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HOST-INDUCED MORPHOLOGICAL VARIATIONS IN THE
STRIGEOID TREMATODE POSTHODIPLOSTOMUM MINIMUM
(TREMATODA: DIPLOSTOMATIDAE). II. BODY MEASUREMENTS
AND TEGUMENT MODIFICATIONS

James R. Palmieri

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 em-
ployed 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 varia-
tion. Of all the experimental definitive hosts used, avian and mammalian are the most suitable for normal develop-
ment of P. minimum. Amphibian and reptilian hosts demonstrate marked variation in the adult worm develop-
ment 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 fresh-
water fishes.

Two subspecies of P. minimum have been reported, based upon the ability of cer-
cariae to penetrate and develop either in centrarchid or cyprinid fish hosts (Hoffman,
1958). The subspecies used in this inv-
estigation is the centrarchid strain (Pal-
mieri 1975).

Since Stunkard's report on intraspecific variation in 1957, several more recent ex-
perimental 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, in-
vestigators such as Dubois (1944, 1955,
1968, and 1970) have delineated species of
strigeoids largely on the basis of host specif-
icity. Recently, however, several in-
vestigators have shown that parasites can
develop within hosts that normally
would be ecologically isolated from in-
volve in the normal life cycle of the
parasite (Blankespoor 1971, Campbell 1972,

The lakes region of northwestern Iowa is an
area rich in conditions requisite for the
production of both snail and fish inter-
mediate hosts of Posthodiplostomum min-
imum. This area also serves as both a feed-
ing 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 meta-
cercariae 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 OsO₄ and 1.0 ml of saturated mercuric chloride) for one minute at 0 ºC. 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), Poliack, 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.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Positive</th>
<th>Negative Species</th>
<th>Total No. Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphibians</td>
<td>17</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>Reptiles</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Birds</td>
<td>18</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Mammals</td>
<td>13</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>TOTAL</td>
<td>56</td>
<td>24</td>
<td>80</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Variable</th>
<th>Mean of Combined Host Class</th>
<th>Standard Deviation</th>
<th>Means</th>
<th>Amphibian</th>
<th>Reptilian</th>
<th>Aves</th>
<th>Mammalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Length (BL)</td>
<td>0.534</td>
<td>0.083</td>
<td></td>
<td>0.517</td>
<td>0.435</td>
<td>0.614</td>
<td>0.589</td>
</tr>
<tr>
<td>Body Width (BW)</td>
<td>0.273</td>
<td>0.043</td>
<td></td>
<td>0.269</td>
<td>0.230</td>
<td>0.288</td>
<td>0.296</td>
</tr>
<tr>
<td>Distance from Anterior Margin of Body</td>
<td>0.188</td>
<td>0.056</td>
<td></td>
<td>0.203</td>
<td>0.120</td>
<td>0.210</td>
<td>0.184</td>
</tr>
<tr>
<td>Margin of Acetabulum (DAA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance from Posterior Margin of</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acetabulum to Posterior Margin of</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body (DPP)</td>
<td>0.260</td>
<td>0.067</td>
<td></td>
<td>0.243</td>
<td>0.198</td>
<td>0.321</td>
<td>0.274</td>
</tr>
<tr>
<td>Ratio of DAA/DPP</td>
<td>0.735</td>
<td>0.238</td>
<td></td>
<td>0.867</td>
<td>0.554</td>
<td>0.664</td>
<td>0.634</td>
</tr>
</tbody>
</table>

1All 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. 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 *Meriones 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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LITERATURE CITED


