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*Posthodiplostomum minimum* (Trematoda: Diplostomatidae). II.  
Body measurements and tegument modifications**

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# The Great Basin Naturalist

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## HOST-INDUCED MORPHOLOGICAL VARIATIONS IN THE STRIGEID TREMATODE *POSTHODIPILOSTOMUM MINIMUM* (TREMATODA: DIPLOSTOMATIDAE). II. BODY MEASUREMENTS AND TEGUMENT MODIFICATIONS

James R. Palmieri<sup>1</sup>

**ABSTRACT.**— Extensive feeding experiments were undertaken to determine if physiological host specificity was a major characteristic of *Posthodiplostomum minimum*. This involved the feeding of experimentally infected sunfish livers containing metacercariae of *P. minimum* to amphibian, reptilian, avian and mammalian hosts. Host-induced morphological variations of adult *P. minimum* were shown to be associated with the genus and class of host employed as well as with the inherent variability of *P. minimum* exclusive of host factors. Morphological characters such as body size and shape and tegumental surface and spines are indicated as undergoing morphological variation. Of all the experimental definitive hosts used, avian and mammalian are the most suitable for normal development of *P. minimum*. Amphibian and reptilian hosts demonstrate marked variation in the adult worm development usually manifested by pronounced size decrease.

*Posthodiplostomum minimum* (MacCallum, 1921) is a strigeoid trematode of the family Diplostomatidae Poirier, 1886. Adults of this species (Fig. 30) parasitize the intestine of piscivorous birds and the metacercarial stage is found in various freshwater fishes.

Two subspecies of *P. minimum* have been reported, based upon the ability of cercariae to penetrate and develop either in centrarchid or cyprinid fish hosts (Hoffman, 1958). The subspecies used in this investigation is the centrarchid strain (Palmieri 1975).

Since Stunkard's report on intraspecific variation in 1957, several more recent experimental studies have shown that size, shape, and position of various organs and structures in helminths may be considerably modified by the host. For many years, investigators such as Dubois (1944, 1955,

1968, and 1970) have delineated species of strigeoids largely on the basis of host specificity. Recently, however, several investigators have shown that parasites can indeed develop within hosts that normally would be ecologically isolated from involvement in the normal life cycle of the parasite (Blankespoor 1971, Campbell 1972, Palmieri 1973, Ulmer 1961, Watertor 1967).

The lakes region of northwestern Iowa is an area rich in conditions requisite for the production of both snail and fish intermediate hosts of *Posthodiplostomum minimum*. This area also serves as both a feeding and nesting area for piscivorous avian hosts needed in maintaining the life cycle of *P. minimum*.

Experimental infections of a variety of amphibian, reptilian, avian, and mammalian hosts with experimentally developed metacercariae of *P. minimum* were carried out

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from 1971 to 1974 at Iowa State University and the Iowa Lakeside Laboratory. Adult *P. minimum* recovered from these ecologically abnormal hosts were examined for host-induced morphological variation.

#### MATERIALS AND METHODS

Three eggs of *Posthodiplostomum minimum*, obtained from a single gravid worm from an experimentally infected chicken 48 hours postexposure, were placed in an embryological watch glass with filtered lake water. Hatching of the miracidia occurred 20 to 21 days later.

A single miracidium was exposed to a laboratory-reared *Physa gyrina* and penetration was observed. This snail was isolated in a one-gallon aquarium and maintained in the laboratory until shedding of cercariae took place (48 days postpenetration). Twice daily for 10 days, contents (shed cercariae) of the one-gallon aquarium were poured into an aquarium containing parasite-free, laboratory-maintained sunfish. Once infected, sunfish were then maintained at room temperature for 45 days. These sunfish livers served as the source of metacercariae for subsequent experimental feedings to definitive hosts.

All definitive hosts which had been exposed to laboratory-developed metacercariae were autopsied from 49 to 96 hours postinfection. Adult worms so obtained were washed in the appropriate Ringer's solution and were prepared for light microscopy or scanning electron microscopy.

*Microscopy.*—Specimens to be examined by scanning electron microscopy were fixed in a modified Parducz (1967) fixative (6.0 ml of 2 percent  $O_3O_4$  and 1.0 ml of saturated mercuric chloride) for one minute at 0 C. All specimens were then washed in distilled water three times at 15-minute intervals. Entire specimens were rapidly dehydrated in ethanol using critical point drying techniques as described by Hearle, Sparrow, and Cross (1972), Cohen and Shaykh (1973), Polliack, Lampen, and de Harven (1973), and Lewis and Nemanic (1973).

Dried specimens were then affixed by electrically conductive aluminum paint to

cleaned brass plates and secondarily affixed to brass specimen holders. Specimens were initially coated with carbon and were subsequently given a double coat of gold-palladium. All specimen coating was done with the aid of an Edwards vacuum evaporator. Coated specimens were viewed and photographed on a Jeolco JSM-S1 scanning electron microscope at an accelerating voltage of 10 KV. All micrographs were recorded on Kodak Ektapan 4162 negative film and developed in a mixture of six parts Kodak D-76 and one part Kodak D-19 for maximum resolution and negative contrast.

*Experimental Infections.*—Definitive hosts were force-fed sunfish livers experimentally infected with over 100 metacercariae of *P. minimum*. Once fed, all hosts were maintained in appropriate cages or aquaria and fed only water. After a suitable developmental period of 49 to 96 hours, these hosts (Table 1) were examined for the presence of *P. minimum* adults, using standard routine laboratory methods. Details of all hosts exposed to cercariae and those fed metacercariae of *P. minimum* have been reported by Palmieri (1976).

#### RESULTS AND DISCUSSION

*Body Measurements.*—Five variables and relationships were analyzed for the body measurements of *P. minimum* (Table 1). These include (1) body length (BL) (from the anterior margin of the forebody to the posterior of the hindbody, exclusive of the extended bursa, when present); (2) body width (BW) (at the widest portion of the forebody); (3) distance from the anterior

TABLE 1. Total number of vertebrate hosts exposed to fish livers experimentally infected with metacercariae of *P. minimum*.

Class	No.		Total No. Hosts
	Positive	Negative	
Amphibians	17	4	21
Reptiles	8	2	10
Birds	18	9	27
Mammals	13	9	22
TOTAL	56	24	80

margin of the forebody to the anterior margin of the acetabulum (DAA); (4) from the posterior margin of the acetabulum to the posterior margin of the hindbody (DPP); and (5) the ratio of DAA/DPP. This relationship (DAA/DPP) was chosen rather than forebody and hindbody lengths because of the lack of uniformity of a distinct demarcation between these regions in some specimens. It was also decided that using the ratio DAA/DPP would minimize the effect of size differences of adults of *P. minimum* because the position of the acetabulum is relatively constant and serves as a more uniform reference point. A complete tabulation of the above data follows in Table 2.

Body size (length and width) of adult *P. minimum* is a significant characteristic in identifying the class of definitive host used for experimental development of adult worms. At the class level, both body size and position of demarcation between the forebody and hindbody regions of *P. minimum* vary significantly. In worms recovered from amphibian and reptilian hosts, very little demarcation can be noted (Figs. 1-3, 9-13). Furthermore, in many worms developed within these poikilothermic hosts, considerable invaginations of the anterior

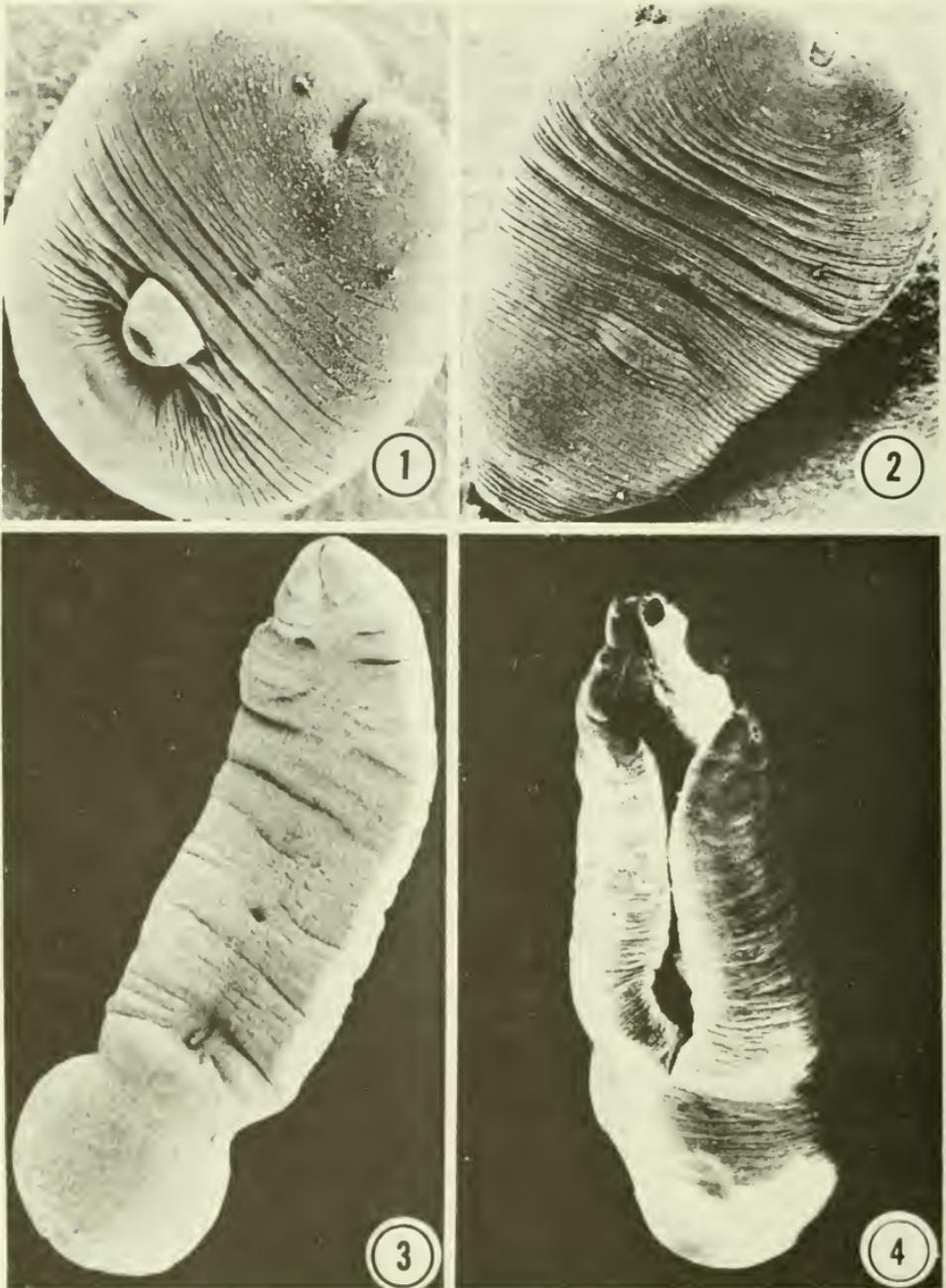
margin of the forebody occur (Figs. 11-12). In many worms the hindbody is not present (Fig. 1-2) or is poorly developed (Fig. 3). In some experimental avian hosts fed metacercariae of *P. minimum*, however, adult worms appear normal and well developed with a well-demarcated forebody and an elongate, cylindrical hindbody (Figs. 6, 8, 14, 16-19). In the most common definitive host for *P. minimum*, the great blue heron, worms recovered from this naturally infected host demonstrate the most characteristic body form (Fig. 19). Those worms recovered from mammalian hosts appear to be more normal in their development than those recovered from amphibian and reptilian hosts but vary somewhat from those recovered from avian hosts in possessing hindbodies whose lengths are reduced in proportion to the forebody (Figs. 4, 5, 7, 15, 17, 18, 20-21).

*Tegumental modifications.*—Several host-induced modifications of the tegument were noted during the course of this study. Scanning electron microscopy of the tegument of *P. minimum* specimens recovered from a variety of experimentally fed hosts revealed that tegumental spines underwent morphological modification. Two regions of the adult worm were selected for observa-

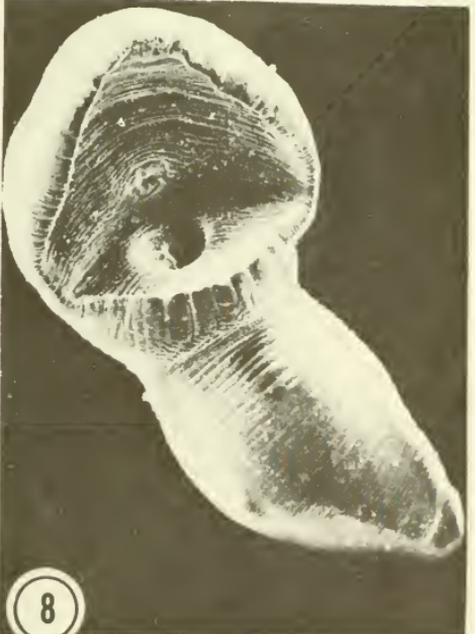
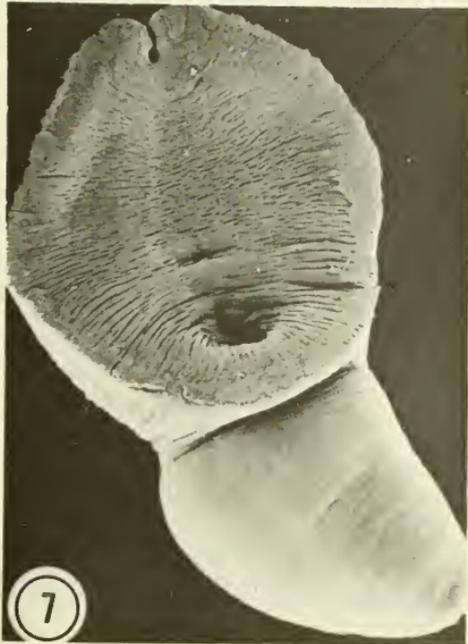
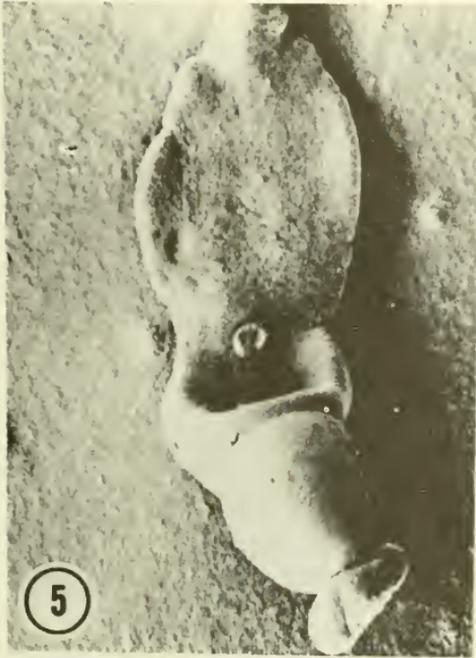
TABLE 2. Body measurements of *P. minimum* from experimental definitive hosts.<sup>1</sup>

Variable	Mean of Combined Host Class	Standard Deviation	Means			
			Amphibian	Reptilian	Aves	Mammalia
Body Length (BL)	0.534	0.083	0.517	0.435	0.614	0.589
Body Width (BW)	0.273	0.043	0.269	0.230	0.288	0.296
Distance from Anterior Margin of Body to Anterior Margin of Acetabulum (DAA)	0.188	0.056	0.203	0.120	0.210	0.184
Distance from Posterior Margin of Acetabulum to Posterior Margin of Body (DPP)	0.260	0.067	0.243	0.198	0.321	0.274
Ratio of DAA/DPP	0.735	0.238	0.867	0.554	0.664	0.634

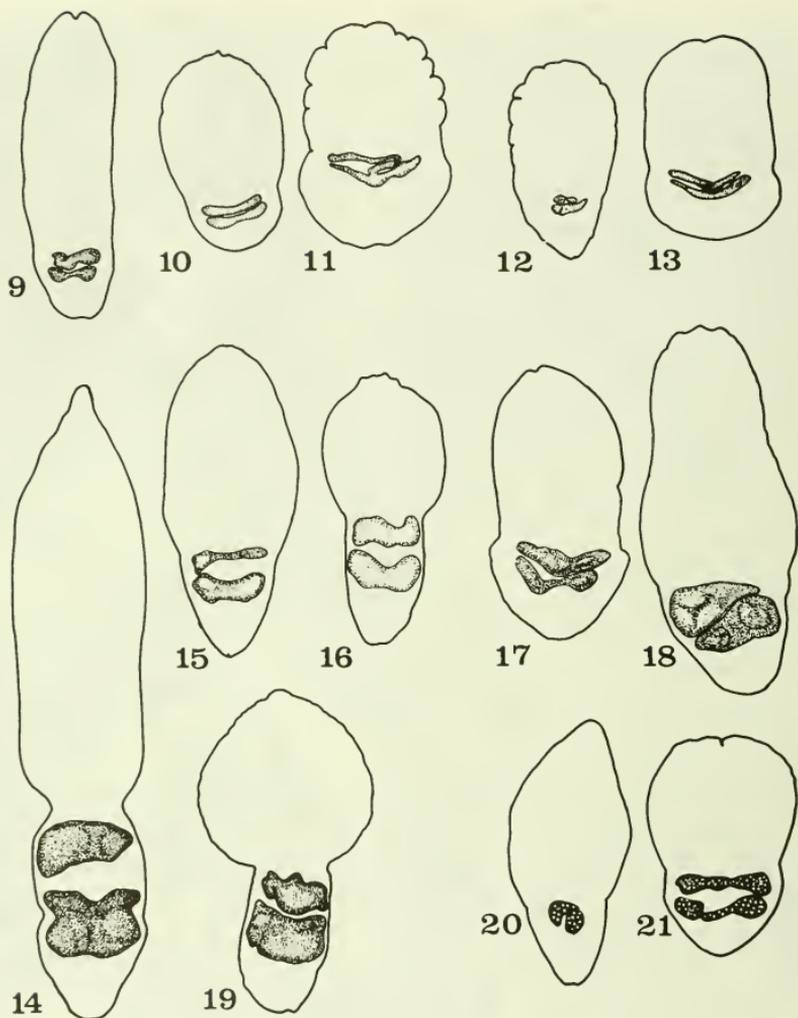
<sup>1</sup>All measurements in mm



Figs. 1-8. Morphological variation of body shape of adult *P. minimum* recovered from vertebrate hosts. Note variations in body shape and hindbody demarcation. All specimens 72 hours old (Scale: 1 inch = .120 mm). Hosts are: 1, *Bufo americanus* Note lack of hindbody; 2, *Amblystoma tigrinum* Note lack of hindbody; 3, *Chry-*

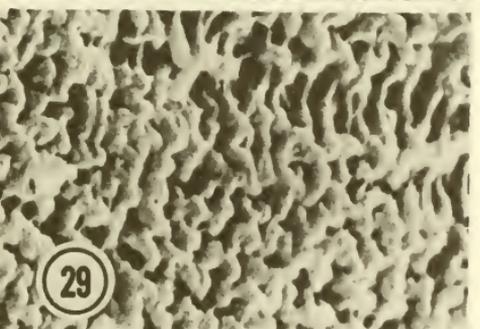
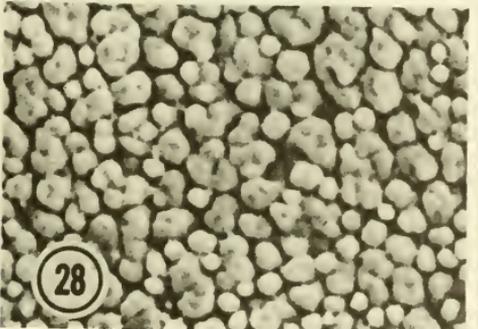
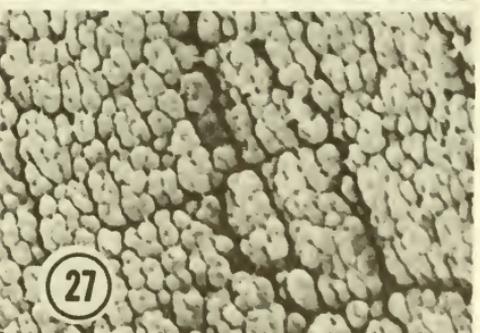
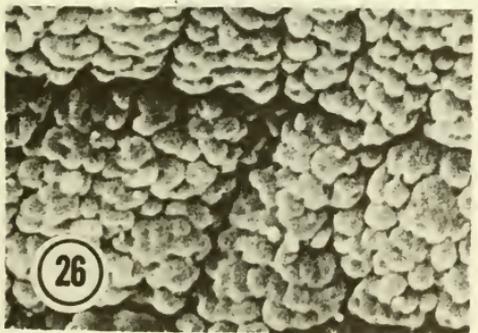
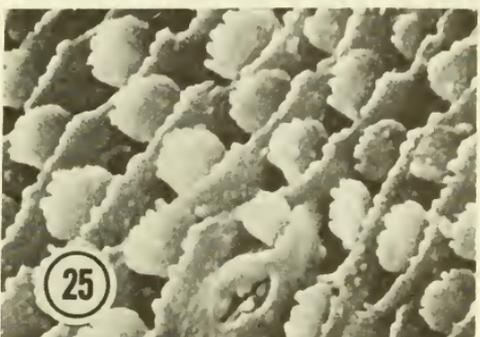
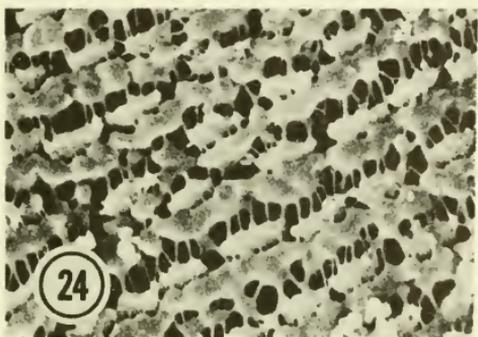
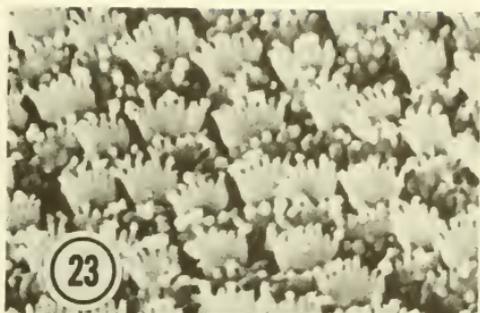
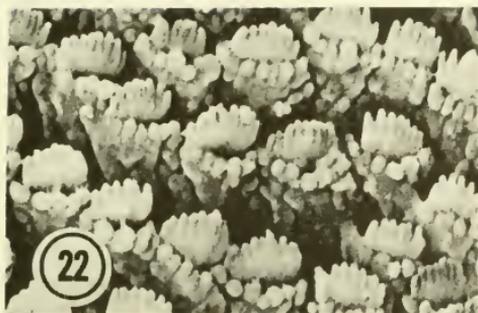


*semys picta* Note bulblike hindbody and extended; 4, *Didelphis marsupialis* Note slightly reduced forebody; 5, *Felis catus* Note large forebody; 6, *Larus argentatus* Note well-developed forebody and hindbody; 7, *Meriones unguiculatus* Note reduced forebody; 8, *Gallus domesticus* Note reduced forebody.



Figs. 9-21. Outline drawings of adult *P. minimum* from amphibian (Figs. 9-11), reptilian (Figs. 12-13), avian (Figs. 14-16, 19), and mammalian (Figs. 17-18, 20-21). All specimens 72 hours old (Scale: 1 inch = 0.1 mm). Hosts are: 9, *Ambystoma tigrinum*; 10, *Bufo americanus*; 11, *Rana pipiens*; 12, *Thamnophis radix*; 13, *Chrysemys picta*; 14, *Larus argentatus*; 15, *Columba livia*; 16, *Zenaidura macroura*; 17, *Meriones unguiculatus*; 18, *Didelphis marsupialis*; 19, *Ardea herodias*; 20, *Canis familiaris*; 21, *Felis catus*.

Figs. 22-29. Morphological modification of the tegument of *P. minimum* due to influences of various vertebrate hosts. Figs. 22-25 from an area lateral to and between the acetabulum and holdfast organ. Figs. 26-29 from an area of the dorsal hindbody (Scale: X = 30,000). Hosts are: 22, *Ambystoma tigrinum* (Note the complex nature of the tegumental spines and surrounding tegumental surface); 23, *Meriones unguiculatus* (Note similarity to Fig. 22 with some loss of complexity of tegumental spines and surface); 24, *Chrysemys picta* (Note that tegumental spines have been greatly reduced and are joined to the tegument by a netlike or weblike process); 25, *Iguana iguana* (Note the reduction of tegumental spine serration and surface tegument); 26, *Chrysemys picta*; 27, *Bufo americanus*; 28, *Rana pipiens*; 29, *Ambystoma tigrinum*.



tion: (1) an area lateral to and equidistant between the acetabulum and holdfast (Figs. 22-25) and (2) a middorsal area on the hindbody (Figs. 26-29). Although no phylogenetic relationships or trends could be discerned, tegumental spination of the ventral forebody surface was reduced from the normal complex structure (Fig. 22). Normal spines are large with serrated margins and are surrounded by tegument containing many surface modifications (Figs. 22-23). In specimens collected from *Chrysemys picta*, spines were so reduced that only a netlike or weblike surface area remained. Such greatly reduced spines are connected to one another as well as to the underlying tegument by filamentous strands (Fig. 24). Tegumental spines examined from most specimens recovered from vertebrate hosts were reduced when compared to others which were more highly developed. There is no apparent relationship between the class of host and complexity of tegumental spine structure. A typical example of a reduced tegumental spine can be found in Figs. 23 and 25, taken from *Iguana iguana* and *Meionectes unguiculatus*, respectively.

On the middorsal hindbody of *P. minimum*, the tegument shows some surface modification ranging from a folded appearance (Figs. 26 and 27) (*Chrysemys picta* and *Bufo americanus*) to one in which bleblike modifications of the tegumental surface predominate (Fig. 28) (*Rana pipiens*). A tegument consisting of irregular ridges (Fig. 29) is also common among worms developed within amphibian hosts. Morphological modification of the tegument and associated surface structures are independent of the class of host used for experimental development of the adult *P. minimum*.

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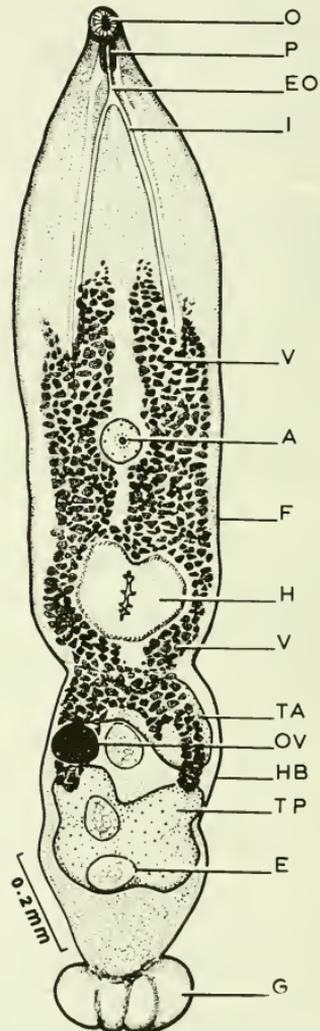


Fig. 30. Diagram of adult *P. minimum* from the gull (*Larus argentatus*) depicting major organs undergoing morphological variation: A—acetabulum; E—egg; EO—esophagus; F—forebody; G—genital bursa; H—holdfast organ; HB—Hindbody; I—intestinal cecum; O—oral sucker; OV—ovary; P—pharynx; TA—anterior testis; TP—posterior testis; V—vitellaria.

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