helm. Coll. No. 81929) have been deposited in the United States National Museum Helminthological Collection, Biosystematic Parasitology Laboratory, Beltsville, MD.

Infection site. Mesenteron between epithelium and peritrophic membrane. The infection centers in the lower half of the ventriculus; however, in cases of heavy infection the mesenteron may appear occluded from the cardiac valve to the pylorus.

Etymology. The specific name *coronata* is given to mark the dense structure of the apical crown that is visible in mature gamonts even under low magnification.

Remarks. *Gregarina coronata* is similar in shape to *G. munieri* (Schneider, 1875) Labbé, 1899 [14, 28] but is distinguished by comparative structure and sporulation biology; *G. coronata* is about one-third smaller, with a shorter protomerite and more rounded posterior margin of the deutomerite than *G. munieri.* These differences are most pronounced in the satellite. The epimerite of *G. munieri* is supported by a conical base attached to the protomerite. This base is lacking in *G. coronata.* Deutomerite growth patterns are not reported from *G. munieri.* Gametocysts differ in the number of oocyst tubes, but are of comparable shape. The gametocyst dimensions of *G. munieri* have not been reported [14, 28]. The oocyst dimensions and structure of *G. munieri* are unknown. *Gregarina munieri* is reported from over 70 host species in the following chrysomelid subfamilies: Clytrinae, Eumolpinae, Chrysomelinae, Galerucinae, Alticinae, Hispinae, and Cassidinae including the following genera: *Timarcha, Chrysomela, Chrysolina, Paraphaedon, Cyrotonus, Galeruca, Rhaphidopalpa,* and *Platycorinus*; however, *D. undecimpunctata* has not been reported as a host species [9–12, 14, 17, 22, 27, 28, 30–35].

Gregarina coronata is distinguished from *G. diabrotica* Wat-

son Kamm, 1918 [35] by both morphological ratios and size; *G. coronata* is longer and narrower with a reduced protomerite. The trophozoites of *G. diabrotica* possess a constricted protomerite that is not apparent in *G. coronata.* The deutomerite growth patterns are not reported for *G. diabrotica.* The gametocysts and oocysts of *G. diabrotica* are unknown. *Gregarina diabrotica* is described from *Acalymma vittata.*

Gregarina coronata is further distinguished from both *G. munieri* and *G. diabrotica* by the consistent presence of an offset cytoplasmic granule (width 2.4) in the posterior third of the protomerite. The cytoplasmic granule is not always readily apparent in fresh preparations; however, it is obvious in permanent mounts and persists in consistent shape and form through a variety of fixatives and stains (stable through alcohol-formalin-acetic acid [36], alcoholic Bouin's fluid [36], neutral buffered formalin [36], Schaudinn's fluid [13]). It was stained a deep crimson in Acid Carmine and light pink in modified Masson's Trichrome stain. While the general cytoplasm gave a strong positive reaction to the Periodic acid-Schiff staining reaction indicating the presence of polysaccharides and mucosubstances, the cytoplasmic granule was not reactive. Nuclear material was consistently Feulgen positive; however, the granule tested Feulgen negative, indicating a lack of nucleic acids. The cytoplasmic granule was likewise not reactive under Defano's method for golgi apparatus.

Gregarina coronata is intermediate in both morphological structure and size between *G. munieri* and *G. diabrotica.* In a short communication, Théodoridès [29] noted that the description of *G. diabrotica* overlapped that of *G. munieri.* The names were synonymized largely based on generalized host relationships and a broad morphological interpretation; however, the morphological ratios of *G. diabrotica* differ from those of *G. munieri* and *G. coronata,* and each taxon has a structurally distinct epimerite. Watson [34] was familiar with the structure of *G. munieri* and identified this species from three host taxa. She clearly distinguished *G. diabrotica* from other known species, declaring the structure of its protomerite to be "unique and a constant and characteristic feature of the species" [35]. The Septatorina tend to be relatively host-specific [19]. Thus the host list of *G. munieri* suggests the existence of a variety of morphologically similar gregarine species that have not been distinguished.

DISCUSSION

The current taxonomic status of the Septatorina is characterized by decreasing stability and utility of individual species descriptions. Levine [19] reported only 919 valid species in the suborder, though roughly 1,400 named species exist in the literature. The genus *Gregarina* held 298 known valid species in the last review [20]. Levine [19] noted the tendency to use this genus as "a collective group name to stand for any gregarine, no matter what its characteristics." The unstable taxonomic status of *G. munieri* and *G. diabrotica* are products of incomplete descriptions of structure and variation throughout their respective life-cycle stages, especially exogenous (gametocyst and oocyst) forms. The synonymy of these species creates a taxon that is overly plastic in structure and size, masking the possible intermediate species which are suggested by the host list of *G. munieri.*

In an effort to strengthen the description of *Gregarina coronata,* we have included the following: full identification of the host taxon, description of three-dimensional oocyst structure, and description of several morphologically distinct stages within the parasite life-cycle. In addition, we have based the description on mature gamonts in association, precluding the accidental elevation of an immature form of a previously described animal to species-status.

Recent studies suggest some fundamental problems with the status quo methods of gregarine description; however, they also suggest additional methods that greatly increase the stability and utility of a new description. Richardson & Janovy [26] demonstrated a significant change in morphological ratios between fresh and permanently mounted specimens of *Actinocephalus carrilynnae* Richardson & Janovy, 1990. A similar change in morphological ratios was demonstrated between fresh and permanently mounted specimens of *Gregarina niphandrodes* Clopton, Percival & Janovy, 1991 [3]. These studies suggest the limited value of septate gregarine type-specimens unless measurements taken from fresh specimens are preserved in the original description. In the context of fresh measurements, preserved specimens retain full value as indicators of the general species shape, especially the structure of the epimerite. They are not good standards for gregarine dimensions and ratios and should not substitute for fresh measurements and ratios in the original description. Clopton et al. [3] demonstrated the utility of three dimensional oocyst structure in the differentiation of four species of *Gregarina* infecting *Tenebrio molitor.* Complete descriptions of the oocyst offer a previously underutilized suite of stable morphometric characters.

The gregarine reported here is readily differentiated from known species. It is the first gregarine described from *D. undecimpunctata howardi,* and is reported as *G. coronata.* The addition of complete oocyst structure to the traditional morphometrics of the endogenous development phases significantly increases the stability of this species description.

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LITERATURE CITED

1. Belton, P. & Grundfest, H. 1962. Potassium activation and K spikes in muscle fibers of the mealworm larva (*Tenebrio molitor*). *Am. J. Physiol.,* **203**:588–594.

2. Branson, T. F., Guss, P. L., Krysan, J. L. & Sutter, G. P. 1975. Corn Rootworms: Laboratory Rearing and Manipulation. USDA-ARS-NC-28. Washington, D.C.

3. Clopton, R. E., Percival, T. J. & J. Janovy, Jr. 1991. *Gregarina niphandrodes* N. Sp. (Apicomplexa: Eugregarinorida) from adult *Tenebrio molitor* (L.) with oocyst descriptions of other gregarine parasites of the Yellow Mealworm. *J. Protozool.,* **38**:472–483.

4. Dufour, L. 1828. Note sur la grégarine, nouveau genre de ver qui vit en troupeau dans les intestines de divers insectes. *Ann. Sci. Nat.,* **13**:366–367.

5. Forbes, S. A. 1896. Insect injuries to the seed and root of Indian corn. *Univ. of Ill. Agric. Exp. Stn. Bull.,* **44**:287.

6. Gurr, E. 1956. A Practical Manual of Medical and Biological Staining Techniques. Interscience Publishers, New York, New York.

7. Jackson, J. J. 1985. *Diabrotica spp. In:* Singh, P. & Moore, R. F. (ed.), Handbook of Insect Rearing, Vol. 1. Elsevier Science Publishers, New York, pp. 237–254.

8. Jackson, J. 1986. Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi. In:* Krysan, J. L. & Miller, T. A. (ed.), Methods for the Study of Pest *Diabrotica.* Springer-Verlag, New York, New York, pp. 49–56.

9. Jolivet, P. 1950. Les parasites, prédateurs et phorétiques des Chrysomeloidea (Coleoptera) de la faune Franco-Belge. *Bull. Inst. Roy. Sci. Nat. Belg.,* **26**:1–39.

10. Jolivet, P. 1953. A pros des parasites et phorétiques de *Timarcha nicaeensis* (Villa). *Bull. Ann. Ent. Soc. Belg.,* **89**:67–68.

11. Jolivet, P. 1954. Les parasites, prédateurs et phorétiques des Chrysomeloidea (Coleoptera). *Bull. Inst. Roy. Sci. Nat. Belg.,* **30**:1–12.

12. Kamm, M. W. 1922. A list of new gregarines described from 1911 to 1920. *Trans. Amer. Micros. Soc.*, **41**:122–152.

13. Kudo, R. R. 1966. Protozoology, 5th ed. Charles C. Thomas; Springfield, Illinois.

14. Labbé, A. 1899. Sporozoa. *Das Tierreich*, **5**:1–180.

15. Lankester, E. R. 1885. Protozoa. *Encycl. Brit.*, 9th ed. **19**:830–866.

16. Léger, L. 1900. Le genre *Eimeria* et la classification des Coccidies. *C. R. Soc. Biol. Paris*, **52**:576–577.

17. Léger, L. & Doboscq, O. 1909. Études sur la sexualité chez les grégarines. *Arch. Protistenk.*, **17**:19–134.

18. Levine, N. D. 1971. Uniform terminology for the protozoan subphylum Apicomplexa. *J. Protozool.*, **18**:352–355.

19. Levine, N. D. 1979. New genera and higher taxa of septate gregarines (Protozoa, Apicomplexa). *J. Protozool.*, **26**:532–536.

20. Levine, N. D. 1988. The Protozoan Phylum Apicomplexa. Chemical Rubber Company Press, Boca Raton, Florida.

21. Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., Lom, J., Lynn, D., Merinfeld, E. G., Page, F. C., Poljansky, G., Sprague, V., Vavra, J. & Wallace, F. G. 1980. A newly revised classification of the Protozoa. *J. Protozool.*, **27**:37–58.

22. Lipa, J. J. 1967. Studies on gregarines (*Gregarinomorpha*) of arthropods in Poland. *Acta Protozool.*, **5**:1–179.

23. Ludwig, K. A. & Hill, R. E. 1975. Comparison of gut contents of adult Western and Northern Corn Rootworms in northeast Nebraska. *Environ. Entomol.*, **4**:435–438.

24. Luna, L. G. 1968. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York.

25. McManus, J. F. A. & Mowry, R. W. 1960. Staining Methods: Histologic and Histochemical. Hoeber Publishing, New York, New York.

26. Richardson, S. & Janovy, J. Jr. 1990. *Actinocephalus carrilynnae* n. sp. (Apicomplexa: Eugregarinorida) from the blue damselfly, *Enallagma civile* (Hagen). *J. Protozool.*, **37**:567–570.

27. Schewiakoff, W. 1894. Über die ursache der fortschreitenden bewegung der gregarinen. *Zeit. Wiss. Zool.*, **58**:340–354.

28. Schneider, A. 1875. Contribution à l'histoire des grégarines d'invertébrés de Paris et de Roscoff. *Arch. Zool. Exp. Gén.*, **4**:493–604.

29. Théodoridès, J. 1954. Identité de *Gregarina diabrotica* Watson Kamm 1918 avec *Gregarina munieri* A. Schneider 1876 (Eugregarina Gregarinidae). *Ann. Parasit. Hum. Comp.*, **29**:596.

30. Théodoridès, J. 1955. Contrabution à l'étude des Phorétiques de Coléoptères Terrestres. *Vie Milieu*, Suppl. No. 4.

31. Théodoridès, J. 1988. Gregarines of Chrysomelidae. *In:* Jolivet, P., Petitpierre, E., & Hsiao, T. H. (ed.), Biology of Chrysomelidae. Kluwer Academic Publishers, Boston, 417–431.

32. Théodoridès, J., Desportes, I. & Jolivet, P. 1975. Grégarines de la Thaïlande. *Ann. Parasit. Hum. Comp.*, **50**:145–159.

33. Théodoridès, J., Desportes, I. & Jolivet, P. 1976. Gregarines from South Korea. *Ann. Parasit. Hum. Comp.*, **51**:161–174.

34. Watson, M. E. 1916. Studies on gregarines. *Ill. Biol. Monogr.*, **2**:213–468.

35. Watson Kamm, M. E. 1918. New gregarines from Coleoptera. *J. Parasit.*, **4**:159–163.

36. Weesner, F. W. 1960. General Zoological Microtechniques. Williams & Wilkins Company, Baltimore, MD.

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