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**PARASITIC MITES OF SURINAM XXXVII.
DEMODOX MARSUPIALI SP. NOV.
FROM DIDELPHIS MARSUPIALIS:
ADAPTATION TO GLANDULAR HABITAT ¹⁾**

by

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ABSTRACT

Demodex marsupiali sp. nov. is described from the pilocerumen-gland complex within the external auditory meatus of *Didelphis marsupialis* Linné, 1758. Pathogenesis is limited to epithelial cell destruction, minor orifice occlusion, and some keratinization. Mites occasionally penetrate into the dermis, without host cellular response.

Ova and immature mites are markedly modified (apomorphic) to match the unusual habitat, whereas adults, especially males, retain many plesiomorphic characters. Supracoxal spines, hitherto thought sensory, of immatures are modified as holdfast structures.

A brief discussion is provided, comparing and contrasting features of this species with other demodecids from marsupial and eutherian mammals.

Demodex marsupiali sp. nov. is the first-described hair follicle mite from the primitive marsupial family Didelphidae, endemic to the New World. It is of special interest because the closely related *Demodex antechini* Nutting & Sweatman, 1970, is known from *Antechinus stuartii* Macleay, 1841, a member of one of the most primitive Australasian families, Dasyuridae (see Kirsch, 1977).

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After a description of this new mite species, its adaptations, and pathogenicity, we discuss the implications, in comparative terms, of *Demodex marsupiali* to resolution of mite-host phylogenies (see Nutting & Desch, 1979) and ectoparasite evolution.

MATERIAL AND METHODS

Several adult *Didelphis marsupialis* Linné, 1758, were obtained (by F.S.L.) in Surinam. Scrapings were made from deep in the external auditory canal, mounted in Hoyer's medium and examined for demodecids. Auditory canals of animals found positive were dissected out, preserved in Bouin's solution and sections prepared using the paraffin technique. Sections (8 μm) were stained in Hematoxylin and Eosin, and Masson's trichrome. Prepared tissue and whole-mounts were studied using light and phase contrast microscopy.

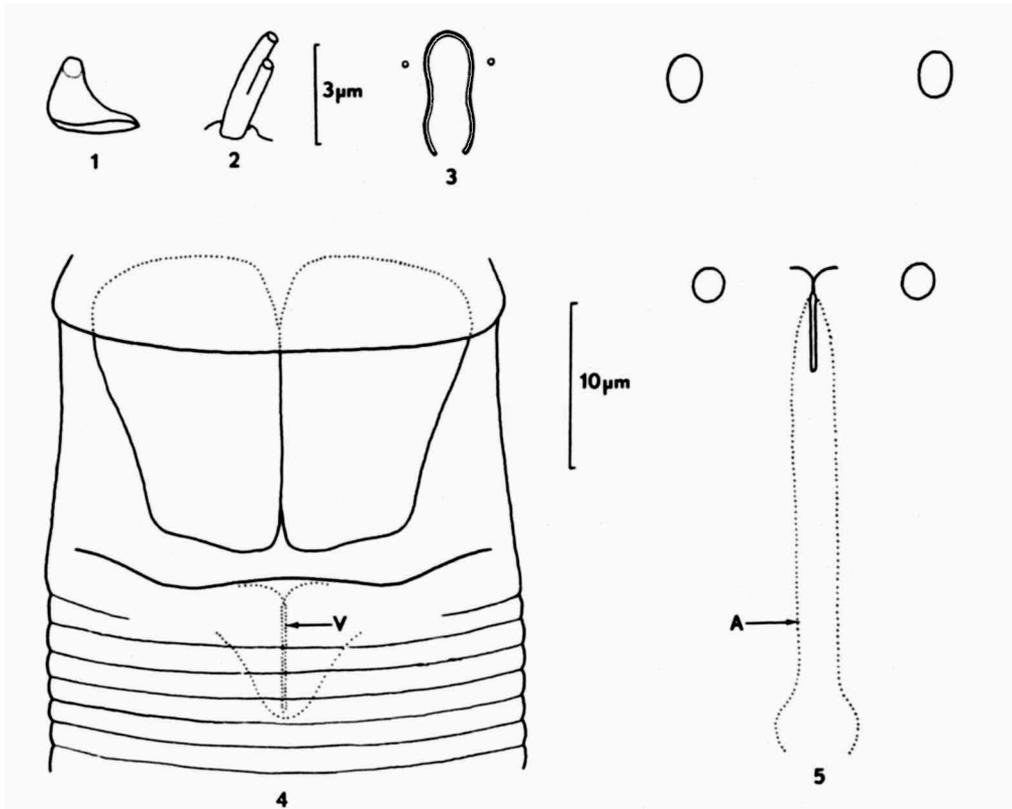
For comparative purposes, several adult *Didelphis virginiana* Kerr, 1792, from Massachusetts were identically treated and sectioned, and hair follicle mites similarly prepared and studied. Mite numbers, especially ova and immatures, of the new species so discovered were inadequate for description.

It became apparent, early in this study, that the specimens of *Demodex marsupiali* do not clear well in Hoyer's medium. Samples of all stages, except ova, were demounted, digested in KOH (5%) and remounted after clearing.

***Demodex marsupiali* sp. nov.**

Description. — Adults elongate, dorsoventrally flattened with marked diastema between legs I and II. Largest specimen, a female, measured $299.2 \times 35.2 \mu\text{m}$. Eggs markedly modified; immatures, rounded in section, with large, lateral (holdfast) supracoxal spines.

Female (pl. 1 fig. 1). — Holotype specimen measured $264 \times 30 \mu\text{m}$, mean of 20 paratypes $260.4 \pm 35.4 \times 32.8 \pm 0.6 \mu\text{m}$ (table 1). Capitulum (extended) nearly square, supracoxal spines wing-shaped (text-fig. 1) with terminus posteriad, outer margin last palpal segment laterally spurred and with four setae (one bifid); pharynx elongate, anteriorly bulbous with lateral setae (text-fig. 3). Podosoma elongate with marked diastema (7 μm) between legs I and II. Legs three-jointed; (# 1 segment enlarged), and terminal segment each with two bifid claws with long shaft spur, and dorsal solenidion on I, II. Opisthosoma terminally rounded and with opisthosomal organ as short (8 μm) mushroom-like sac above a pore 10 μm from opisthosomal terminus. Annulae marked. Podosomal diastema tri-annulate and all coxae widely separated antero-posteriorly by ventral plates but narrowly



Figs. 1-5. Diagrammatic representations of critical taxonomic features of *Demodex marsupiali* sp. nov. 1, right supracoxal spine of adults; 2, same of larva; 3, pharynx with subgnathosomal setae; 4, female genitalia (v = vulva); 5, male genitalia with podosomal tubercles (A = aedeagus).

TABLE I

Measurements (in micrometers) of life stages of *Demodex marsupiali* sp. nov.

Adult female (n = 20)	Ova (n = 5)
capitulum 19.7 × 19.8 μm	112.2 × 26.8 μm
podosoma 90.1 × 29.5 μm	Larva (n = 4)
opisthosoma 162.1 × 32.8 μm	175.5 × 25.9 μm
Total 260.4 ± 35.4* μm × 32.8 ± 0.6 μm	
Adult male (n = 20)	Protonymph (n = 5)
capitulum 18.5 × 22.2 μm	206.3 × 31.2 μm
podosoma 76.8 × 32.9 μm	Nymph (n = 18)
opisthosoma 154.0 × 30.9 μm	262.4 × 36.5 μm
Total 236.7 ± 30.5* μm × 30.9 ± 0.9 μm	

* Large standard deviation due, in part, to unequal preservation of gnathosomal retractor muscles.

separated midline. Genitalia a simple ($3.5 \times 0.5 \mu\text{m}$) elongate slit behind closed opisthosomal annulus (text-fig. 4). A ventral anteriorly-opening genital flange (operculum) serves as entryway to the vulva.

Male (pl. I fig. 2). — Allotype measures $244 \times 34 \mu\text{m}$, mean of 20 paratypes $236.7 \pm 30.5 \times 30.9 \pm 0.9 \mu\text{m}$. Body contours and external structures other than genitalia almost identical to those of female. Opisthosomal organs, six short stamen-like structures from a common base, each ($10 \mu\text{m}$ long) with expanded tip, opening $8 \mu\text{m}$ from tip of opisthosoma. Dorsal transverse inscriptions marked (and behind genital opening). Aedeagus long ($27 \mu\text{m}$), slender ($2.4 \mu\text{m}$), sheathed anteriorly and with expanded truncate base. Opening through slit at level between legs II and III. Podosomal tubercles oval, posterior pair on either side of genital opening, anterior pair at mid-level legs I (text-fig. 5).

Ovum (pl. I fig. 3). — Expanded anteriorly, including nipple ($41.8 \times 26.8 \mu\text{m}$) mid-section $36.4 \mu\text{m}$ and slender tapering posterior $36.2 \mu\text{m}$. Total length $112 \mu\text{m}$. In two instances, small anterior nipple ruptured in preparation as though operculate.

Larva (pl. I fig. 4). — Capitulum nearly as wide as body ($24 \mu\text{m}$ vs $26 \mu\text{m}$), with prominent ($4.4 \mu\text{m}$) peg-like dorsolateral, supracoxal spines (text-fig. 2). Prominent reflexed palpal setae (one large bifid), three pairs of stubby appendages apparently without claws and three pairs of mammiform ventral scutes: anterior rounded, posterior pairs more triangular. Measurements $176 \times 26 \mu\text{m}$.

Protonymph (pl. I fig. 5). — Very similar to larva except body to $6 \mu\text{m}$ wider than capitulum, and approximately $206 \mu\text{m}$ long $\times 30 \mu\text{m}$ wide. Legs as in larva except two pairs trifold subterminal claws each. Scutes as in larva, anterior pair oval, posterior two pairs sub-triangular. Supracoxal spines as larva.

Nymph (pl. I fig. 6). — Similar in all respects but larger ($262 \times 36 \mu\text{m}$) than protonymph, with four pairs of stubby legs ($9 \mu\text{m}$ long anteriorly, diminishing in length to $7 \mu\text{m}$ posteriorly) and four pairs of ventral triangularly mammiform scutes. Two pairs of trifold claws per leg, dorsolaterally subterminal. Supracoxal spines as in larva. Possibly two size groups representing sexual dimorphism.

Diagnosis. — Most similar, especially males, to *Demodex antechini* from *Antechinus stuartii*. Differs from this last, in larger adults (length $200+$ μm vs $195-$ μm), markedly large ($3+$ μm) opisthosomal annuli vs small ($2-$ μm or indistinct), without marked sexual dimorphism vs marked, and opisthosomal organs mushroom-like vs flower-like in females (see Nutting & Sweatman, 1970).

Host. — *Didelphis marsupialis* Linné, 1758, collected (F.S.L.) in Surinam at Lelydorp (10.i.1970; 13.vii.1971), Paramaribo (31.i.1970) and Coronie (12.i.1970).

Habitat. — All stages in atrium and/or lumen of cerumen glands in external auditory meatus of host.

Types. — Holotype and allotype specimens, both from Paramaribo hosts, sent to Rijksmuseum van Natuurlijke Histories, Leiden. Paratypes, all from Paramaribo, will be deposited in: U.S. National Museum of Natural History, Washington, D.C.; British Museum (Natural History), London; Institut de Médecine Tropicale, Prince Léopold, Antwerp; Institute of Acarology, Columbus, Ohio; Field Museum of Natural History, Chicago, Illinois; Zoologisches Institut und Zoologisches Museum, Hamburg; Institute of Parasitology, Academy of Sciences, Prague; and in collections of authors at the University of Massachusetts, Amherst (W.B.N.) and Catholic University, Nijmegen (F.S.L.). Specimens from Lelydorp and Coronie are also stored in the last two collections.

Life cycle (reconstructed from sections and whole mounts). — Ova are deposited, anterior toward fundus, in the atrial cavity, and hatch to larvae which move into a lumen of the gland complex, feeding on the thin renewing stratified lining of the walls and cells of the gland. They ecdyse to protonymphs, nymphs and adults *in situ*, each of these apparently feeding. Adults move back into the gland complex atrium (pl. 2 fig. 1) where they align with the central hair shaft. These last are, we believe, the only stages which transfer between glands or to other hosts.

Adaptation. — The habitat of *Demodex marsupiali* is unusual (pl. 2 fig. 2) and apparently a primitive modification of the pilosebaceous complex. A small orifice (22-46 μm dia.) surrounded by thick (10-15 μm) stratified epithelium and usually with single protruding hair, leads into an expanded thin-walled (5 μm) atrium (77-84 μm). The hair shaft traverses this atrial cavity and extends centrally, lodging in an epithelial-lined portion of the hair follicle. Several (three to eight) thin-walled canals (lumen 22-42 μm dia. \times 250-275 μm long) fan out laterally from the atrial fundus with expanded termini weakly filled (less than one-third) with holocrine gland cells.

Immature stages are located in these canals with mouthparts in the fundus, the cylindrical shapes and multiple holdfast structures (including unusually large and somewhat lateral supracoxal spines, large reflexed palpal setae, prominent ventral scutes and subterminal claws) admirably match this area. Moulting to adults occurs here, but most adults, and all ova, are found in the atrium: the former often with opisthosomae protruding from the atrial

orifice and gnathosomae burrowed into the follicular epithelium.

Ova are laid, and found anteriorly down, in the atrium: large anteriorly probably subvert extrusion through the attenuated orifice. Except for the marked gap between legs I and II, expanded segment 1 of the legs and spacing between epimeral plates, the adults are unmodified compared with those of more recently evolved hosts.

Retention of the primitive (?) opisthosomal organs in both sexes and marked modification of immatures may be related to the fact that only the immatures survive and feed extensively in these primitive sebaceous glands whereas adults feed on the follicular (including atrial) epithelium.

As in many demodecids the sex ratio (1 male : 3.7 females) suggests arrhenotoky, and that this is a primitive feature of the genus assuring interhost transfer in demodecids.

Pathogenesis. — All immatures destroy glandular cells during feeding, even in some instances as deep as the dermis. Penetration of the dermis does not evoke a host cellular response even in heavily infested host animals. Adults also feed on the atrial and follicular lining, in some cases to the dermis without host cellular reaction. In two instances, adults plugged the pilocerumen orifice causing moderate distention of the atrium and glandular ducts. This last, however, appeared little different from distention due to dried cerumen plugs occasionally noted in sectioned material. Some keratinization is evident in areas adjacent to adult claws. *Demodex marsupiali* is apparently a minor pathogen.

Discussion. — Although *Demodex marsupiali* is present in a primitive marsupial (Didelphidae, *Didelphis marsupialis* (see Gardner, 1973)) it resides in a specialized habitat, the cerumen glands, in contrast to *Demodex*

	<i>D. marsupiali</i>	<i>D. antechini</i>
HABITAT	— glandular — immatures. follicular — adults, ova.	— hair follicle — all stages.
MORPHOLOGY		
Ova	— tadpole-like (large)	— spindle-shaped (small)
Larva	— large capitulum, holdfast supracoxal spines, rounded in cross-section.	— small capitulum, small supracoxal spines, dorso-ventrally flat.
Nymphs	— holdfast spines, rounded in cross-section.	— small spines; dorso-ventrally flattened.
Adults	— female longer than male, opisthosomal organs (female) mushroom-like. Male staminate six and unequal in length. Opisthosoma rounded.	— male longer than female, opisthosomal organs (female) flower-like. Male staminate four, equal and with opisthosoma acuminate

antechini from the hair follicle habitat (Nutting & Woolley, 1965) of the less primitive host (Dasyuridae: *Antechinus stuartii* (see Marshall, 1977)). Items of marked difference include the features (Nutting & Sweatman, 1970) listed prepage.

It appears that, as suggested before (Nutting & Desch, 1979), the hair follicle dwelling demodecids, as *Demodex antechini*, represent a more primitive condition than glandular dwelling species, as *Demodex marsupiali*. Modification of opisthosomal organs in the latter combined with marked modification of ova and immature morphology, as here detailed, representing recently derived (apomorphic) characters, whereas adults retain more primitive (plesiomorphic) characteristics. For terms, see Hennig, 1966.

This is further borne out by comparison with a new demodecid from *Didelphis virginiana* also located in the cerumen glands. Adults, although smaller in all features, are nearly identical to *Demodex marsupiali* but immatures much less modified. Genitalia are distinctive in this *Demodex* species and without ventral genital operculum in females.

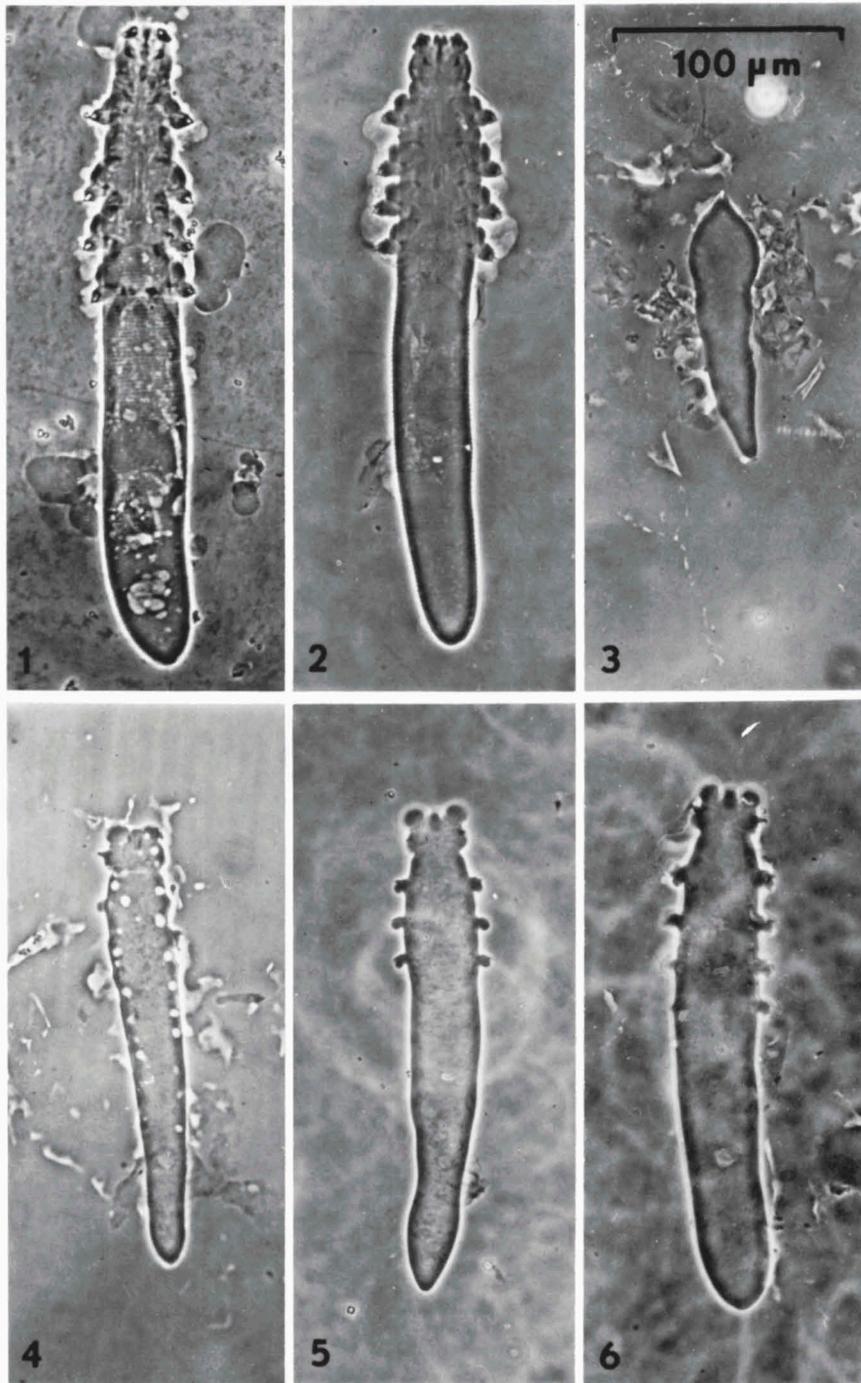
In comparison with eutherian demodecids, a few rodent (e.g., *Demodex* sp. from *Onychomys leucogaster* Wied-Neuwied, 1841) species have a small diastema between legs I and II. None of these are resident in glandular habitats, and also, as in other follicle-dwelling species, have spindle- to oval-shaped ova, dorsoventrally depressed immatures, and moderate to small supracoxal spines in the immatures. The presence of opisthosomal organs in both sexes of *Demodex marsupiali* is, we believe, a primitive characteristic shared by some rodentine demodecids and absent, even here, in species in which the adult mites survive in sebaceous or sudoriferous glands.

In conclusion, *Demodex marsupiali* would seem to be the most primitive, yet-known, gland-dwelling demodecid with adults retaining shared primitive (symplesiomorphic) marsupial hair-follicle-dwelling features, ova apomorphic and markedly modified for retention in the follicular atria, and immatures apomorphic and greatly modified for survival in an unusually modified, and primitive sebaceous gland.

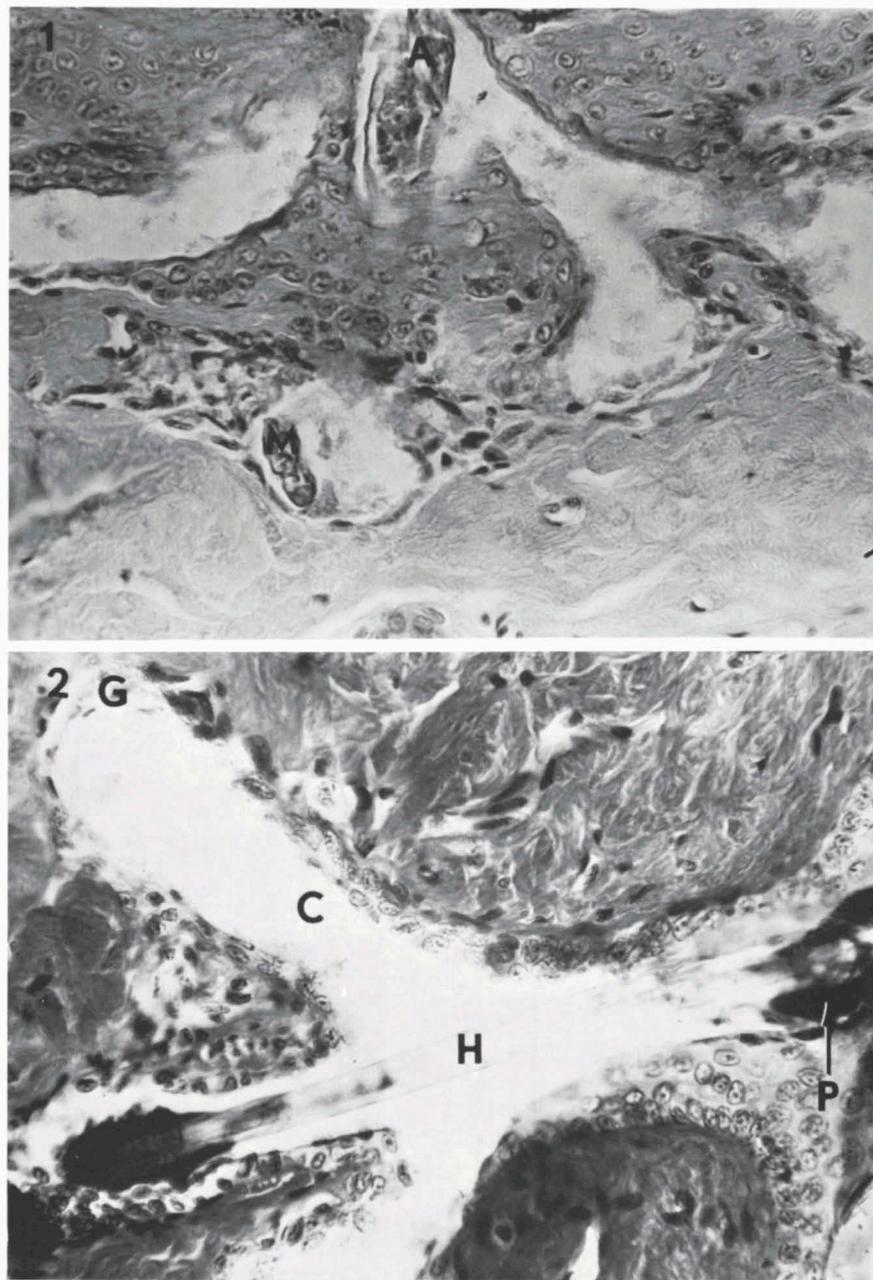
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Figs. 1-6. Phase contrast photomicrographs of stages in the life cycle of *Demodex marsupiali* sp. nov. 1, adult female; 2, adult male; 3, ovum; 4, larva; 5, protonymph; 6, nymph. All of same magnification (see 3).



Figs. 1-2. Photomicrographs of skin sections through pilocerumen gland complex of *Didelphis marsupialis*, HaE, $\times 440$. 1, A = adult mite paralleling hair shaft and in atrium, M = other mites in gland canal; 2, P = cerumen plug, H = hair shaft through center of atrium, G = weak holocrine glands. Note thick orifice epithelium, thin atrial epithelium and hair bulb embedded in follicular epithelium.