Rev. Biol. Trop., 17(1): 27-89, 1970

A contribution to the morphology of the eggs and nymphal stages of Porocephalus stilesi Sambon, 1910 and Porocephalus clavatus (Wyman, 1847) Sambon, 1910 (Pentastomida)*

by

Mario Vargas V.**

(Received for publication January 29, 1968)

The systematic position of the genus *Porocephalus* Humboldt, 1809 has been shifted from time to time. SAMBON (21) placed this genus in the order Porocephalida, family Porocephalidae, subfamily Porocephalinae, section Porocephalini. FAIN (6) creates the suborder Porocephaloidea that includes the family Porocephalidae, with genus *Porocephalus.* FAIN also (4) adds a new species, *Porocephalus benoiti,* to the four classically accepted in this genus, *P. crotali, P. stilesi, P. subulifer* and *P. clavatus.*

Adults of *Porocephalus crotali* (Humboldt, 1808) Humboldt, 1811 are recorded according to PENN (20) in the Neotropical Region from *Crotalus durissus terrificus* (Laurenti), 1758. In the same region, *Porocephalus stilesi* Sambon, 1910 is found in *Lachesis muta,* (L.) 1758, *Bothrops jararaca* Wied, 1824, *Bothrops alternatus* Duméril, Bibron et Duméril 1854, *Bothrops atrox,* (L.) 1758, *Bothrops jararacussu* Lacerda, 1884, and *Helicops angulatus* L. 1758. The third species, *Porocephalus clavatus* (Wyman, 1847) Sambon, 1910 is found in *Boa (Constrictor) constrictor,* L. 1758, *Boa (Constrictor) imperator* Daudin 1803, *Epicrates angulifer* Bibron, 1840, *Epicrates (Cenchria) cenchris,* L. 1758, *Epicrates (Cenchria) crassus* Cope, 1862 and *Eunectes murinus,* (L.) 1758 (HEYMONS 14). In the Nearctic Region *Porocephalus crotali* is found in *Crotalus atrox* Baird & Girard, 1853, *Crotalus horridus,* L. 1766, *Crotalus adamanteus* Pal. de Beauvoir,

---

* Part of a thesis submitted by the author to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1968.

** Present address: Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica.

1799, and *Crotalus durissus durissus,* (L.) 1758 and from the water mocassin *Agkistrodon piscivorus* (Lacépède), 1787 (20).

In the Ethiopian Region, *Porocephalus subulifer* is found in *Mehelya (Simocephalus) poensis* Smith, *Mehelya savorgnani* (Mocquard), *Mehelya (Simocephalus) lamani* (Lönnb) and young adults in *Bitis, Causus* and *Naja* (?) (17). *Porocephalus benoiti* Fain, 1960 is reported from ophidians, possibly *Naja* (6). GIGLIOLI (9) and HEYMONS (14) point out the problems in the recognition of species of the genus *Porocephalus* because of the obsolete distinctions among them, except for host records. The former also stresses the variability induced by the size of the host. Furthermore, GIGLIOLI (9) concludes that *P. stilesi* is synonymous with *P. clavatus.*

The characters commonly used to separate adults of *Porocephalus* species are length, width of head, and number of body segments. Not only can different hosts influence the size of these parasites, but also size is related to age (SELF and MCMURRY, 23).

Preliminary examination of specimens from Costa Rica clearly indicates that body size and the number of segments might not be suitable characters to separate the three "species" of *Porocephalus* considered in this study. The difficulties encountered in separating species using these characters can be easily shown (Table 1). In 29 specimens of *Porocephalus* collected from a *Lachesis muta muta* (14 males and 15 females), it was found that they could well fit into any of the three species according to measurements and number of segments in both males and females (Table 2), although *Porocephalus stilesi* has been the only recorded species collected from *Lachesis muta muta.* The same is true with two groups of *Porocephalus* collected from *Boa constrictor imperator,* one consisting of two mature specimens (one male, one female), and the other of five specimens (three males, two females). According to host distinctions these would all be *Porocephalus clavatus,* the only species of *Porocephalus* recorded from this snake.

The purpose of this study is to examine the morphology of the eggs and nymphal stages of *Porocephalus stilesi* and the eggs of *Porocephalus clavatus* to determine whether there are distinguishable characters that can be used to separate these two species and *P. crotali.*

Biological data from the rearings are presented as a possible contribution to the problem of species distinctions in this group.

MATERIAL AND METHODS

EGGS. The eggs used in this study were taken from two adult females of *Porocephalus stilesi* from *Lachesis muta muta* caught at Puerto Viejo, Heredia, Costa Rica and from one female of *Porocephalus clavatus* from a *Boa constrictor imperator* caught at La Fortuna, Limón, Costa Rica (Figs. 42, 43).

The adult female pentastomids were kept at about 10 C., in 0.4 % saline solution in Petri dishes. Infective (mature) eggs were produced by these specimens for a few days. The eggs then remained viable for about ten months

TABLE 1

Specific diagnoses of adult Porocephalus.

Species	Body segments (no.)		Length (mm)		Width of head (mm)		Author
	Male	Female	Male	Female	Male	Female	
P. crotali	32-34	30-35	25-57	50-65	3	4 -8	Hett, 1924
	40-41	39-40	30-35	60-70	3.1-1.4	7.8-9.2	Self & McMurry, 1948
	34-37	ca-37	23-34	60			Heymons, 1935
P. clavatus	35	43	25-36	75-86	2	4.5	Sambon, 1922
	40-47	39					Giglioli, 1927b
	35-43	38-44	25-36	40 72			Heymons, 1935
	35-39	34-38	27-38	57-88	3 -4	6	....
P. stilesi	45	50	30-38	80-96	3.5-4	5 -6	Sambon, 1922
	43	42					Giglioli, 1927b
		36-38		109			Heymons, 1935
	36-40	33-41	25-30	45-80			....
P. subulifer		38-40		60-65		3 -4.5	Sambon, 1922
		35-40	17-22	52-80		3.7	Fain, 1961
P. benoiti		37-39		40-41		2.3	Fain, 1961

TABLE 2

Length (in mm) and number of body segments of males and females of Porocephalus stilesi *and* P. clavatus *from Costa Rica.*

	Males		Females	
Specimen Number	Length	Body Segments	Length	Body Segments
		Porocephalus stilesi		
1	30	39	74	39
2	29	39	78	40
3	26	38	79	41
4	29	40	70	40
5	29	38	74	39
6	29	38	79	37
7	26	39	77	36
8	26	38	80	33
9	26	40	65	37
10	27	38	70	39
11	25	40	68	36
12	25	40	70	37
13	28	36	70	39
14	27	39	59	38
15			45	39
		Porocephalus clavatus		
1	27	37	57	38
2	35	39	82	34
3	37	35	88	35
4	38	38		

at room temperature. Whole eggs, whole embryos dissected from the eggs and ruptured embryos were employed, in order to study the various structures. Eggs and embryos are ruptured by rotatory squashing under a cover slip on a slide in a minimum amount of water. Methylene blue was added to facilitate observation of minute details and structures. Drawings were made with a camera lucida and photomicrographs with normal light or phase contrast microscopy.

In view of the apparent impossibility of distinguishing the eggs of *P. stilesi* from those of *P. clavatus* on the general aspect of the eggs (and embryos), comparative measurements were made, using a compound microscope and an ocular micrometer.

The standard deviation was calculated for each of the structures measured in both species. Tests of significance at 5% were made for the differences between measurements of all the structures of the eggs of *P. clavatus* and *P. stilesi.* The available data (3) were also analyzed statistically to compare *P. crotali* with *P. stilesi* and *P. clavatus.*

NYMPHAL STAGES: Mature eggs of *P. stilesi* were fed to adult male albino rats. A total of 30 rats were routinely inoculated with about 50 eggs each. The dissecting schedule worked out by ESSLINGER (2) for *P. crotali* was used for *P. stilesi.* After several attempts to collect and observe the first nymphal stage, the dose of infective eggs was increased by giving the rats a dose of eggs on three successive days. The small size of the first nymphal stage makes it very difficult to handle and to distinguish. For the dissection of each rat a similar procedure was followed: the different organs were separated, placed in water, and the material checked with the stereomicroscope for encapsulated forms. The capsules were separated from adjacent tissues and the nymph dissected out from the capsule. The first two nymphal stages were placed in Bouin's fixative; the third to sixth stages in Railliet and Henry's fixative. The first and second nymphal stages were mounted whole in PVA.

Every specimen was pierced with an insect *minute nadel* to facilitate penetration of the PVA and to prevent the collapse of the body. Specimens were mounted ventral side up to allow study of the oral armature.

Sclerotized structures of the third to sixth nymphal stages were studied by dissecting the specimens and mounting only the anterior region which bears the oral armature, hooks, and some of the body rings. These structures show, when so mounted, the relative position and distance from each other and can be easily measured and photographed. Only with the sixth nymphal stage was it necessary to dissect the oral armature and hooks separately because of the difficulties in positioning for measurement. Artificial gastric juice at 37 C was used to clean all nymphal capsules from surrounding tissues.

Nymphs from third to sixth stages were mounted immediately in PVA after an adequate elimination of body tissues. The mounted material was kept for a week before measuring to allow the various structures to become stained and clarified. Measurements were made, for each nymphal stage, of oral armature components, hook length and basal width, distance from the oral and body rings. Body length and width of the specimens were measured under the low power of a compound microscope, using a hanging drop slide and a small amount of Bouin's fixative. The length and width of nymphal stages III, IV, V and VI were taken with a stereomicroscope. Measurements of sclerotized structures of all nymphal stages were made under a compound microscope.

Tests of significance at the 5 % level were made for the differences between measurements of the outer and inner pairs of hooks of nymphs V and VI of *P. stilesi.*

RESULTS

Data are presented on the eggs of *P. stilesi* and *P. clavatus* and on the nymphal stages of *P. stilesi.*

EGG: Eggs of *Porocephalus* are superficially similar to those of many cestodes (Fig. 10).

Egg membranes: The intact egg has a thin flexible and permeable envelope or membrane, the bladder-like envelope (Fig. 10). This envelope is subspherical in most cases and measures 132.1 ± 19.9 μ by 125.1 ± 21.2 μ in *P. stilesi* and 144.4 ± 49.9 μ by 140.1 ± 49.5 μ in *P. clavatus.* Next, very close to the embryo, there is an outer and an inner shell (Fig. 11). The outer shell is about 1 μ thick, hyaline and inelastic, and is impervious to stains such as methylene blue and neutral red. The outer shell is very strong, as opposed to the inner shell which collapses almost immediately after its rupture. The outer shell measures 105.5 ± 10.2 μ by 88 ± 10.4 μ in *P. stilesi* and 105.8 ± 8.2 μ by 92.1 ± 6.6 μ in *P. clavatus.* The inner shell is very elastic and about 2 μ thick, measuring 94.6 ± 10.8 μ by 80.6 ± 11.2 μ in *P. stilesi* and 88.8 ± 14.6 μ by 71.3 ± 10.9 μ in *P. clavatus.*

When the outer shell is broken by pressure, a layer of gelatinous material is easily observed surrounding the inner shell. Its thickness varies from 5 to 15 μ in both species, and it is impervious to stains. A part of the inner shell is the so-called facet, facette dorsale, pore or operculum (Figs. 14, 15). It consists of a ring-like aperture in the inner shell, and oriented toward the embryo, it extends as a cone-like structure with the base touching the cuticle of the embryo. Usually this cone is set at such an angle that when the facette is viewed from above, the base of the cone is anterior (toward the anterior pole of the egg) and the aperture or pore is posterior to it. The pore of the facette measures 4.6 ± 1.2 μ in *P. stilesi,* and 5.4 ± 0.5 μ in *P. clavatus.* The cone-like structure is very difficult to measure due to its peculiar position. Sometimes its wall appears rent by a longitudinal slit.

The embryo: The embryo is bilaterally symmetrical. Its general shape is oval. The body of *P. stilesi* measures 121.8 ± 18.7 μ by 70.9 ± 10.1 μ and that of *P. clavatus* 108.2 ± 24.2 μ by 71.1 ± 15.9 μ. The embryo is easily recognized by the presence of a perforating apparatus at the anterior pole, two pairs of legs with well developed paired claws on each leg, and a two-pointed tail at the posterior pole. The cuticle shows some irregular transversal infoldings.

On the dorsal side of the embryo and slightly anterior to the median position is a structure called the dorsal organ (Figs. 16, 17). This organ is internal but contiguous to the cuticle of the embryo. It consists of a vesicle that measures 7.4 ± 1.9 μ by 4.4 ± 2.2 μ in *P. stilesi* and 6.4 ± 2.9 μ by 6.5 ± 2.5 μ in *P. clavatus;* and a crescent-shaped plate (in lateral view) that measures 7.7 ± 2.6 μ by 2.1 ± 0.2 μ in *P. stilesi,* and 8.2 ± 3.1 μ by 2.4 ± 0.8 μ in *P. clavatus.* Usually the vesicle is oriented toward the anterior pole of the egg and the plate toward the posterior pole. If the plate is observed very carefully it can be recognized not as a solid structure but as having an opening connecting with the vesicle. Thus the whole dorsal organ can be compared with a hat, with the vesicle forming the crown and the crescent the brim. No connections were found between the dorsal organ and the facette, but in most cases both structures are associated in such a way that the vesicle of the dorsal organ is pressed into the base of the cone-like facette.

At the dorsolateral surface of the body, slightly anterior to the base of the fore pair of legs, there is a pair of ring-like structures called by most workers stigmata, but in this paper called body-rings, with a diameter of 4.7 ± 1.4 μ for *P. stilesi,* and 5.4 ± 1.5 μ for *P. clavatus.*

The penetrating or boring apparatus is placed in an anteroventral position and is composed of a median blade and two lateral Y-shaped structures (Fig. 18). The median blade has a length of 25.9 ± 3.3 μ in *P. stilesi,* and 24.6 ± 3.0 μ in *P. clavatus.* It is slightly curved toward the body with muscles attached to the proximal end, and the distal end protrudes upward from the body. The blade is not always bilaterally symmetrical, sometimes one side is broader near the distal end; at this point the maximum width for both species is 4.1 ± 1.3 μ. In the distal half of the blade and on the median line there is a thin longitudinal slit-like area.

The base or proximal stem of the lateral Y-shaped structures is 6.1 ± 2.0 μ in length in *P. stilesi,* and 8.4 ± 3.0 μ in *P. clavatus.* Of the two branches arising from it, the inner is 7.1 ± 1.8 μ in *P. stilesi,* and 7.0 ± 1.2 μ in *P. clavatus;* this branch is closer to the median blade of the penetrating apparatus and slightly curved toward it. The outer branch, which is 10.9 ± 1.8 μ in length in *P. stilesi* and 9.7 ± 1.9 μ in *P. clavatus,* is larger than the inner and slightly curved. Both branches show a subterminal knob-like enlargement and a thin slit-like area at the center of this knob, and both end in a pointed blade. Arising from the base of the inner branch, and between the inner and outer branches, there is a sclerotized bar that runs forward and disappears beyond the apex of the outer branch (Fig. 18). This bar shows a more or less straight inner border and a slightly curved outer border. The width increases, especially beyond the apex of the inner branch. There are muscles attached to the proximal end of the stem of the Y-shaped structures and to the distal end of the bar that is placed between the inner and outer branches.

The mouth ring, oral plate or oral armature, U-shaped in ventral view, and composed of one transverse and two lateral bars is located on the mid-ventral

TABLE 3

Comparative measurements (in microns) of egg and embryo of Porocephalus crotali, P. clavatus *and* P. stilesi (size of samples in parenthesis)

Structure	*P. crotali*	*P. stilesi*	*P. clavatus*
EGG:			
External envelope	135— 170 av. 151 (12)*** 118 ± 17 × 114 ± 5 (45)*	132.1 ± 19.9 × 125.1 ± 21.2 (50)	125 (?)** 144.4 ± 49.9 × 140.1 ± 49.5 (50)
Outer shell	114—128 × 99—106 av. 123 × 125 (12)*** 102 ± 8 × 83 ± 2 (45)*	105.5 ± 10.2 × 88.0 ± 10.4 (50)	40—100 × 42— 80 (?)** 105.8 ± 8.2 × 92.1 ± 6.6 (50)
Inner shell		94.6 ± 10.8 × 80.6 ± 11.2 (50)	88.8 ± 14.6 × 71.3 ± 10.9 (50)
Gelatinous coat	5—10 (?)*	5—15 (10)	5—15 (10)
Facette pore		4.6 ± 1.2 (12)	5.4 ± 0.5 (15)
Facette body	8 ± 0.9 × 9 ± 1.2 (10)*		
EMBRYO:			
Body length	137 ± 7*	121.8 ± 18.7	108.2 ± 24.2
Body width	71 ± 5*	70.9 ± 10.1	71.1 ± 15.9
Oral armature:			
Lateral bar	13 ± 1.4*	5.8 ± 5.3	9.2 ± 3.3
Transverse bar	6.2 ± 1.1*	3.2 ± 2.8	4.8 ± 1.1
Penetrating apparatus:			
Median spear, length	25 ± 1.6*	25.9 ± 3.3	24.6 ± 3.0
Median spear, max. width	3-4*	4.1 ± 1.3	4.1 ± 1.3

Lateral forks, stem		6.1 ± 2.0	8.4 ± 3.0
Lateral forks, inner branch		7.1 ± 1.8	7.0 ± 1.2
Lateral forks, outer branch		10.9 ± 1.8	9.7 ± 1.9
Body rings:			
Diameter	5-6*	4.7 ± 1.4	5.4 ± 1.5
Dorsal organ:			
Plate diameters	10 ± 1 × 8 ± 1*	7.7 ± 2.6 × 2.1 ± 0.2	8.2 ± 3.1 × 2.4 ± 0.8
Vesicle diameters	(whole organ)	7.4 ± 1.9 × 4.4 ± 2.2	6.5 ± 2.5 × 6.4 ± 2.9
Tail:			
Length	25 ± 1*	17.5 ± 5.5	19.7 ± 4.7
Width at base	21 ± 2*	20.5 ± 2.1	19.9 ± 5.0
Width at distal end	15 ± 3*	15.9 ± 5.2	11.4 ± 4.1
Depth of notch	8 ± 3*	2.9 ± 1.4	3.4 ± 3.0
Legs:			
Length of claws	11 ± 0.6*	11.0 ± 1.7	11.1 ± 1.7
Claw plate diameter	10 ± 0.9*	10.3 ± 0.0 × 5.5 ± 1.7	8.5 ± 5.1 × 8.3 ± 4.0
Dorsal support	10 ± 0.9*	10.1 ± 1.3	13.2 ± 2.6
Basal support	15 ± 1.2*	15.5 ± 0.1	14.1 ± 2.1
Plate bean		4.6 ± 1.5 × 2.0 ± 0.3	4.2 ± 1.3 × 2.3 ± 0.8
Giant cells:			
Nuclei	5*		

* Esslinger (3)
** Giglioli (8)
*** Penn (20).

side of the embryo, between the first pair of legs (Fig. 13). Each lateral bar measures 5.8 ± 5.3 μ in length in *P. stilesi* and 9.2 ± 3.3 μ in *P. clavatus.* The transverse bar measures 3.2 ± 2.8 μ in length in *P. stilesi,* and 4.8 ± 1.1 μ in *P. clavatus.* When seen in profile the oral armature is J-shaped, due to the fact that the lateral bars curve toward the ventral side of the embryo, very near the end at which the transverse bar is placed. The transverse bar is also slightly concave, the concavity directed toward the ventral side of the embryo.

The embryo has two pairs of lateroventral legs placed at the anterior half of the body; both pairs are close together (Fig. 12). The legs are equal and unsegmented, and each has a pair of hollow claws that arise from a common and slightly sclerotized membranous area, they measure 11.0 ± 1.7 μ in length in *P. stilesi,* and 11.1 ± 1.7 μ in *P. clavatus,* curve ventrally at the tips and end in a very fine point.

On each leg there is a cone-like structure formed by a sclerotized plate which is open ventrally (Fig. 19). At the apex of the cone there is a slightly curved sclerotized bar with the concavity directed toward the posterior pole of the embryo. This extension looks like the apodemes present in sarcoptiform mites. The cone-like structure is apparently formed by a dorsal support that measures 10.1 ± 1.3 μ in *P. stilesi,* and 13.2 ± 2.6 μ in *P. clavatus,* and by a basal support with a length of 15.5 ± 0.1 μ in *P. stilesi,* and 14.1 ± 2.1 μ in *P. clavatus.* The oval membranous area or plate that supports the claws measures 10.3 ± 0.0 μ by 5.5 ± 1.7 μ in *P. stilesi,* and 8.5 ± 5.1 μ by 8.3 ± 4.0 μ in *P. clavatus.* Very close to the distal end of the cone-like structure of each leg and in lateral position (directed toward the anterior pole) there is a small oval or reniform plate just anterior to the base of the cone bar; this plate measures 4.6 ± 1.5 μ by 2.0 ± 0.3 μ in *P. stilesi,* and 4.2 ± 1.3 μ by 2.3 ± 0.8 μ in *P. clavatus.*

Dorsad and slightly anterior to the bases of the fore and hind pairs of legs there is a small external unciform process that is clearly visible when the embryo moves its legs.

The tail is subterminal and attached to the body on the ventral side (Figs. 20, 21). It has a wide base and a two-pointed end. The tail has a width at the base of 20.5 ± 2.1 μ in *P. stilesi,* and 19.9 ± 5.0 μ in *P. clavatus;* the width at the tip is 15.9 ± 5.2 μ in *P. stilesi,* and 11.4 ± 4.1 μ in *P. clavatus;* the length is 17.5 ± 5.5 μ in *P. stilesi* and 19.7 ± 4.7 μ in *P. clavatus;* the depth of the notch is 2.9 ± 1.4 μ en *P. stilesi,* and 3.4 ± 3.0 μ in *P. clavatus.* There are no papillae, hairs or spines on the tail of either species.

Table 3 summarizes the data for the eggs and embryos of *Porocephalus crotali,* and of *P. stilesi* and *P. clavatus* as measured in this study (3, 8, 20).

From a total of 32 tests of significance (5 % level) for the differences between measurements of the various structures of the eggs of *P. clavatus* and *P. stilesi* of Costa Rica, it was found that the differences were significant for the following 24 structures: external envelope (both diameters), outer shell (both diameters), inner shell (both diameters), facette pore, length of body, length of median blade of the boring apparatus, length of stem of the boring apparatus, length of the outer branch of the boring apparatus, body rings, smaller diameter of dorsal organ plate, vesicle of dorsal organ (both diameters), length of tail, width of tail at distal end, plate bearing claws (both diameters), dorsal and basal leg supports, reniform leg plate (smaller diameter), and oral armature (both lateral and transverse bars).

From the 14 tests between the available data for eggs of *P. crotali* and those of *P. stilesi,* seven were significant as follows: length of body, length of tail, notch of tail, transverse bar of the oral armature, external envelope (both diameters), and the outer shell (smaller diameter).

From the 14 tests made for *P. crotali* and *P. clavatus,* eight were significant: tail length, tail notch, tail width at distal end, oral armature (both lateral and transverse bars), external envelope (smaller diameter), outer shell (smaller diameter) and length of body.

The tests were significant at 5 % level for the differences between the three possible combinations: *P. crotali - P. stilesi, P. crotali - P. clavatus,* and *P. stilesi - P. clavatus* for the following structures: external envelope (smaller diameter), outer shell (smaller diameter), length of body, length of tail, and transverse bar of the oral armature. In other words, from the tests made for 14 different characters of the three species of *Porocephalus,* only five were significant at the 5 % level.

Activity of the embryo: The movements of the embryo can be seen through the egg shells, but for convenience the embryo was dissected out of the envelopes. The median blade of the penetrating apparatus is moved forward and backward, coordinated with the movements of the legs. The blade can be pushed out of the body, exposing nearly half its length, while the lateral Y-shaped structures are almost immobile. Usually the median blade of the penetrating apparatus is completely pushed out when the posterior pair of legs is at the beginning of the stroke. The movements of the claws are also coordinated with those of the legs. When the leg is moved forward, the claws are retracted and close together, then protract and spread apart when the leg is moved in its backward stroke.

The legs are moved in a peculiar way, first the fore pair, then the hind legs. For each pair, the stroke takes about ten seconds. Five seconds after the fore legs start their movements, the posterior pair begins its stroke.

The tail and the anterior end of the body have some limited movement, to which well-developed striated muscles contribute. There is an internal current of liquids through the body cavity.

NYMPHAL STAGES: Six nymphal stages of *Porocephalus stilesi* were found. Capsules containing nymphs were found in different organs and tissues of the albino rats in decreasing abundance according to the following sequence: seroses, omenta, lungs, liver and tissues of the walls of the ribs and backbones (Figs. 44, 45).

All nymphal stages were confirmed by the finding of the skins of the previous stages within the enveloping capsule formed by the host tissues.

The skins appear more or less disintegrated but can be recognized by the characteristics of the oral armature, mouth hooks and body rings (Fig. 41). When the nymph is in its first stage, the larval skin can be found and recognized by the presence of the boring apparatus, oral armature, accessory apparatus of the legs, and the claws (Fig. 24). An enlargement of the testes can be seen externally in nymphal stages IV, V and VI.

Nymphs V and VI were stimulated to leave their capsules with artificial gastric juice; hence it was not necessary to dissect them from the capsules.

Nymphal stage I (Fig. 25): Body oval, 0.167 ± 0.018 mm by 0.112 ± 0.019 mm (15 specimens), without appendages or detectable segmentation, with a pair of anterodorsal body rings of 8.9 ± 2.1 μ by 7.4 ± 0.8 μ (11 specimens).

The oral armature is ventrally placed, horseshoe-shaped, with a U-like plate that surrounds the oral opening and a flap hanging from the posterior border of the U-shaped plate. The arms of the U have their free ends oriented forward. The arms are more or less irregular in the anterior half, showing a thin area or an opening near the inner border. The free ends of the arms are irregular and very difficult to observe.

The dimensions of the oral armature (Fig. 1), are:

Ring, inner transversal diameter	(A): 7.9 ± 2.3 μ (12 specimens)
Ring, outer transversal diameter	(B): 13.5 ± 2.8 μ (12 specimens)
Flap, transversal diameter	(D): 5.3 ± 1.7 μ (7 specimens)
Ring, inner longitudinal diameter	(E): 13.6 ± 4.6 μ (12 specimens)
Flap, longitudinal diameter	(G): 5.4 ± 2.2 μ (6 specimens)

Nymphal stage II (Fig. 26): Body oval, 0.336 ± 0.030 mm by 0.256 ± 0.038 mm (30 specimens), without appendages or detectable segmentation; there are 21.4 ± 5.7 body rings in the anterodorsal region of the body (18 specimens); the rings measure 12.0 ± 2.6 μ by 9.2 ± 1.9 μ (18 specimens).

The oral armature is ventrally placed, horseshoe-shaped and with three distinguishable regions (Fig. 31): (a) a ring-like sclerotized piece or plate that surrounds the oral opening, only partly visible in live specimens; (b) a flap or sclerotized piece, suspended from the posterior end of the ring-like plate, embedded in tissue and not visible in live specimens; (c) a curved band, covering the posterior part of the ring-like plate and mesh-like in appearance due to irregular sclerotization; the band is embedded in tissue and cannot be seen in live specimens; it passes above the flap plate.

The dimensions of the oral armature (Fig. 2), are:

Ring, inner transversal diameter	(A): 21.0 ± 3.7 μ (20 specimens)
Ring, outer transversal diameter	(B): 31.6 ± 3.6 μ (20 specimens)
Flap, transversal diameter	(D): 15.0 ± 5.0 μ (20 specimens)
Ring, inner longitudinal diameter	(E): 33.2 ± 2.6 μ (20 specimens)
Flap, longitudinal diameter	(G): 11.3 ± 4.9 μ (16 specimens)
Mesh, longitudinal diameter	(H): 7.5 ± 3.8 μ (11 specimens)

Nymphal stage III (Fig. 27): Body elongate-oval, slightly concave ventrally, measuring 0.798 ± 0.163 mm by 0.371 ± 0.10 mm (20 specimens), without detectable segmentation, and with dorsolateral body rings in the anterior two thirds of the body. The rings measure 12.2 ± 2.6 μ by 9.7 ± 3.7 μ (Fig. 41) (12 specimens).

The oral armature, located ventrally, is ring-shaped, and is made up of the following (Fig. 32): (a) a ring-like plate; (b) a flap hanging from the posterior border of the ring-like plate, embedded in tissue that cannot be seen in live specimens; and (c) a curved band surrounding the posterior two thirds of the ring-like plate, and retaining the mesh-like appearance described in nymphal stage II. The band is also embedded in tissue and cannot be seen in live specimens.

The dimensions of the oral armature (Fig. 3), (12 specimens) are:

Ring, inner transversal diameter	(A): 45.0 ± 5.0 μ
Ring, outer transversal diameter	(B): 64.1 ± 5.2 μ
Mesh, outer transversal diameter	(C): 81.4 ± 10.1 μ
Flap, transversal diameter	(D): 27.0 ± 3.5 μ
Ring, inner longitudinal diameter	(E): 55.0 ± 14.6 μ
Ring, antero-longitudinal diameter	(F): 11.6 ± 11.6 μ
Flap, longitudinal diameter	(G): 19.8 ± 5.7 μ

Two minute cone-shaped hooks can be seen on either side of the oral armature (Fig. 36). The inner pair is placed on a line parallel to the anterior border of the ring-like plate and at each side of the ring, approximately 77 μ and 88 μ away from the ring (2 specimens), average 82.5 μ. The outer pair is placed behind the line of the inner pair, more or less on the transverse diameter of the ring plate. The distance from the outer pair to the inner is between 88 μ and 104.5 μ (2 specimens), average 90.3 μ. The length of the outer hooks (2 specimens) is 4.8 μ. The basal width of the outer hooks (2 specimens) is 2.4 and 4.8 μ, average 3.6 μ. The length of the inner pair of hooks (2 specimens) is 4.8 μ and the basal width of the inner hooks (2 specimens) 4.8 μ.

Nymphal stage IV (Fig. 28): Body elongated, strongly curved ventrally, measuring 3.141 ± 0.8 mm in length, with a width at head of 0.621 ± 0.0022 mm; at mid-body of 0.600 ± 0.18 mm (15 specimens) The body shows slight segmentation, anterior end strong, blunt and tapering posteriorly. Body rings cover the dorsal and lateral sides of the body completely, and are not present in the ventral region; they measure 11.5 ± 1.5 μ by 9.2 ± 1.8 μ (15 specimes).

The oral armature is ventrally placed, with a ring-like plate increasing in diameter toward the anterior border (Fig. 33).

The ring is apparently incomplete at the anterior border. A flap-like projection at the posterior border hangs from the ring-like plate, extending backward; the basal portion is sclerotized and distinct, while the marginal and distal portions are weaker and gradually become indistinct. A mesh-like band surrounds the ring-like plate and extends almost to the anterior side of the ring. The mesh and flap are imbedded in tissue and cannot be seen in live specimens.

The dimensions of the oral armature (Fig. 4), (15 specimens) are:

Ring, inner transversal diameter (A): 75.1 ± 10.8 μ
Ring, outer transversal diameter (B): 97.1 ± 13.6 μ
Mesh, outer transversal diameter (C): 139.7 ± 18.8 μ
Flap, transversal diameter (D): 46.6 ± 10.8 μ
Ring, inner longitudinal diameter (E): 73.7 ± 22.6 μ
Ring, antero-longitudinal diameter (F): 42.5 ± 16.3 μ
Flap, longitudinal diameter (G): 51.7 ± 18.8 μ

Two minute, cone-shaped hooks can be seen at each side of the oral armature (Fig. 37). The inner pair is placed on a parallel line slightly posterior to the anterior border of the ring-like plate. The distance between the oral ring and the inner pair is from 88 to 110 μ (7 specimens), average 101.4 μ. The outer pair of hooks is placed slightly behind the line of the inner pair, more or less on the transverse diameter of the ring plate. The distance between the outer pair and the inner pair of hooks is from 44 to 137.5 μ (5 specimens), average 105.6 μ. The length of the outer pair is 9.2 ± 5.0 μ (7 specimens), and the basal diameter of the same hooks 5.4 ± 2.2 μ (7 specimens). The length of the hooks of the inner pair is 9.0 ± 2.6 μ (7 specimens) and the basal diameter of the same hooks 5.1 ± 1.3 μ (7 specimens).

Nymphal stage V (Fig. 29): Body elongated, strongly curved ventrally, 5.336 ± 3.1 mm long with a width at head of 1.15 ± 0.03 mm; at mid-body of 1.07 ± 0.275 mm (19 specimens).

The body shows a well defined segmentation; the anterior end is blunt, and tapers posteriorly. Body rings are found all over the body, measuring 10.1 ± 0.6 μ by 8.6 ± 0.5 μ (18 specimens).

The oral armature, ventrally placed. is U-shaped, and consists of: (a) a U-shaped sclerotized plate with its free ends facing forward; (b) a flap-like structure hanging from the posterior border of the U-shaped plate; and (c) a band formed by a mesh-like, weakly sclerotized plate surrounding the U-shaped plate and the flap-like structure (Figs. 5, 34).

The dimensions of the oral armature (Fig. 5), (19 specimens) are:

U-shaped plate, inner transversal diameter	(A): 119.2 ± 24.5 μ
U-shaped plate, outer transversal diameter	(B): 152.6 ± 35.3 μ
Flap, transversal diameter	(D): 74.5 ± 15.5 μ
U-shaped plate, inner longitudinal diameter	(E): 163.5 ± 21.0 μ
Flap, longitudinal diameter	(G): 92.5 ± 52.1 μ

Two pointed hooks can be seen at each side of the oral armature (Fig. 38). The inner pair is placed on a straight line from each free end of the U-shaped plate. The outer pair is on a straight line slightly posterior to the line of the inner pair. The distance from the U-shaped plate to the inner pair is between 164.5 and 235.0 μ (10 specimens), average 190.8 μ. The distance from the inner to the outer pair of hooks is between 188.0 and 329.0 μ (10 specimens), average 256.2 μ. The length of the outer pair of hooks is 11.4 ± 11.4 μ, its basal width is 21.4 ± 9.0 μ (31 specimens). The length of the inner pair of hooks is 36.5 ± 11.4 μ, its basal width is 22.1 ± 9.7 μ (38 specimens).

Both the outer and the inner pairs of hooks are slightly curved, the concavity directed toward the posterior end of the body (Fig. 8).

Nymphal stage VI (Fig. 30): Body elongated, vermiform, slightly curved ventrally, 13.1 ± 0.5 mm in length, with a width at head of 2.0 ± 0.2 mm; at mid-body of 1.9 ± 0.11 mm (25 specimens).

The body shows a well defined segmentation; segments number 34.7 ± 0.4 μ (25 specimens) and there are a number of segments (about 3-7, average 3.8) not completely detectable in some part of its perimeter; the anterior end of the body is blunt, the posterior end tapering. Body rings are placed all over the body, being 12.4 ± 06 μ by 11.4 ± 0.4 μ in diameter (18 specimens).

The oral armature is ventrally placed, (Fig. 35) and consists of: (a) a U-shaped plate with its free ends directed forward; (b) a tongue-like sclerotized plate hanging from the posterior border of the U-shaped plate and directed backward. The curving mesh-like plate observed in earlier stages is entirely lacking.

In lateral view, the oral armature shows a wing projection from each of the free ends of the U-shaped plate, the wings directed inward. The tongue-like plate is also directed inward on an angle of about 125°.

The dimensions of the oral armature (Fig. 6), (7 specimens) are:

U-shaped plate, inner transversal diameter	(A): 109.4 ± 33.0 μ
U-shaped plate, outer transversal diameter	(B): 143.0 ± 43.5 μ
Tongue-like plate, transversal diameter	(D): 137.6 ± 40.2 μ
U-shaped plate, inner longitudinal diameter	(E): 221.6 ± 68.2 μ
Tongue-like plate, longitudinal diameter	(G): 227.6 ± 82.1 μ

The oral armature in lateral view (Fig. 7) presents the following dimensions (2 specimens): Wing, longitudinal diameter (I), 211.5 μ; U-shaped arms, lateral diameter (J), 51.7 - 70.5 μ, average 61.1 μ.

Two curved, pointed hooks are present on either side of the oral armature (Figs. 39, 40). The inner pair is placed on a line slightly anterior to the free ends of the arms of the oral armature. The outer pair is placed in a line slightly anterior to the inner pair of hooks. The distance between the U-shaped plate and the inner pair of hooks is 209-380 μ, average 260 μ (6 specimens). The distance from the inner to the outer pair of hooks is between 285 and 380 μ (6 specimens), average 326 μ.

The dimensions of hooks (Fig. 9) are as follows (24 specimens):

Outer pair,
Diameter from point of hooks perpendicular to its base (V): 62.1 ± 51.8 μ.
Depth of hook's curvature (W): 105.8 ± 20.4 μ.
Diameter from point of hook to its opposite base (X): 289.1 ± 34.0 μ.
Basal diameter of base of hook (Y): 325.3 ± 60.0 μ.
Diameter from base to top of fulcrum of hook (Z): 367.7 ± 48.7 μ.

Inner pair,
Diameter from point of hook perpendicular to its base (V'): 67.4 ± 30.0 μ.
Depth of hook's curvature (W'): 98.3 ± 18.1 μ.
Diameter from point of hook to its opposite base (X'): 290.4 ± 43.9 μ.
Basal diameter of base of hook (Y'): 321.0 ± 68.5 μ.
Diameter from base to top of fulcrum of hook (Z'): 361.1 ± 63.1 μ.

The outer pair of hooks presents the so-called accessory or auxiliary hooks which are 141.9 ± 39.4 μ long. These are extensions of the integument that forms part of the fulcrum of the outer hooks. In live specimens they are very pointed and easily detected when the outer hooks are well protracted. In preserved specimens, especially if the outer hooks are retracted, they are more difficult to distinguish. Hooks mounted in PVA at first appear pointed but after several days the shape appears changed and in some cases their identity is lost.

Tests of significance were made between the length and width of the outer and inner pairs of hooks of nymphs IV and V. The tests for the width were not significant in nymphs IV and V nor were they for length of the hooks of nymph IV. The length of the hooks of nymph V was significant at a 5 % level. From the tests of significance for the diameters V-V', W-W', X-X', Y-Y', and Z-Z', of the hooks of nymph VI, only the diameter W-W', was significant at a 5 % level.

The sex of nymphal stage VI can be easily recognized because of the presence in the males of a three-lipped genital opening near the posterior border of the oral armature. Females have the genital opening at the posterior end of the body.

Table 4 shows the dissection schedule used to obtain the nymphal stages of *P. stilesi.* The periods of appearance for each nymphal stage are given, and ESSLINGER'S (2) data for *P. crotali* are included for comparison.

Dissections of capsules of less than 8 days always show the larva or "primary larva" surrounded by tissues. The larva at this time can be easily mounted in PVA for observation (Figs. 22, 23).

Table 5 summarizes the data for the six nymphal stages of *Porocehalus stilesi* as obtained from laboratory infections of albino rats.

DISCUSSION AND CONCLUSIONS

EGG: Description of the eggs of *Porocephalus* species are scarce; apparently nothing has been published on the morphology of the eggs of *P. stilesi* and little on those of *P. clavatus.*

Membranes: The egg membranes or envelopes have been described by several authors. VAN BENEDEN (1) describes and illustrates three envelopes for *P. clavatus.* FONSECA (7) shows a crude schematic figure of the egg and embryo of *P. clavatus,* but gives no descriptions. GIGLIOLI (8) points out the characteristics of the egg of *P. clavatus* and mentions an external envelope of about 125 μ, and an inner oval-shaped envelope of 40-100 μ by 42-80 μ in diameter. PENN (20) illustrates an egg of *P. crotali* and a newly hatched larva. He shows the inner and outer shells and an external envelope, but he mentions only an inner oval-shaped vitelline membrane and an outer membranous shell. ESSLINGER (3) describes four membranes for *P. crotali*: an outer shell membrane, remarkably flexible and permeable; then an "inner shell complex" that includes a middle shell membrane, an inner shell membrane and a gelatinous layer between the middle and the inner shells.

Both PENN (20) and GIGLIOLI (8) overlook the inner shell, which is detected only when the second shell is ruptured. On the other hand, ESSLINGER (3) considers as a membrane the layer of gelatinous material between the outer and inner shells of *P. crotali* eggs. This interpretation is difficult to follow. There does not seem to be a fourth membrane but only a layer of gelatinous material that surrounds the inner shell, seen as soon as the middle shell breaks. At least this is the case with eggs of *P. stilesi* and *P. clavatus.*

Embryo morphology: The embryo of the pentastomids has very interesting

TABLE 4

Nymphal stages of Porocephalus stilesi, *albino rat dissecting schedule, periods (in days) after ingestion of eggs, nymphal stages found, and periods (in days) for each nymphal stage.*

Specimen number	Days after ingestion of eggs by rats	Nymphal stage found	Periods (in days) for each nymphal stage	*P. crotali**
1	8-9-10	I	Nymph I=8-20	8
2	13	I		
3	13-14-15	I		
4	18-19-20	I		
5	22-23-24	II	Nymph II=22-32	19
6	28-29-30	II		
7	32	II		
8	34	III	Nymph III=34-36	28
9	36	III		
10	40	IV	Nymph IV=40-51	39
11	42	IV		
12	44	IV		
13	48	IV		
14	51	IV		
15	61	IV-V	Nymph V=61-66	50
16	66	V		
17	73	VI	Nymph VI=73-110	79
18	78	VI		
19	92	VI		
20	110	VI		

NOTE: rats Nº 21-30 not included in this table were kept for reinoculation of snakes.
* After Esslinger (2).

TABLE 5

Summary of data for the nymphal stages of Porocephalus stilesi *obtained from laboratory infections of albino rats.*

STRUCTURE			NYMPH I			NYMPH II			NYMPH III			NYMPH IV			NYMPH V			NYMPH VI		
			$\bar{X}$	S	$S_{\bar{x}}$	$\bar{X}$	S	$S_{\bar{x}}$	$\bar{X}$	S	$S_{\bar{x}}$	$\bar{X}$	S	$S_{\bar{x}}$	$\bar{X}$	S	$S_{\bar{x}}$	$\bar{X}$	S	$S_{\bar{x}}$
BODY	length (mm.)		0.167	0.018	0.005	0.336	0.030	0.006	0.798	0.163	0.036	3.141	0.800	0.200	5.336	3.100	0.600	13.10	1.700	0.030
	width at half body (mm.)		0.112	0.019	0.005	0.256	0.038	0.007	0.371	0.100	0.023	0.600	0.180	0.050	1.070	0.275	0.060	1.90	0.500	0.097
	number of segments														35-39 av.37			34.7	4.0	0.8
	number of body rings or stigmata		2			21.4	5.8	1.4	>100			>100			>100			>100		
	diameter of body rings or stigmata (in μ)		7.4 8.9	0.8 2.1	0.2 0.6	12.0 9.2	2.6 1.9	0.6 0.5	12.2 9.7	2.6 3.7	0.7 1.1	11.5 9.2	1.5 1.8	0.4 0.5	10.1 8.6	1.8 1.4	0.4 0.3	12.4 11.4	1.7 1.4	0.4 0.3
ORAL ARMATURE (in μ)	diameters (ventral view)	A	7.9	2.3	0.7	21.0	3.7	0.8	45.0	5.0	1.4	75.1	10.8	2.8	119.2	24.5	5.6	109.4	33.0	12.5
		B	13.5	2.8	0.8	31.6	3.6	0.8	64.1	5.2	1.5	97.1	13.6	3.5	152.6	35.3	8.1	143.0	43.5	16.4
		C							81.4	10.1	2.9	139.7	18.8	4.8						
		D	5.3	1.7	0.6	15.0	5.0	1.1	27.0	3.5	1.0	46.6	10.8	2.8	74.5	15.5	3.6	137.6	40.2	15.2
		E	13.6	4.6	1.3	33.2	2.6	0.6	55.0	14.6	5.9	73.7	22.6	5.9	163.5	21.0	4.8	221.6	68.2	25.8
		F							11.6	11.6	3.4	42.5	16.3	4.2						
		G	5.4	2.2	0.9	11.3	4.9	1.2	19.8	5.7	1.6	51.7	18.8	4.8	92.5	52.1	12.0	227.6	82.1	31.0
		H				7.5	3.8	2.6												
	diameters (lateral view)	I																211.5 av.211.5		
		J																51.7-70.5 av.61.10		
HOOKS (in μ)	outer pair	length							4.8 av.4.8			9.2	5.0	1.9	32.2	11.4	2.0			
		width							2.4-4.8 av.3.6			5.4	2.2	0.8	21.4	9.0	1.6			
	inner pair	length							4.8 av.4.8			9.0	2.6	1.0	36.5	11.7	1.8			
		width							4.8 av.4.8			5.1	1.3	0.5	22.1	9.8	1.6			
	OUTER PAIR	V																62.1	34.1	7.0
		W																105.8	20.3	4.1
		X																289.1	34.0	7.0
		Y																325.3	60.0	12.2
		Z																367.8	47.8	9.8
	INNER PAIR	V'																67.4	30.0	6.1
		W'																98.3	18.1	3.7
		X'																290.4	43.9	9.0
		Y'																321.0	68.5	14.0
		Z'																361.9	63.1	12.9
	auxiliary	length																141.9	39.4	12.5
	distance between	oral arm. inner pr.							77-88 av.82.5			44-137.5 av.105.6			164.5-235.0 av. 190.8			209-380 av.260		
		inner pr. outer pr.							88-104.5 av.96.3			88-110 av.101.4			188-329 av.256.2			285-380 av.326		

NOTE: See text for meaning of diameters included in this table.

and important structures that have strong taxonomic and evolutionary implications.

Dorsal organ: SCHUBART (22) first observed an organ in the egg of *Linguatula serrata (Pentastomum taenioides)* which is now called the dorsal organ. He described the egg of this pentastomid as covered by three envelopes, the inner one being provided with a small opening or facette. From his drawings the presence of a ring-like structure as part of the inner shell and a second formation which is present on the mid-dorsal region of the embryo can be inferred. This second structure is not mentioned in the text but is labelled the "star-like body" in one of the drawings. LEUCKART (16) adds more information on the dorsal organ of *Linguatula serrata* and describes it. During early development, there is a point of connection between the embryo and the inner envelope of the egg (the former embryonic cuticle). This connection remains for some time, but later there is a complete separation between the inner envelope and the embryo. The dorsal organ is now divided into two parts; one remains as a part of the inner membrane and constitutes the facette, the other fuses with the chitinous cuticle of the embryo and forms the "dorsal cross" or "star-like body" of Schubärt.

In mature eggs of both *P. stilesi* and *P. clavatus* these two structures usually appear separate, due, as LEUCKART (16) points out, to the rotation of the embryo over its long axis. STILES (25) was the first to study the dorsal organ of *Pentastomum proboscideum (=Porocephalus clavatus?).* He describes the structure as a double depression and mentions that the facette has the same size as a glandular stigma. GIGLIOLI (8) describes a tube-like structure for *P. clavatus* that arises from the facette and extends toward the embryo. It is not a simple tube, but has an ampullar dilation. He also claims that the embryo is in direct communication with the large space filled with hayline liquid between the external and the "internal" envelopes. In view of these structural peculiarities, it seems to him that the role of the external envelope and its contents is not only to act as a simple organ of protection or "flotteur" but probably as a source of nutrient, the amount of which is reduced as the embryo increases in size.

As previously pointed out, Giglioli describes only two envelops for the egg of *P. clavatus.* This interpretation is entirely incorrect because he overlooks the median envelope. Nor are his conclusions true about the direct communication between the embryo and the hyaline liquid within the bladder-like envelope; the space within the external envelope as a source of nutrients for the embryo seems unlikely. In an attempt to interpret Giglioli's drawing of the facette (sagittal view), we have two alternatives: it is based either on an immature or on a mature egg. In relation to the latter possibility, it seems that what Giglioli calls an "envelope interne" is really both the median and the inner egg shells; his "pedicule" is a point in which the gelatinous coat around the inner shell is interrupted, forming a funnel-like channel. Finally a plate-like structure that appears in his drawing, but that he does not describe, is actually the opening of the facette.

More recently ESSLINGER (3) adds some information on the dorsal organ of *P. crotali* in which the facette is described as composed of an eccentric solid distal body in the inner shell. Frequently one or two inward projections of the body or neck are seen. In the intact egg, this body is oriented with its free end toward the head of the embryo. It seems that the formation Esslinger describes as a solid distal body is present in *P. stilesi* and *P. clavatus* as a hollow cone-like structure. Neither the projections nor the vesicle he mentions could be recognized. Instead, in some cases, the wall of the cone is broken at some point, thus appearing as projections.

NOC (18), by means of photography, points out that the facette (?) or dorsal cross of *Armillifer armillatus* is nothing but the folded opening of a pouch, and that the cross-like or star-like appearance is really caused by the folds of that pouch.

As already mentioned, a star-like appearance of the structure on the dorsal region of the embryo is not a characteristic of *Porocephalus stilesi* nor of *P. clavatus.*

HEYMONS (12, 13) reports the dorsal organ in *Raillietiella.* He also describes a remarkable feature. In fresh material, behind a "dorsal ring" structure that corresponds to the "dorsal cross" of others, he finds a group of five or six cells arranged in two rows. These cells have large, rounded nuclei and a granular content. OSCHE (19) describes in *Reighardia sternae* a group of similar giant glandular cells of club-like shape whose necks reach the chitinous vesicle. When stained with methyl-green-acetic acid, these cells are easily recognized by their characteristic large nuclei. The cellular group is so voluminous that it compresses the midgut, located below these cells. Thus the cells are surrounded by a cup-like formation of the intestinal walls.

There seem to be, then, two groups of pentastomids, one including *Raillietiella* and *Reighardia* (Cephalobaenida) in which, according to the available descriptions, there is a group of giant cells placed behind the vesicle; and the second (Porocephalida), including *Linguatula, Armillifer* and *Porocephalus,* in which the group of giant cells is apparently absent. These cells could not be recognized in mature eggs of *P. stilesi* and *P. clavatus.* The former group is considered primitive, the latter, more advanced. A comparative study, including other species in the same or other genera, would help determine whether this character can be used to separate the two groups, and whether the presence or absence of these cells has any evolutionary significance.

The function of the dorsal organ is not known; many theories have been advanced in order to explain its presence. LEUCKART (16) eliminates the idea of a micropile because at the time when fertilization occurs, neither the dorsal organ nor the inner shell are formed. NOC (18) speculates that it is a glandular organ that plays a role in the preservation of the embryo when it is coming out of its envelopes in the digestive tube of the new host. GIGLIOLI (8) believes that it is an organ not only for protection but also very probably a food reserve for the embryo. HEYMONS (12, 14) at first formulates the hypothesis of an apparatus that enables the embryo to break the inner shell in a purely mechanical way; later he assumes that its cells act as molt glands producing a substance that disolves the inner shell. He also believes that this organ is a precursor of the stigmatic glands of the adults. The facette appears to be either for the secretion of substances from the interior of the egg to the space between the inner and the medial shells or for the process of excretion. OSCHE (19) observes that sometimes material can be seen coming out through the pore of the facette of *Reighardia,* and that this material swells. Regarding the glandular cells, he believes that they are probably glandular in function and that the close contact between the glandular cells and the intestine makes it possible for them to participate in the process of excretion. He points out, however, that the only thing evident is that this organ represents the only connection between the enclosed embryo and the surrounding environment.

ESSLINGER (3) suggests, as previous workers did, that this structure may be involved in the elaboration of the gelatinous layer. Perhaps this is true for *P. stilesi* and *P. clavatus;* in both, the gelatinous coat surrounding the inner shell is interrupted by a tiny "tube" just below the aperture of the dorsal organ. Perhaps it represents a point in which for some reason the material is still a sol, not a gel.

Penetrating apparatus: The penetrating or boring apparatus of the pentastomid embryo has been mentioned, described or illustrated in different species by different authors; VAN BENEDEN (1), STILES (25) and GIGLIOLI (8) in *P. clavatus;* SCHUBART (22) and LEUCKART (16)) in *Linguatula serrata;* NOC (18) in *Armillifer armillatus;* HEYMONS (13) in *Raillietiella kochi;* KEEGAN (15) in *Kiricephalus coarctatus;* ESSLINGER (3) in *Porocephalus crotali;* LEUCKART (16) in *Sebekia oxycephala;* FAIN (6) in *Cubirea pomeroyi* and *Raillietiella boulengeri.*

All the above workers except FAIN (6), in *Raillietiella boulengeri,* agree that the boring apparatus of all the mentioned species is formed by an anteroventral median stylet, shaped like a spearhead. The stylet, according to LEUCKART (16), is below the mouth opening of the embryo, but all others agree that this structure is anterior to the mouth. The length of the stylet varies from 7 μ in *Linguatula serrata* and 9 μ in *Sebekia oxycephala* (16), to 17 μ and 17.6 μ in *P. clavatus* (8, 25), 23 μ in *Kiricephalus coarctatus* (15) and 25 ± 1.6 μ in *Porocephalus crotali* (3). For the last two species the width of the stylet is given as 4 μ at its thickest part. On the other hand, the same authors describe a pair of accessory structures (lateral forks of ESSLINGER, 3) placed laterally to the median stylet. These accessory structures have been usually described as two pairs of Y-shaped spines placed one pair behind the other, the stalk of the anterior pair being attached to the aperture of the angle of the hind pair.

FAIN (6) describes the boring apparatus of *Raillietiella boulengeri* as formed by a median two-pointed stylet constricted behind the bifurcation, about 24.5 μ in length, the two points being about 8 μ in length. The maximum width is about 8.5 μ near the base of the points. There are three pairs of lateral stylets about 10 to 15 μ in length and 2 μ in width, with their bases at the level of the constriction. This description does not agree with HEYMONS' drawing of *Raillietiella kochi* (14).

Apparently the medial stylet and the accessory structures are not anatomically in relation in any species described, except in *Kiricephalus coarctatus,* for which KEEGAN (15) describes a transverse bar from which both the medial stylet and the accessory structures arise.

Active movements of the medial stylet are noted by VAN BENEDEN (1) and ESSLINGER (3), who describe less pronounced movements in the accessory structures. It would be of interest to know how the boring apparatus of *Kiricephalus coarctatus* can move, as the medial stylet and the accessory structures are fused to the transverse bar.

The present observations on the structure of the penetrating apparatus coincide with ESSLINGER'S (3), although a complete comparison was not possible, since this author mentions only the length and width of the median spear.

Oral armature: There are some differences in this structure among the species studied by different authors. In Cephalobaenida, the oral armature is ring-shaped in *Raillietiella boulengeri* and *R. kochi.* In the former species the ring is trapezoidal in shape, very large (about 26 μ in length, 10 μ in maximum width) (6), and in the latter the ring is slightly shorter and more convex at the anterior border (14). In the Porocephalida the oral armature is U-shaped, the arms extending toward the anterior pole of the embryo. Length, width and position of the arms vary from species to species. For example, the length of the oral armature of *Sambonia lohrmanni* is 12 μ; of *Leiperia cincinnalis,* 9-10 μ; in *Porocephalus subulifer,* 8-9 μ; in *Armillifer armillatus,* 12 μ (6); in *Porocephalus crotali* it is 13 ± 1.4 μ (2). The length of the oral armature in *P. stilesi* and *P. clavatus* is respectively 5.8 ± 5.3 μ and 9.2 ± 3.3 μ.

In *Armillifer armillatus* the arms are parallel, while in *Leiperia cincinnalis, Cubirea pomeroyi* and *Porocephalus subulifer* the arms diverge slightly as they extend forward (6); this is also true in *P. crotali* (3), *P. stilesi* and *P. clavatus.*

Legs: The legs of pentastomid embryos have been carefully studied to determine whether they are typical arthropod legs. This is important in decisions as to which phylum pentastomids belong in.

VAN BENEDEN (1) reports that *P. clavatus* has two pairs of jointed legs, each with one basal article and a second, movable article which bears the claws. LEUCKART (16) indicates that in *Linguatula serrata* the legs are not bisegmented, but small, club-shaped protuberances. To him, the error of considering the legs as bisegmented was due to the presence of a forked, chitinous structure or accessory apparatus. This structure had two prongs directed outward and a basal arm directed inward. At the distal end of each leg there is a strong chitinous ring with the claws. The ring forms a joint with the accessory apparatus. STILES (25) points out that in *Pentastomum proboscideum* the chitinous ring is united to the claws but that it is completely separated from a three-pronged fork (accessory apparatus). Two of the prongs are of the same size, but shorter than the third. In this species the ring with the claws can be retracted into a pocket formed at the extremity of the leg; this pocket is supported by the three prongs of the fork. Accordingly, LEUCKART'S (16) idea that the fork and the epimeres of the acarines are analogous is not very convincing. The septation of the legs, as interpreted by VAN BENEDEN (1) does not exist; rather, the basal part with the accessory apparatus is the first segment, and the chitinous ring with the claws, the second.

SAMBON (21) divides the pentastomids into two subfamilies, the Raillietiellinae, with six legs and the Porocephalinae, with four. NOC (18) describes a type of pentastomid embryo with three transverse slits delimiting four segments, two of which bear a pair of legs, while Raillietiellini and *Armillifer* embryos have more slits and segments, with the caudal extension being considered as a third pair of legs. He suggests a relationship between the pentastomids and the acarines because of the same number of legs in each group. GIGLIOLI (8) points out the four-legged condition of *P. clavatus*. HEYMONS (14) mentions the four-legged condition of the pentastomid embryo and the presence in the walls of the legs of the three-pronged accessory apparatus which embraces the retractable chitinous ring and claws. He agrees that the two-segmented condition of the legs as described by early authors does not exist in arthropods. The embryonic legs, as such, are completely unjointed, only the distal, final piece bearing the claws (onychium) can be considered as a distal septum, STILES' (25) second segment. KEEGAN (15) points out that the claws of *Kiricephalus coarctatus* are jointed at their bases and rest upon a chitinous ring, supported by a three-pronged shaft.

OSCHE (19) describes eggs of *Reighardia sternae* obtained from the uterus of a live specimen. He found the embryos to have two pairs of typical, ventral, clearly jointed, clawless legs (II and III) and in front of these a third pair (I), directed forward, smaller and more distinctly jointed still. The accessory apparatus was lacking in all three pairs. A pair of preoral lateral antenna-like appendages could also be recognized. He considers these features a very primitive condition, corresponding to the early embryo of arthropods.

FAIN (6) describes the legs of *Raillietiella boulengeri* as consisting of a large, basal chitinous ring or "coxa", articulated with which is a plate or "tarsus", likewise annular, bearing two large, subequal claws. The "coxal" diameters calculated from his drawings are about 26-30 μ by 15-18 μ. The "tarsal" diameters are about 11-13 μ by 5-6 μ and the length of the claws is about 17-18 μ for the larger and 12-15 μ for the smaller.

ESSLINGER (3) points out that in *Porocephalus crotali* the legs are unsegmented. He recognizes the structures previously seen by others, such as the claws and the chitinous ring. The latter structure is considered an expansion of the claws, forming a rigid plate. He interprets the accessory apparatus as a hollow cone open at the base and along the ventral wall. His most important contribution is the view that the accessory apparatus, previously described as a fork-like structure with two or three prongs, really consists of ventrolateral and dorsal thickenings of the wall of a cone. The movements of the plate, claws and legs are achieved by striated muscles. Extension of the claws is achieved by a hydraulic mechanism. The ventral margin of the plate hinges on the cuticle and lateral supporting structures.

To date, then, the only study that shows the jointed condition of the pentastomid legs more clearly is that of OSCHE (19) in *Reighardia sternae;* but in this case the observations are based on eggs taken directly from the uterus in an early stage of development, in which the claws and accessory apparatus are not yet developed. Similar studies are needed to confirm the presence of primordia of articulated legs in other groups, using immature eggs for this purpose. If the primordia of articulated legs were recognizable at least in other members of the Cephalobaenida, the most primitive group, the arthropod relationship of these animals would be established. The embryos used in the present study were too advanced in development for comparison with Osche's specimens.

Tail: The tail of the pentastomid is folded in the egg beneath the ventral surface of the embryo. Shape, size, and special structures vary among the genera. In *Sebekia oxycephala* there is no tail and the body is rounded posteriorly (14). In *Reighardia* the tail is rounded and does not possess any special structure (19). In *Kiricephalus coarctatus* (15) it is quite stubby and possesses a rounded end with the surface covered with papillae and tiny spurs on either side (15). In *Linguatula serrata* the tail is more slender than the body, comprising the posterior half of the body, and showing at the distal margin from one to five pairs of slender spurs (16). In *Armillifer armillatus* the tail shows a tapering and terminally spined process (21). However NOC (18) shows for the same species a bifid tail (porocephalid type) instead of the spined type of Sambon. FAIN (6) describes the tail of *A. armillatus* as bifid and relatively large, with two straight, narrow, apical, chitinous stylets.

In *Raillietiella kochi* the tail shows two furcae which were considered by early workers as a third pair of legs in spite of the fact that these do not have any resemblance to them, and that there are no unguis musculature system or other structures typical of the pentastomid legs. Each furca ends in a long chitinous seta (13). In *Raillietiella boulengeri* FAIN (6) indicates the same bifid condition and the presence of a large and slightly curved hook at the end of each tail piece. In *Leipeira cincinnalis* he describes the tail as ending in two small, round, slightly chitinous protuberances; in *Sambonia lohrmanni* the tail is moderately large, ending in two fine points, and *Cubirea pomeroyi* has a short tail, ending in two slightly chitinous points similar to *Porocephalus subulifer.*

VAN BENEDEN (1), describing the embryo of *Porocephalus clavatus* mentions that the body ends posteriorly in a bifurcation. In his drawings the tail is shown as bifid but without hairs on its posterior margin. STILES (25) points out that in *Pentastomum proboscideum (= Porocephalus clavatus?)* the tail is variable in size and shape. It is a bifid structure that might have at the posterior margin some hairs directed posteriorly. GIGLIOLI (8) also describes the bifid tail of *P. clavatus* but does not mention the presence of hairs. ESSLINGER (3) also indicates the absence of hairs in *P. crotali.*

In our specimens it was found that neither *P. stilesi* nor *P. clavatus* have hairs on the tail. The presence of hairs on the posterior margin of the *Porocephalus* tail cannot be taken as a good character, either to separate the species of this genus as ESSLINGER (3) suggests, or to separate genera. The presence of such structures in these three species has not been reported. Furthermore, STILES (25) describes the presence of hairs in his *Pentastomum proboscideum* as an alternative condition. In the African species, *Porocephalus subulifer*, FAIN (6) describes the tail as short and with two small weak triangular chitinous points.

Thus, the evolutionary line apparently shows a well developed furcate tail in the most primitive groups of pentastomids, the Cephalobaenida, with the bifid condition less pronounced in the higher Porocephalida. In the latter group, there is some variation in the number and arrangement of the spurs and the size of the tail.

General comments: It is accepted that the measurable variations among members of the same species of parasites may be due to different factors such as geographic distribution and host. An attempt is made to compare statistically the available data for eggs of the three American species of *Porocephalus*, *P. crotali*, *P. stilesi* and *P. clavatus*, collected from samples from different regions and hosts. It is quite possible that the differences found are due not only to the above factors but to others, such as plasticity of the animal, (body length, tail length and width, tail notch, external envelope), age of the eggs, and to the fact that the samples came from different females of *Porocephalus*. Neither the statistical nor the morphological analysis have given a good character that can be used satisfactorily to separate these so-called species of *Porocephalus*. It is necessary to continue this kind of study to learn more about the differences due to geographic variation and to host.

NYMPHAL STAGES: The post-embryonic development of *Porocephalus* species was only partially known until ESSLINGER (2) gave a detailed description of all nymphal stages of *P. crotali* from experimental infections in albino rats. STILES (25) and HEYMONS (14), in studies of *Pentastomum proboscideum* (= *Porocephalus clavatus?*), describe four nymphal stages recovered from experimentally infected mice. Three stages correspond to encysted "larva" and one stage to the free migratory "larva". The first nymphal stage of *P. clavatus* is characterized by two glandular pores or stigmata. The oral ring is not closed but is horseshoe shaped, forming a chitinous structure. The second nymphal stage is reached four and a half weeks after the eggs are fed to mice. This stage measures 0.8 to 1 mm in length and can be compared in size and shape, more or less, with the third nymphal stage of *Linguatula*. The body is strongly curved toward the ventral side and is circular in transverse view. Numerous glandular pores (stigmata) are disposed in 18 rows limited to the dorsal region and sides of the anterior half of the body, two in the first row, increasing in number to 14, then decreasing to six about the middle of the body. The oral ring in this stage is closed, its dimensions being 50 by 36 μ. In this stage, the genital pore of the male is immediately posterior to the mouth, and in the female 13 μ anterior to the anus. The third nymphal stage, with a length of about 2 mm, is reached at six-and-a-half weeks. The number of glandular pores that extend to the ventral side varies from 16 to 44 per row.

The fourth stage appears in mice about 18 and one-half weeks after infection. STILES (25) mentions that in one mouse, after about four months, most of the nymphs were in their capsules and a few had migrated and were free in the abdominal cavity. Their maximum length was 13 mm. The "abdominal segments" show crowns of small protuberances. Stiles also mentions that in the first and second stages there are no invaginations for the mouth hooks; these invaginations appear in the third stage. In the wider segments, the body pores are arranged in two rows, those in the head region being larger than the others.

ESSLINGER (2) reports in detail seven stages of *P. crotali* in the intermediate hosts (albino rats). The first is the "primary larva" or infective stage that hatches from the egg. This form migrates through the wall of the small intestine and comes to rest in the viscera and mesenteries. This stage is followed by six molts, resulting in the nymphal stages I, II, III, IV, V and VI; the last stage is infective to the definitive reptilian host.

Comparison of our nymphs of *P. stilesi,* reared in the laboratory, with ESSLINGER'S (2) data for *P. crotali* shows the same number of nymphal stages for each species. No basic differences were found between the six nymphal stages of *P. crotali* and those of *P. stilesi* with regard to body shape, length and width, mouth hooks and oral armature.

There are some slight differences to note between the two species. The oral armature was measured by ESSLINGER (2) in living specimens of *P. crotali.* However, it is nearly impossible to see the details of this structure in living specimens. In the present study the measurements were taken from mounted specimens; thus it was possible to observe more details and to measure other dimensions for such structures, as the flap or tongue-like extension of the oral ring and the mesh-like structure that surrounds it (Figs. 1-9). The hooks of nymph V of *P. crotali* are described by Esslinger as being straight; in *P. stilesi* both pair of hooks are slightly curved if seen laterally, but from any other angle they appear to be straight. It is preferable to use the term "cone-like hooks" for the hooks in the third nymphal stage instead of Esslinger's "cuticular papillae". The number of body segments reported by Esslinger for nymphal stage V of *P. crotali* is different from *P. stilesi,* perhaps because he counts the number of segments in living specimens and the *P. stilesi* counts are based on the number of rows of body rings observed on cleaned nymphal skins.

Their number is difficult to determine in living specimens as the segments are not easy to follow because of the movements and contractions of the body. Body rows are better defined on the mid-ventral line of the skin.

Comparing the nymphal stages of *Pentastomum proboscideum* (= *Porocephalus clavatus?)* (25), *P. crotali* (2) and *P. stilesi,* it appears that Stiles' nymphal stages, I, II, III and IV correspond with stages I, III, IV and VI respectively of *P. crotali* and *P. stilesi.* Apparently Stiles overlooked stages II and V.

The nymphal stages of the pentastomids show very interesting characters that can be used for taxonomic purposes.

In *Raillietiella,* the "larva" (infective nymph) differs markedly from that of the porocephalids in the following details: structure of the mouth hooks, presence of stylets at the anterior border of the body, parapodial lobes, and absence of crowns of spines on the "abdominal" segments; the four mouth hooks are each composed of two blades, placed side by side and arising from a common base (6). GRETILLAT *et al.* (10) find the same general characters in other *Raillietiella* nymphal forms. The number, shape, or size of the stylets may prove of some value in the separation of species. On the other hand, in the last nymphal stage of some of the porocephalids, the hooks are double; but in this case, an accessory hook is placed dorsally to the principal hook and is never identical to it. Besides, there are neither parapodial lobes nor a supporting ring around the hooks, instead there is a fulcrum that articulates with the hooks.

There are rows of spines or protuberances on the posterior border of each of the "abdominal" segments. Among the more primitive genera of Porocephalida, the infective nymph has double hooks, except in *Sambonia,* in which the accessory hooks are replaced by a chitinous formation arising from the cuticle that separates the fulcrum and the base of the hook. The infective *Porocephalus* nymph has a double outer pair of hooks, while the inner pair is single. The condition of the accessory hooks has been studied by SELF (24) and apparently is very similar to the hooks of *Sambonia.*

It seems, then, that the evolutionary line for the structure of the mouth hooks of the infective nymph of pentastomids begins with the presence of two pairs of double hooks. In the next step only the outer pair has an accessory or auxiliary hook and the inner pair is single; and lastly, both the outer and inner pairs are single.

The number of post-embryonic stages of pentastomids is of great interest because of the taxonomic implications. It is possible that the number of stages is a useful character to separate pentastomids into groups. Only a few workers have given some information on such numbers: LEUCKART (16) describes nine nymphal stages in *Linguatula serrata,* STILES (25), four in *Porocephalus clavatus* and FAIN and MORTELMANS (5), five in *Sambonia lohrmanni.* Finally both *P. crotali* (2) and *P. stilesi* have six. It would be very interesting to know if other species of the same genus and of other genera show the same number of nymphal stages.

Another important question is whether a last molt of the infective *Porocephalus* nymph (VI) occurs in the reptile host before the adult stage. In one instance a nymph taken from the lung of a *Boa constrictor imperator* was molting.

It could not be confirmed that the nymphal forms of *Porocephalus clavatus* (?) (described as *P. stilesi* from *Lachesis muta)* migrate into the testicular parenchyma of the albino rats, as FONSECA (7) described. In fact, there are many nymphs encapsulated in tissues that surround the testes but the capsules were never seen in the parenchyma.

The length and width of the body and of the oral ring of the six nymphal stages of *P. crotali* (according to ESSLINGER, 2) and *P. stilesi* are compared in tables 6 and 7. Significance tests between these measurements of the two species are as follows: differences in the length of the body for all nymphal stages of *P. crotali* and *P. stilesi* were significant at the 5 % level; the body

TABLE 6

Comparison between the length and width (in mm) of the nymphal stages of Porocephalus crotali(*) *and* P. stilesi *as obtained from experimentally infected albino rats.*

Stage number	Length		Width	
	P. crotali	*P. stilesi*	*P. crotali*	*P. stilesi*
Nymph I	0.201 ± 0.007	0.167 ± 0.018	0.136 ± 0.008	0.112 ± 0.019
Nymph II	0.406 ± 0.033	0.336 ± 0.030	0.256 ± 0.017	0.256 ± 0.038
Nymph III	0.928 ± 0.031	0.798 ± 0.09	0.421 ± 0.021	0.371 ± 0.05
Nymph IV	2.11 ± 0.13	3.141 ± 2.54	0.570 ± 0.03	0.60 ± 0.017
Nymph V	6.59 ± 0.52	5.34 ± 3.1	1.11 ± 0.10	1.07 ± 0.275
Nymph VI	11.9 ± 0.4	13.1 ± 0.5	1.5 ± 0.0	1.9 ± 0.11

(*) Data as given by Esslinger (2)

TABLE 7

Comparison between the length and width (in microns) of the oral ring of the nymphal stages of Porocephalus crotali (*) *and* P. stilesi *as obtained from experimentally infected albino rats.*

Stage number	Length		Width	
	P. crotali	*P. stilesi*	*P. crotali*	*P. stilesi*
Nymph I	21.4 ± 1.4	13.6 ± 4.6	14.6 ± 0.6	13.5 ± 0.3
Nymph II	40.0 ± 2.1	33.1 ± 2.6	33.4 ± 2.7	31.6 ± 3.6
Nymph III	77.4 ± 1.8	55.0 ± 14.6	69.3 ± 1.9	64.1 ± 5.2
Nymph IV	138 ± 3	73.7 ± 22.6	105 ± 3	97.1 ± 13.6
Nymph V	203 ± 8	163.5 ± 21.0	155 ± 6	152.6 ± 35.3
Nymph VI	236 ± 14	221.6 ± 68.2	163 ± 14	143 ± 43.5

(*) Data as given by Esslinger (2)

width was significant for stages I and VI, but not for stages II, III, IV and V; the length of the oral ring was significant for all stages except for nymph VI; the width of the oral ring was significant for stages I, II, III, IV and VI.

The interpretation of these results is difficult, perhaps because of differences in techniques of fixation, other variations in preparing and observing specimens, geographical distribution, and hosts.

ACKNOWLEDGMENTS

Thanks are due to Dr. B. V. Travis for his invaluable counsel and encouragement. The author is also indebted to Drs. F. Montero-Gei, A. Ruiz, M. Chinchilla, B. Fernández, R. Umaña, R. L. Rodríguez; to Major H. Flowers, and to Messrs. G. Zúñiga, A. Zúñiga, G. Muñoz, and M. Gómez who in one way or another contributed to make this research possible.

The following contributed financially to this project: International Cooperation Administration, Agency for International Development, Organization for Tropical Studies, Costa Rican Ministry of Public Health, University of Costa Rica, and Allied Chemical and Dye Co.

SUMMARY

The morphology of the egg and nymphal stages of *Porocephalus stilesi* and the eggs of *Porocephalus clavatus* were examined to determine if there are distinguishable characters that can be used to separate these two species and *Porocephalus crotali.*

Eggs of *P. stilesi* were taken from adult females collected from *Lachesis muta muta;* eggs of *P. clavatus* were taken from females collected from *Boa constrictor imperator.* Both hosts were caught in Costa Rica.

Nymphal stages of *P. stilesi* were dissected out from adult male albino rats which had been fed eggs from the former collection.

The following structures of the egg and embryo of *P. stilesi* and *P. clavatus* were studied and measured: membranes, facette, dorsal organ, penetrating apparatus, oral armature, legs, and tail. Statistical comparisons were made between these and *P. crotali.* Some observations were also made on the activity of the embryo.

The morphology of the six nymphal stages of *P. stilesi* was studied. Data include measurements for body length, width, oral armature, hooks, body rings and number of segments and periods at which each nymphal stage was observed after the ingestion of eggs by the rats. A statistical comparison is made between the length and width of the body and of oral armature of *P. crotali* and *P. stilesi.*

The differences between measurements of the three possible combinations *P. crotali-P. stilesi, P. crotali-P. clavatus* and *P. stilesi-P. clavatus* were significant at the 5 % level for the following egg structures: external envelope (smaller diameter), outer shell (smaller diameter), length of body, length of tail, and transverse bar of the oral armature.

The differences between the measurements of *P. crotali* and *P. stilesi* nymphs were significant at the 5 % level for the body width of stages I and VI and for all body lengths of the six nymphal stages; for the length of the oral ring for all stages except for nymph VI; and for the width of the oral ring for all stages except V.

As all three species of *Porocephalus* came from samples from different regions and hosts, it is very possible that the differences found are due to factors such as geographic distribution, hosts, plasticity of the embryo and nymph, age of eggs and nymphs, differences in techniques of fixation, variations in preparing and observing specimens, and to the samples from different females of *Porocephalus*.

Neither the statistical nor the morphological analyses have given good characters that can be used to separate these so-called species of *Porocephalus* satisfactorily.

RESUMEN

Se describe la morfología del huevecillo y estadíos ninfales de *Porocephalus stilesi* y los huevecillos de *P. clavatus* en búsqueda de caracteres diferenciales que puedan ser usados para separar estas dos especies entre sí, así como éstas de *P. crotali.*

Los huevecillos de *P. stilesi* fueron obtenidos de hembras encontradas en *Lachesis muta muta;* los de *P. clavatus* de hembras provenientes de *Boa constrictor imperator;* ambos reptiles de Costa Rica.

El estudio de las estructuras se hizo con huevecillos intactos así como mediante la disección del embrión.

Para estudiar los estadíos ninfales de *P. stilesi* se dieron, mediante vía oral, huevecillos maduros a ratas albinas, machos. Las ratas fueron disecadas y los distintos órganos colocados en agua, y revisados por formas encapsuladas. Las cápsulas se separaron de los tejidos adyacentes, y las ninfas disecadas de sus cápsulas. Las ninfas se fijaron y luego se montaron en forma total o sólo algunas de sus partes.

El estudio del huevecillo incluyó las siguientes estructuras: membranas, faceta, órgano dorsal, aparato penetrador, armadura oral, patas y cola. Se tomaron medidas de todas las estructuras mencionadas en las dos especies y fueron comparadas estadísticamente con *P. crotali.* También se hicieron algunas observaciones sobre la actividad del embrión.

En el estudio morfológico de los seis estadíos ninfales de *P. stilesi* se tomaron medidas de longitud y anchura del cuerpo, armadura bucal, ganchos, anillos del cuerpo y número de pseudosegmentos. Se da información sobre los períodos requeridos para la aparición de los distintos estadíos ninfales a partir de la ingestión de los huevecillos.

Se incluyen fotografías que muestran todas las estructuras descritas y estudiadas, tanto para los huevecillos, como para los estadíos ninfales.

Las diferencias entre las medidas de las tres combinaciones posibles, *P. crotali-P. stilesi, P. crotali-P. clavatus* y *P. stilesi-P. clavatus,* fueron significativas al 5 % para las siguientes estructuras del huevecillo: envoltura externa (diámetro menor), cáscara externa (diámetro menor), longitud del cuerpo, longitud de la cola y barra transversal de la armadura bucal.

Asimismo las pruebas de significancia al 5 % para las diferencias entre las medidas de las ninfas de *P. crotali* y *P. stilesi* fueron significativas para el ancho del cuerpo de los estadíos I y VI y para la longitud de los seis estadíos ninfales; también para la longitud del anillo oral de todos los estadíos excepto la ninfa VI y para el ancho del anillo bucal excepto el de la ninfa V.

Debido a que las tres especies de *Porocephalus* estudiadas provinieron de diferentes regiones y huéspedes, es muy posible que las diferencias encontradas sean debidas a factores tales como distribución geográfica, huéspedes, plasticidad del embrión y ninfa, edad de los huevecillos y ninfas, diferencias en las técnicas de fijación, variaciones en la preparación y observación de los especímenes y a las muestras de distintas hembras de *Porocephalus.*

Ni el análisis estadístico ni el morfológico dieron caracteres adecuados que pudieran ser usados para separar estas llamadas especies de *Porocephalus* satisfactoriamente.

LITERATURE CITED

1. VAN BENEDEN, P. J.
1849. Recherches sur l'organisation et le développement des Linguatules (Pentastoma, Rud.) suivies de la description d'une éspece nouvelle provenant d'un mandrill. *Ann. Sci. nat. Par. Zool.,* 3 s, 11: 313-348 figs. 1-18.

2. ESSLINGER, J. H.
1962a. Development of *Porocephalus crotali* (Humboldt, 1808) (Pentastomida) in experimental intermediate hosts. *J. Parasitol.,* 48(3): 452-456 12 figs. 1 table.

3. ESSLINGER, J. H.
1962b. Morphology of the egg and larva of *Porocephalus crotali* (Pentastomida). *J. Parasitol.,* 48(3): 457-462 8 figs.

4. FAIN, A.
1960. Diagnoses de deux nouveaux Pentastomides du Congo Belge. *Revue Zool. Bot. afr.,* 61 (1-2): 117-118.

5. FAIN, A. & J. MORTELMANS
1960. Observations sur le cicle évolutif de *Sambonia lohrmanni* chez le Varan. Preuve d' un développement direct chez les Pentastomida. *Bull. Acad. Belg. Cl. Sci.,* 5 s., 46(6): 518-531 13 figs.

6. FAIN, A.
1961. Les Pentastomides de l'Afrique centrale. *Ann. Mus. r. Afr. cent. Sér.* 8⁰. *Zoologie,* 92: 1-115.

7. FONSECA, F. DA.
1939. Observacçoes sobre o ciclo evolutivo de *Porocephalus clavatus,* especialmente sobre o seu orquidotropismo em cobaias. *Mem. Inst. Butantan,* 12: 185-190 4 plts.

8. GIGLIOLI, G. O.M.
1927a. Observations sur la morphologie de l'oeuf et de l'embryon chez *Porocephalus clavatus* (Wyman 1845, Sambon, 1910). *Bull. Soc. Path. exot.,* 20(3): 260-270 6 figs.

9. GIGLIOLI, G. O. M.
1927b. Notes on some neo-tropical species of tongue worms. Human Porocephaliasis in Tropical America. *J. Trop. Med. Hyg.,* 30(3): 33-39 5 figs.

10. GRETILLAT, S., E. BRYGOO & C. A. DOMERGUE
1962. Pentastomes de reptiles malgaches. *Ann. Parasit. hum. comp.,* 37(3): 295-313 23 figs.

11. HETT, MARY L.
1924. On the family Linguatulidae. *Proc. Zool. Soc. Lond.,* 107-159 13 figs.

12. HEYMONS, R.
1926a. Ueber das Dorsalorgan der Pentastomiden. *Sber. Ges. naturf. Freunde Berl.,* (1-10): 22-24.

13. HEYMONS, R.
1926b. Beiträge zur Kenntnis der Gattung *Raillietiella Samb.* (Pentastomida). *Zool. Anz.,* 67: 45-56 5 figs.

14. HEYMONS, R.
1935. Pentastomida. *In* H. G. Bronns. *Klassen und Ordnungen des Tierreichs. 5 Band: Arthropoda.* IV Abteilung: Arachnoidea I Buch: Pentastomida. p. 1-268. Leipzig.

15. KEEGAN, H. L.
1943. Observations on the pentastomid, *Kiricephalus coarctatus* (Diesing) Sambon. 1910. *Trans. Amer. microsc. Soc.,* 62(2): 194-199 4 figs.

16. LEUCKART, R.
1860. *Bau und Entwicklungsgeschichte der Pentastomen. Nach Untersuchungen besonders von* Pent. taenioides *und* P. denticulatum. VI + 160 6 pls. Leipzig & Heidelberg.

17. NICOLI, R. M.
1963. Phylogénese et systématique le phylum des Pentastomida. *Ann. Parasit. hum. comp.,* 38: 483-516.

18. NOC, F.
1923. Sur l'embryon acariforme et les stades larvaires des Linguatulidés. *Bull. Soc. Path. exot.,* 16: 340-346 6 figs., 1 pl.

19. OSCHE, G.
1959. "Arthropodencharaktere" bei einem Pentastomiden Embryo *(Reighardia sternae*). *Zool. Anz.,* 163: 169-178 3 figs.

20. PENN, G. H.
1942. The life history of *Porocephalus crotali,* a parasite of the Louisiana muskrat. *J. Parasitol.,* 28(4): 277-283 2 figs.

21. SAMBON, L. W.
1922. A Synopsis of the family Linguatulidae. *J. trop. Med. Hyg.*, 25: 188-266; 391-428 figs.

22. SCHUBART, T. D.
1853. Ueber die Entwickelung des *Pentastoma taenioides*. *Z. wiss. Zool.*, 4(1): 117-118 12 figs. pl. VII-VIII.

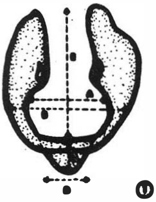
23. SELF, J. T. & F. B. MCMURRY
1948. *Porocephalus crotali* Humboldt (Pentastomida) in Oklahoma. *J. Parasitol.*, 34(1): 21-23 4 figs.

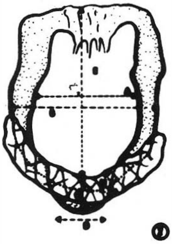
24. SELF, J. T.
1951. The auxiliary hooks in *Porocephalus crotali* Humboldt. *Trans. Amer. microsc. Soc.* 70(3): 255-256 1 fig.

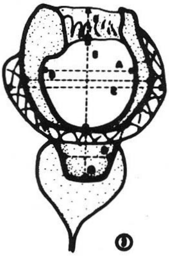
25. STILES, C. W.
1891. Bau u. Entwicklungsgeschichte v. *Pentastomum proboscideum* Rud. und *Pentastomum subcylindricum* Dies. *Z. wiss. Zool.*, 52: 85-157 2 pls.

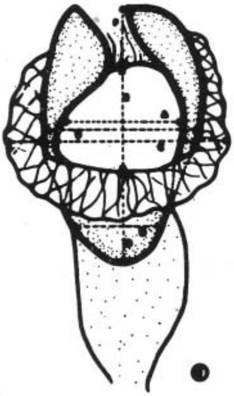
FIGURES

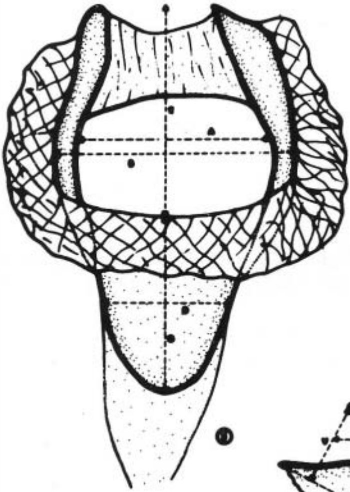
Figs. 1-9. Standard measurements of oral armatures and hooks of *Porocephalus stilesi* nymphs (not drawn to scale).
Fig. 1. Oral armature of nymph I (ventral view).
Fig. 2. Oral armature of nymph II (ventral view).
Fig. 3. Oral armature of nymph III (ventral view).
Fig. 4. Oral armature of nymph IV (ventral view).
Fig. 5. Oral armature of nymph V (ventral view).
Fig. 6. Oral armature of nymph VI (ventral view).
Fig. 7. Oral armature of nymph VI (lateral view).
Fig. 8. Hook of nymph V (lateral view).
Fig. 9. Outer hook of nymph VI (lateral view).











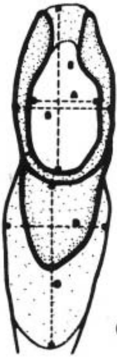
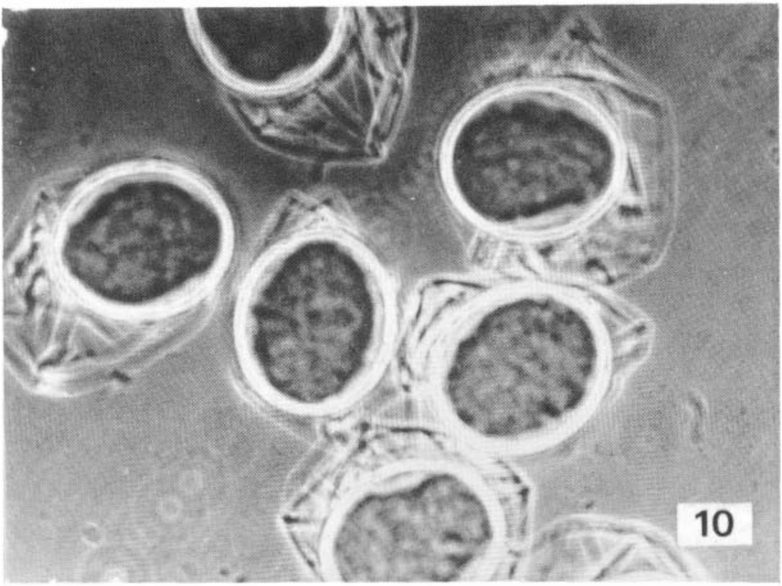
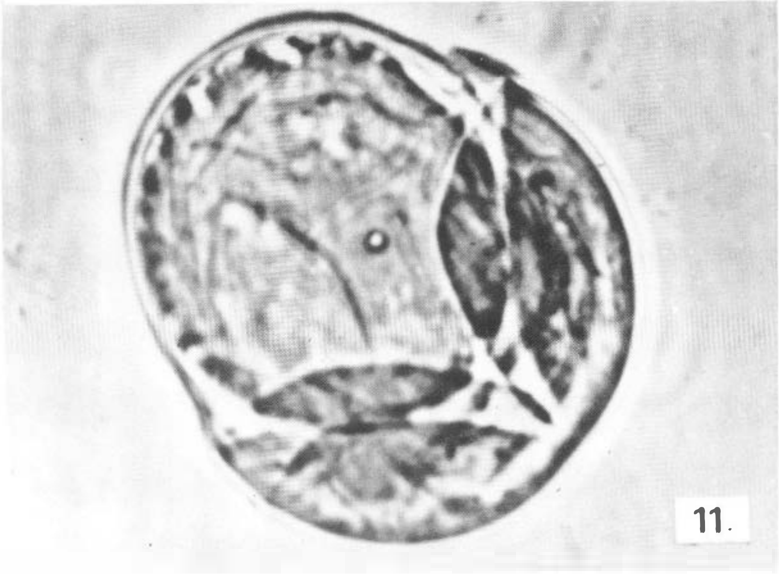


Fig. 10. Eggs of *Porocephalus stilesi* (general aspect, note external bladder-like envelope). 230 X.

Fig. 11. Egg of *Porocephalus stilesi* showing outer and inner shells (note split on outer shell). 650 X.



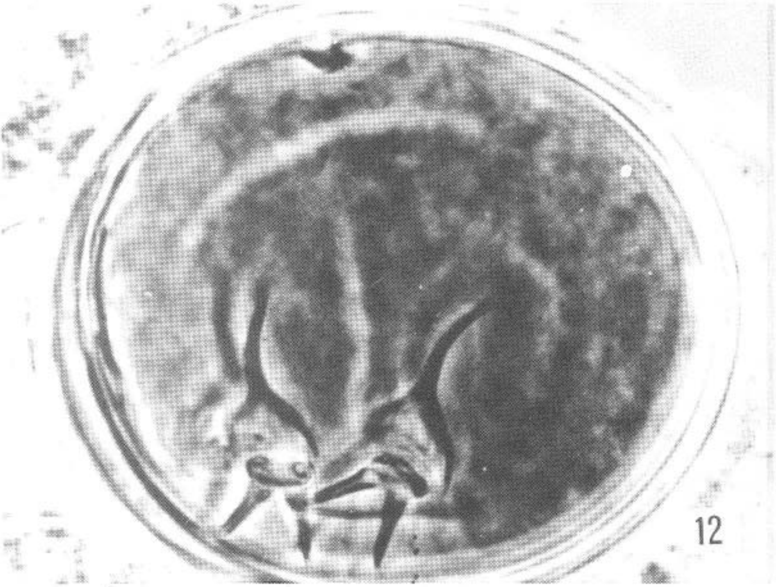
10



11.

Fig. 12. Egg of *Porocephalus stilesi* showing the embryo (lateral view) (note dorsal organ, claws, plate bearing claws and accessory apparatus). 900 ×.

Fig. 13. Egg of *Porocephalus stilesi* (ventral view) (note embryo penetrating apparatus, oral ring and legs). 1,000 ×.



12

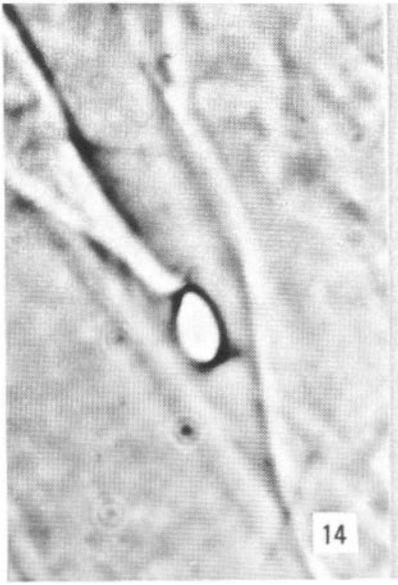
13.

Fig. 14. Facette pore of embryo of *Porocephalus stilesi.* 2,000 ×.

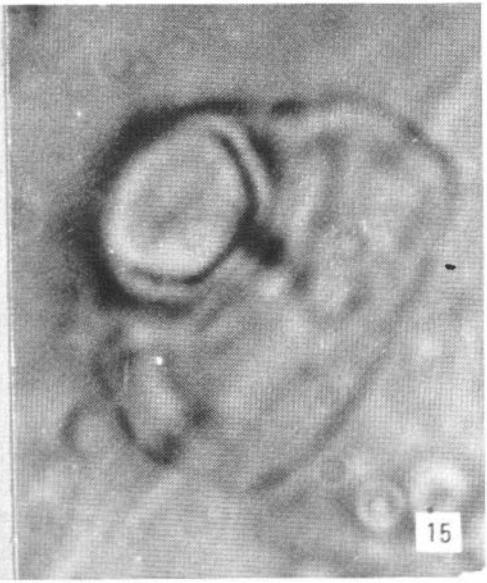
Fig. 15. Pore and facette body of embryo of *Porocephalus stilesi.* 5,000 ×.

Fig. 16. Facette and dorsal organ of embryo of *Porocephalus stilesi* (lateral view). 4,000 ×.

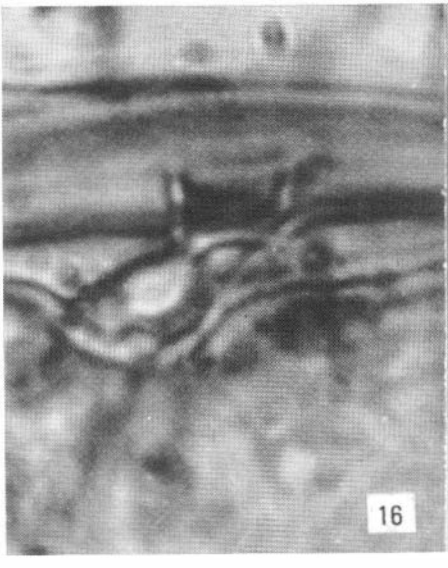
Fig. 17. Plate and vesicle of dorsal organ of embryo of *Porocephalus stilesi.* 5,300 ×.



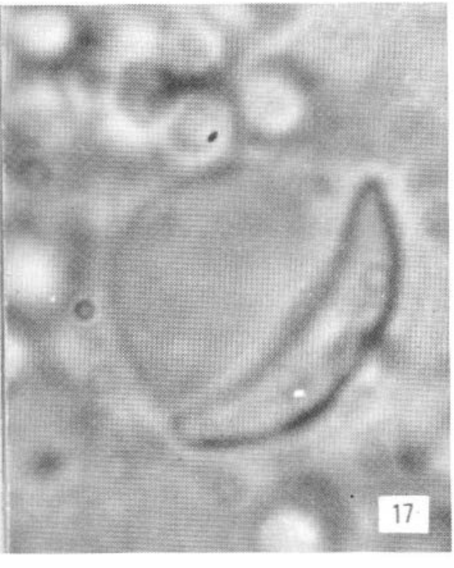
14



15



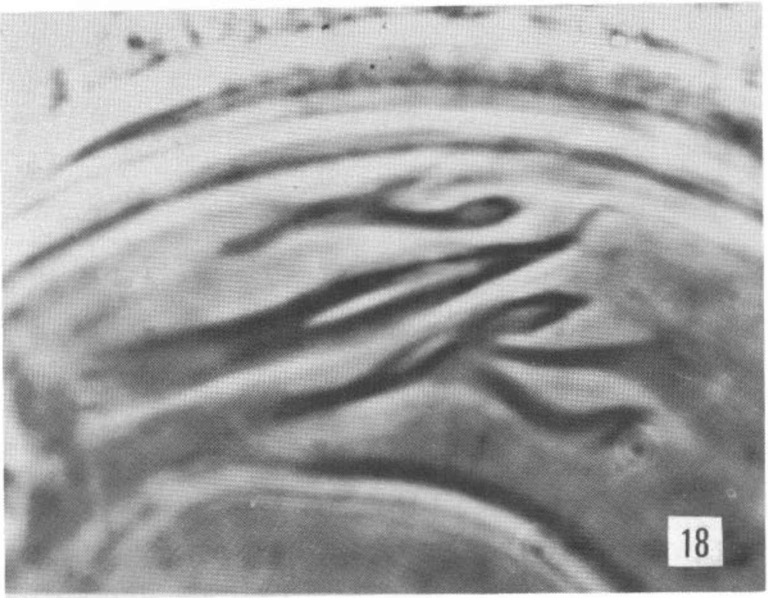
16



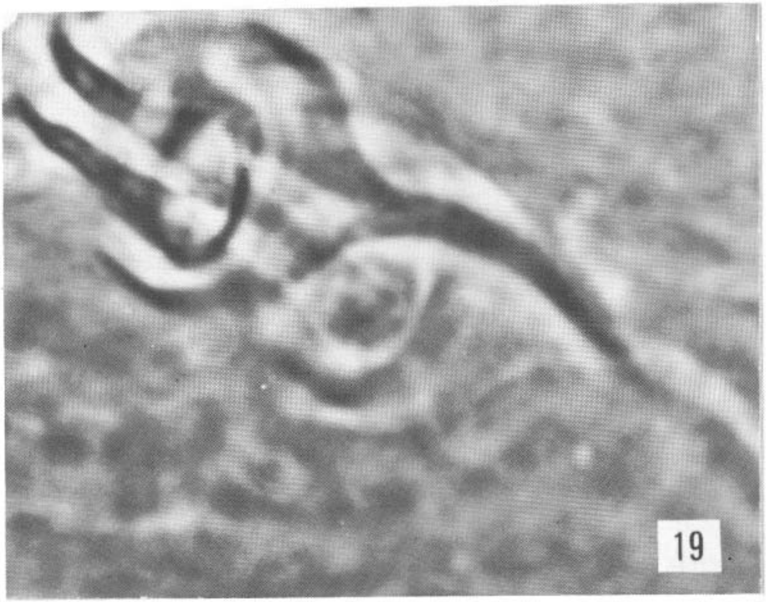
17

Fig. 18. Penetrating apparatus of the embryo of *Porocephalus stilesi* within the egg (anteroventral view) (note median blade and lateral forks). 2,800 ×

Fig. 19. Claws and accessory apparatus of leg of embryo of *Porocephalus stilesi.* 2,800 ×.



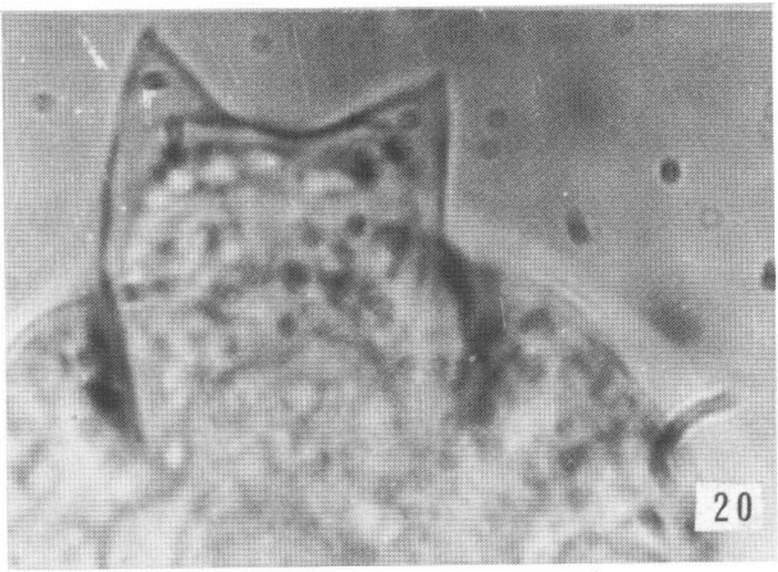
18



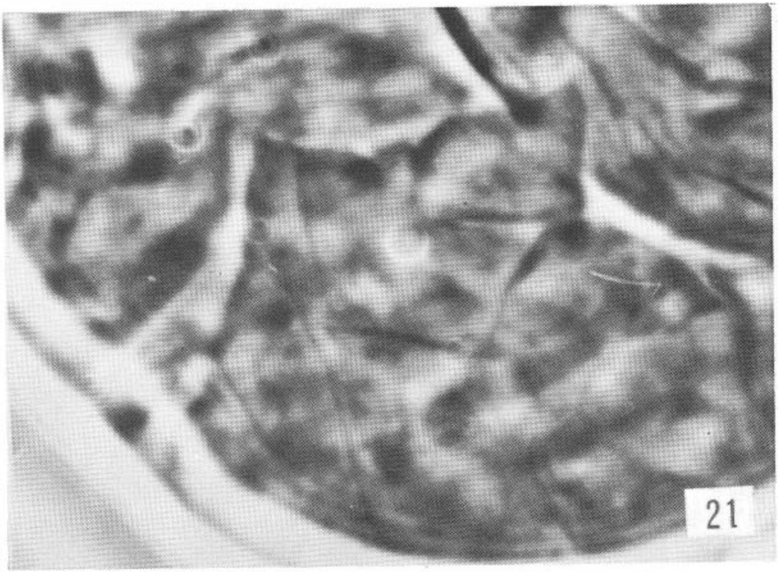
19

Fig. 20. Tail of embryo of *Porocephalus stilesi* (ventral view). 2,500 ×.

Fig. 21. Folded tail of embryo of *Porocephalus stilesi* within the egg. 2,600 ×.



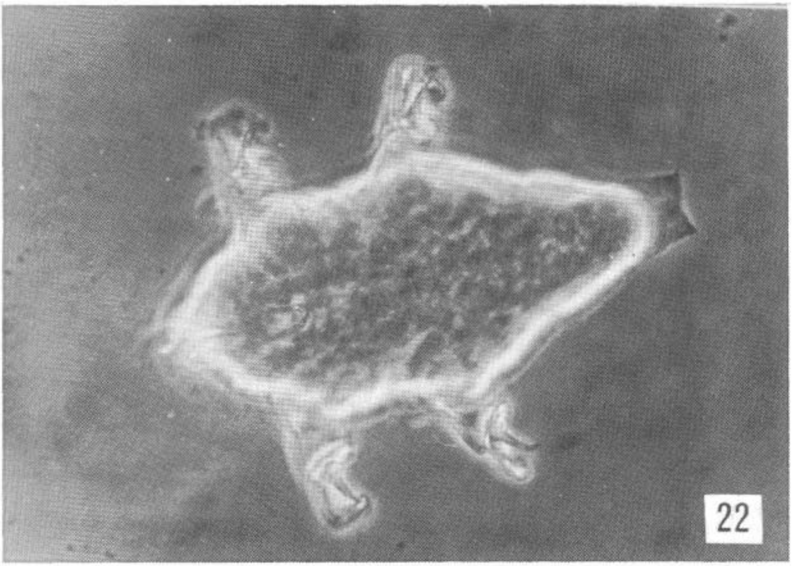
20



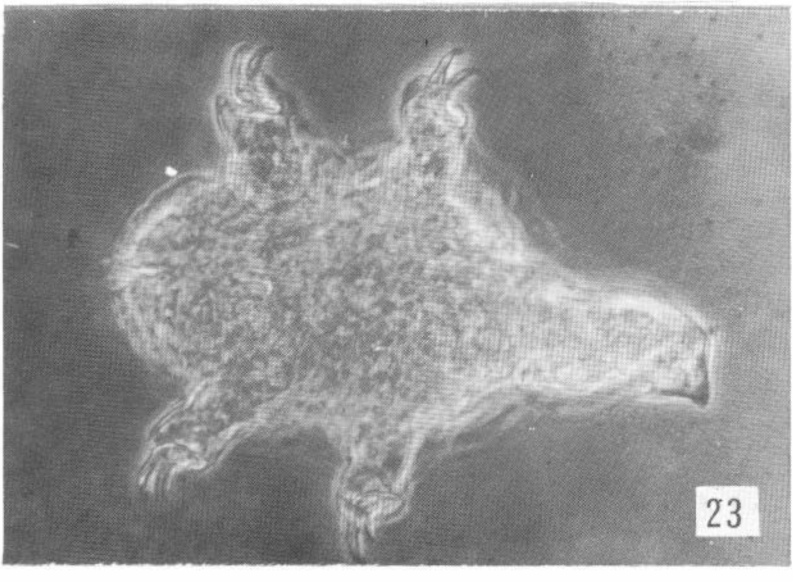
21

Fig. 22. Larva of *Porocephalus stilesi* (ventral view). 580 ×.

Fig. 23. Larva of *Porocephalus stilesi* (ventral view). 660 ×.



22

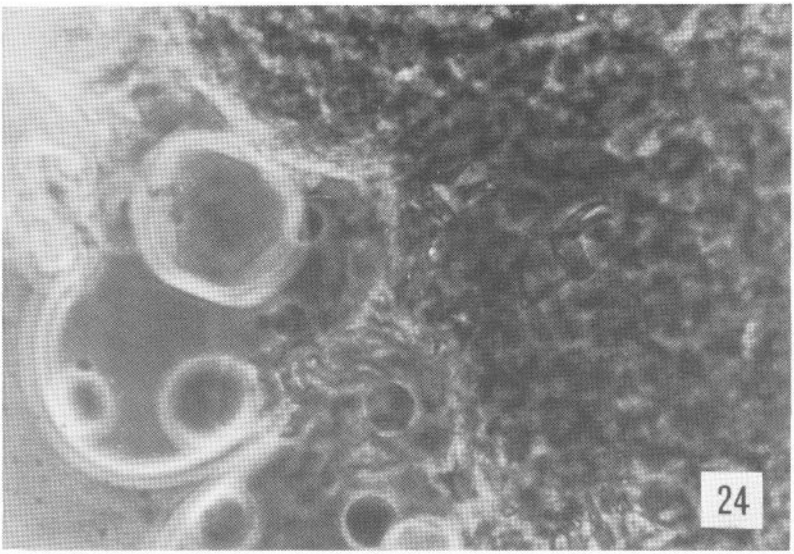


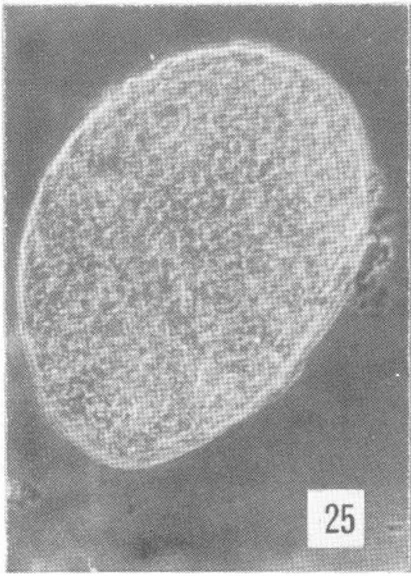
23

Fig. 24. Larval remains of encapsulated form of *Porocephalus stilesi* (note the remains of two legs and penetrating apparatus). 800 ×.

Fig. 25. First nymphal stage of *Porocephalus stilesi* (lateral view). 350 ×.

Fig. 26. Second nymphal stage of *Porocephalus stilesi* (latero-ventral view). 230 ×.


24


25

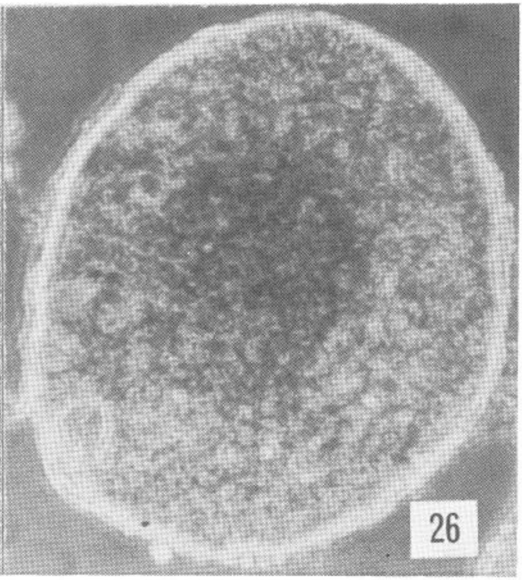
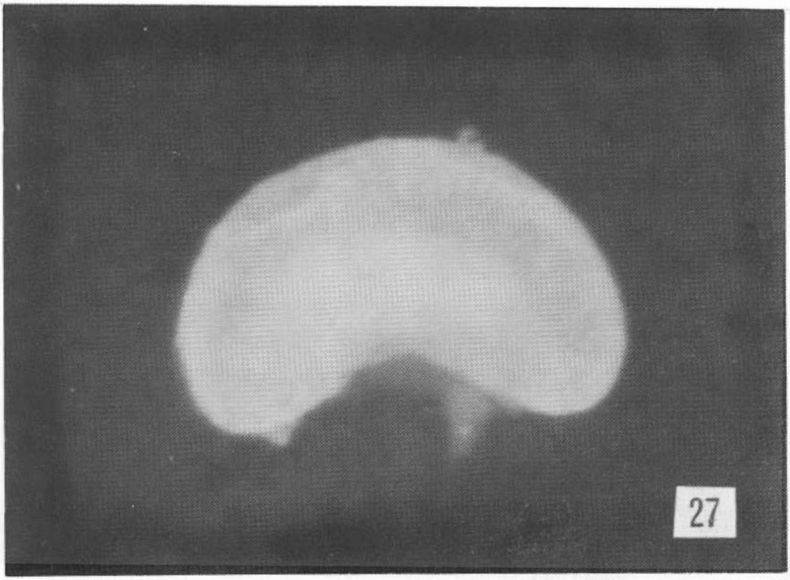
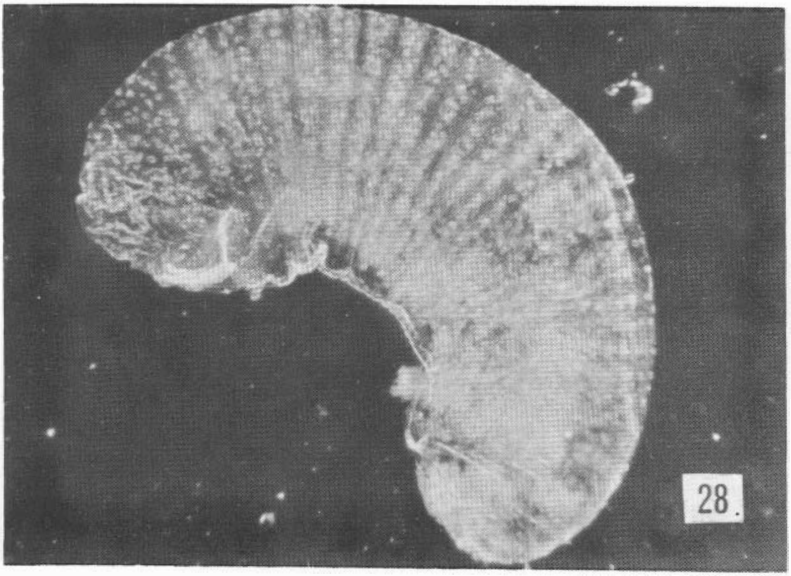

26

Fig. 27. Third nymphal stage of *Porocephalus stilesi* (lateral view). 150 ×.

Fig. 28. Fourth nymphal stage of *Porocephalus stilesi* (lateral view). 50 ×.



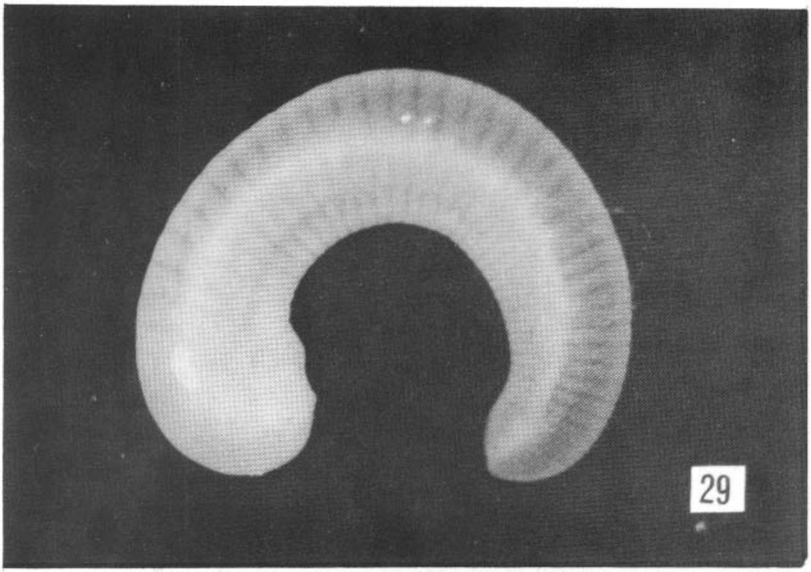
27



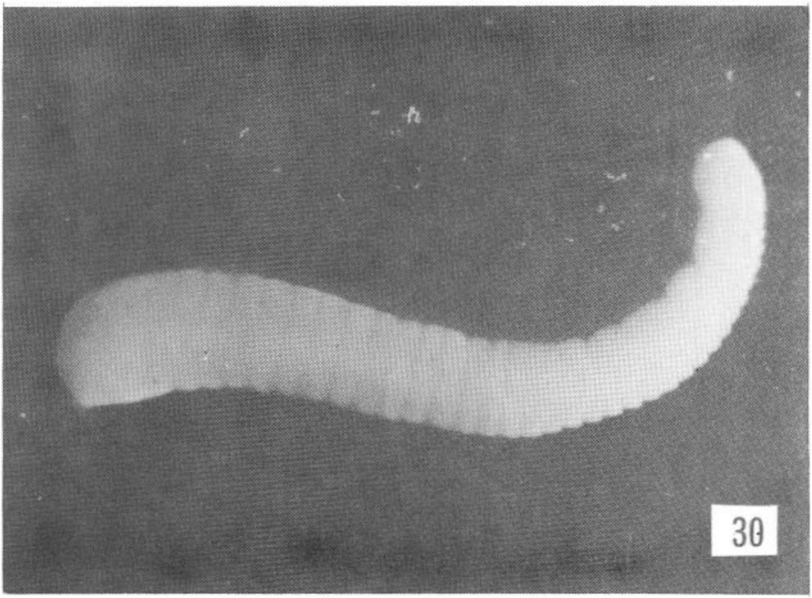
28.

Fig. 29. Fifth nymphal stage of *Porocephalus stilesi* (lateral view). 40 ×.

Fig. 30. Sixth nymphal stage of *Porocephalus stilesi* (lateral view). 9 ×.



29



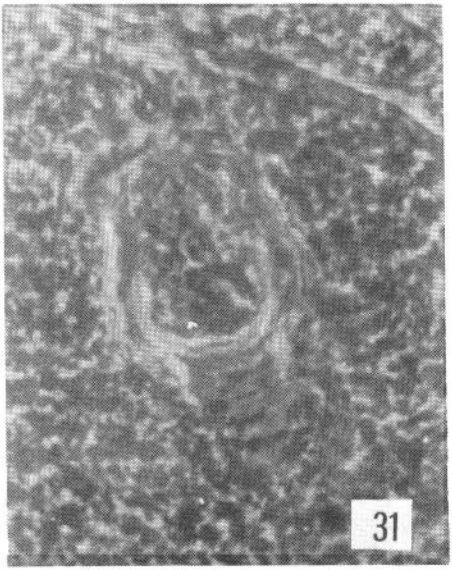
30

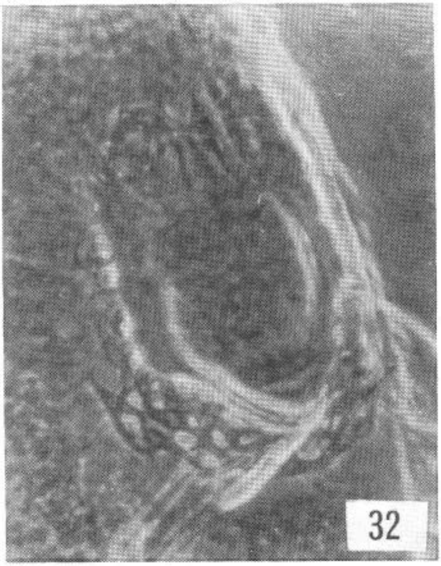
Fig. 31. Oral armature of nymph II of *Porocephalus stilesi* (ventral view). 750 ×.

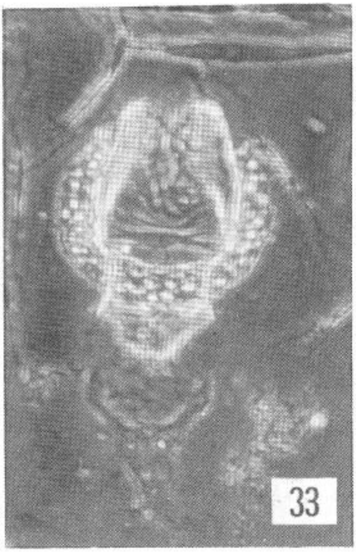
Fig. 32. Oral armature of nymph III of *Porocephalus stilesi* (lateroventral view). 450 ×.

Fig. 33. Oral armature of nymph IV of *Porocephalus stilesi* (ventral view). 200 ×.

Fig. 34. Oral armature of nymph V of *Porocephalus stilesi* (ventral view). 200 ×.


31


32


33

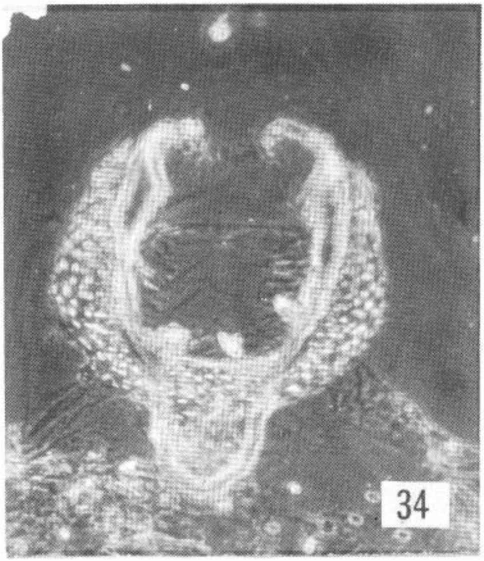
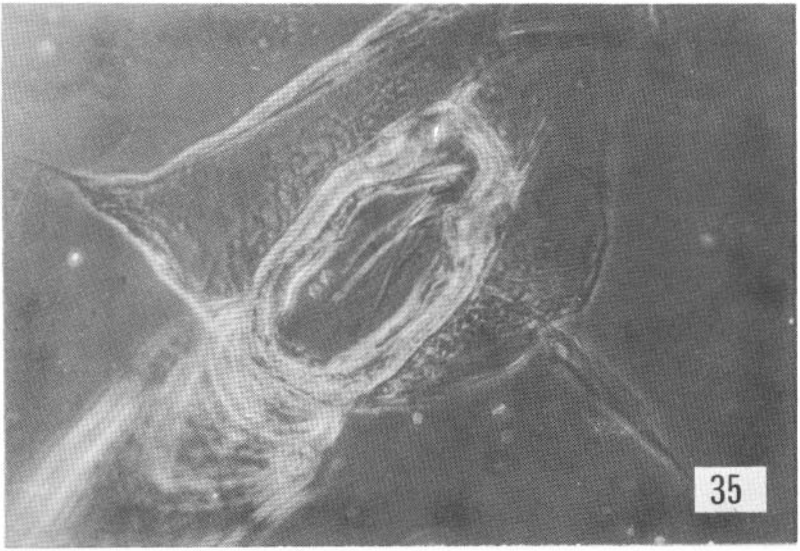

34

Fig. 35. Oral armature of nymph VI of *Porocephalus stilesi* (ventral view). 150 ×.

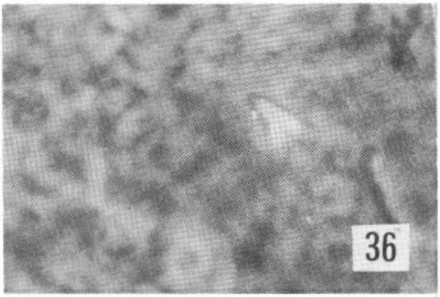
Fig. 36. Mouth hook of nymph III of *Porocephalus stilesi* lateral view) 1,600 ×.

Fig. 37. Mouth hook of nymph IV of *Porocephalus stilesi* (lateral view). 800 ×.

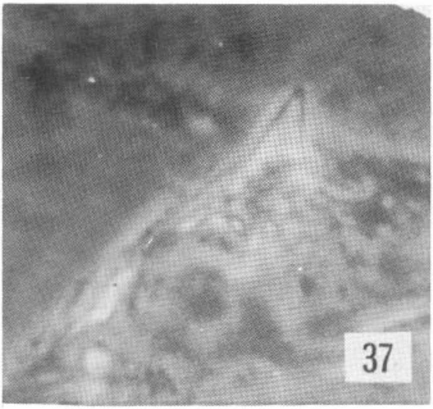
Fig. 38. Mouth hook of nymph V of *Porocephalus stilesi* (lateral view). 610 ×.



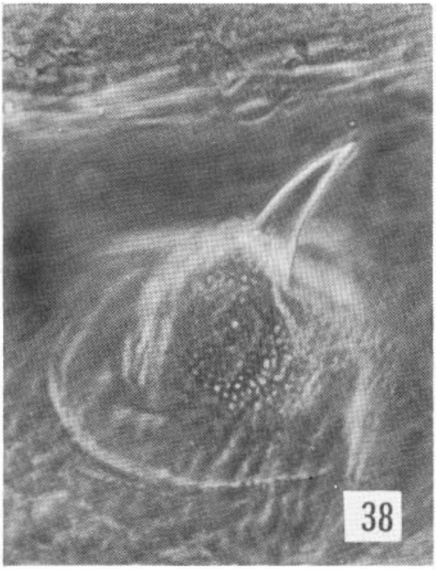
35



36



37



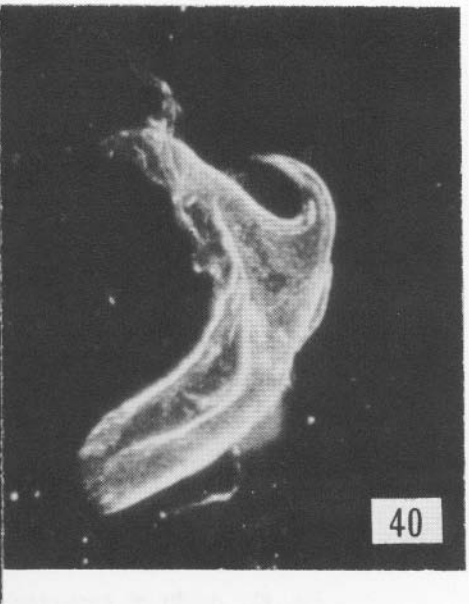
38

Fig. 39. Inner mouth hook of nymph **VI** of *Porocephalus stilesi* (lateral view). 96 ×

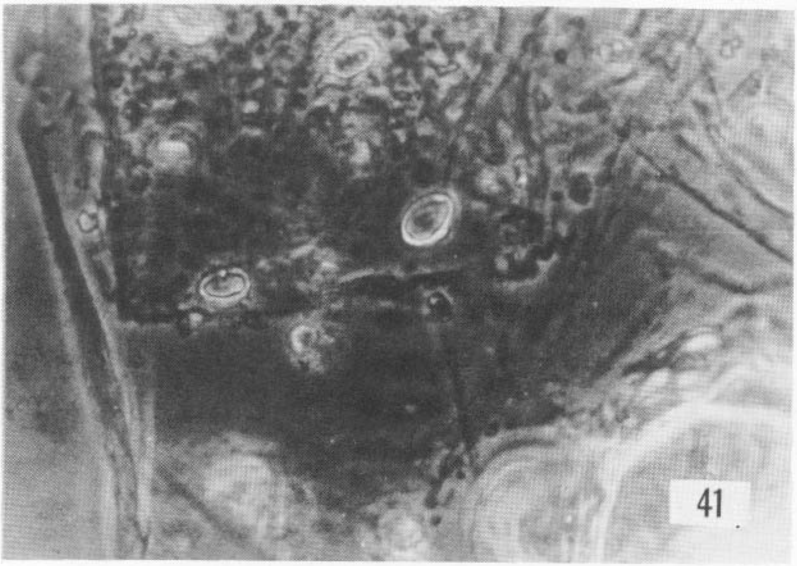
Fig. 40. Outer mouth hook of nymph **VI** of *Porocephalus stilesi* (lateral view) (note auxiliary hook). 90 ×.

Fig. 41. Body rings of nymphal stage III of *Porocephalus stilesi*. 660 ×.

39



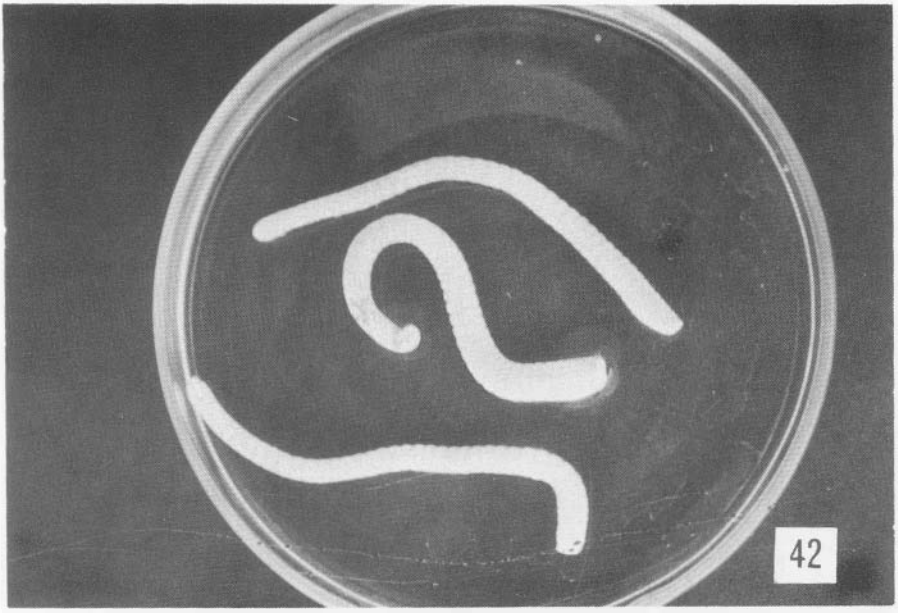
40



41

Fig. 42. Adults of *Porocephalus stilesi.* 0.9 ×.

Fig. 43. Dissection of *Boa constrictor imperator* showing adults of *Porocephalus clavatus* in lungs and air sacs.


42

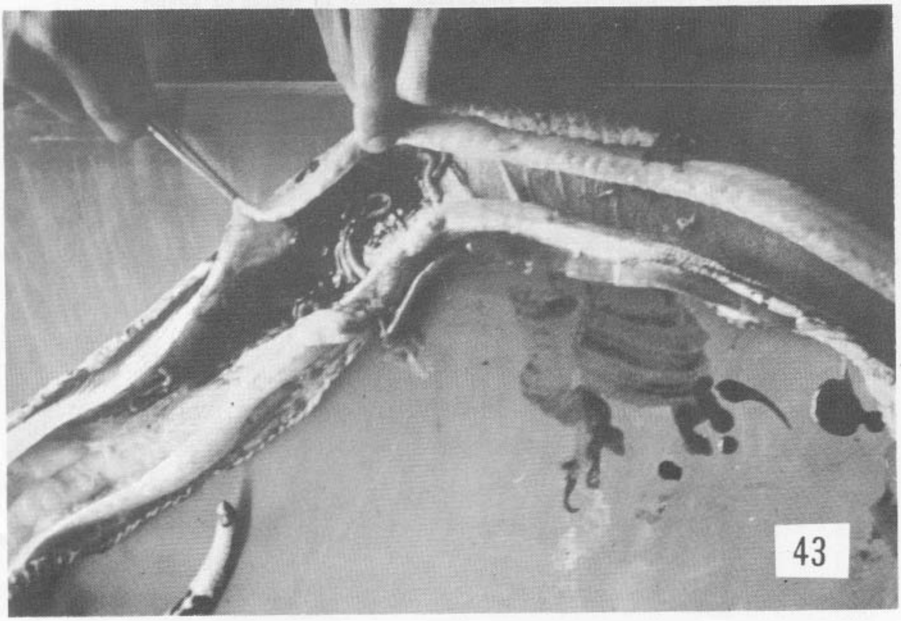
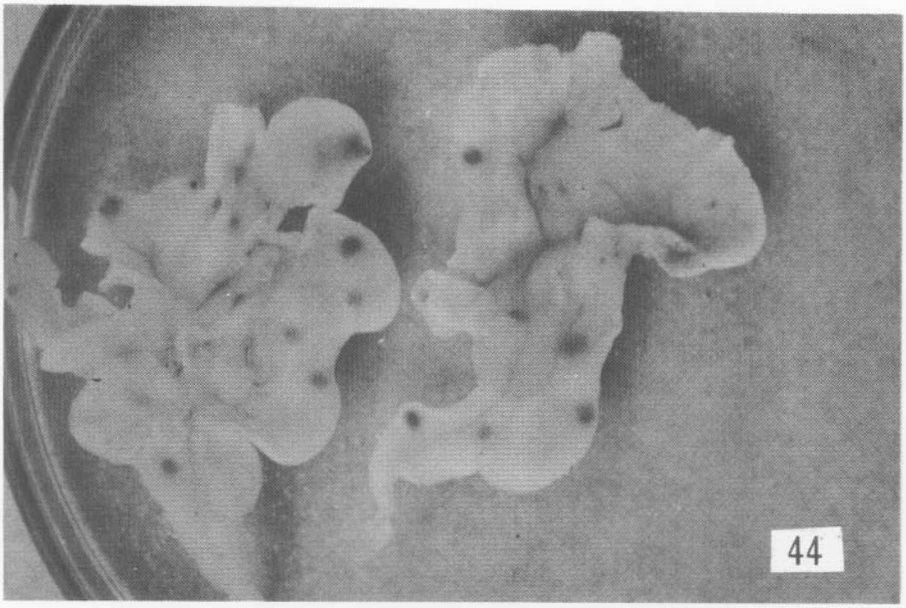
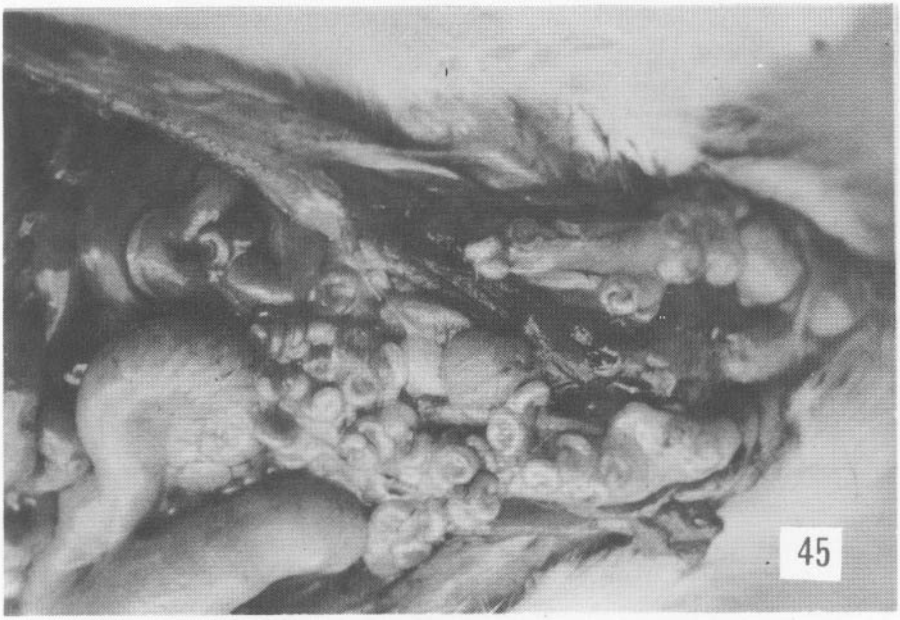

43

Fig. 44. Encapsulated forms of *Porocephalus stilesi* in seroses of albino rat (about 3rd or 4th nymphal stage). 2 ×.

Fig. 45. Nymphs VI of *Porocephalus stilesi* in tissues of albino rat (many forms in seroses and tissues surrounding the testes). 1 ×.



44



45