Onderstepoort J. Vet. Res. (1966), 33 (1), 287–296
Printed in the Republic of S. Afr. by the Government Printer, Pretoria

A DESCRIPTION OF THE FIRST STAGE LARVA OF *OESTRUS AUREOARGENTATUS* RODHAIN AND BEQUAERT (1912) OBTAINED BY ARTIFICIAL MATING (DIPTERA: OESTRIDAE)

E. M. NEVILL and P. A. BASSON, Veterinary Research Institute, Onderstepoort

Introduction

Specific oculo-vascular myiasis (uitpeuloog) in domestic animals is caused by the invasion of an aberrant host by the first stage larvae of *Gedoelstia* spp. (Basson, 1962). These and other oestrid flies have various antelopes, such as blue wildebeest [*Connochaetes taurinus* (Burchell, 1823)] and hartebeest [*Alcelaphus buselaphus* (Pallas, 1766)] as natural hosts and frequently occur in their nasal cavities and sinuses. Studies on certain aspects of the disease, however, were hampered by the difficulty of producing sufficient cases artificially. This was due to the fact that, in spite of being reared successfully these flies refused to mate in captivity. Adult *Gedoelstia* flies are rarely seen in their natural habitat and their capture proved to be extremely difficult. In an attempt to overcome this problem the methods of artificial mating adopted successfully by Weintraub (1961) for *Hypoderma* spp. were tried with both *Gedoelstia* spp. and *Oestrus* spp. reared from larvae collected from antelopes. These methods included flying the insects on cotton thread leashes. In *Hypoderma* spp. flying seemed to activate their mating response and copulation could be successfully initiated. A second method of inducing copulation was to decapitate the male and hold it in an appropriate copulatory position in direct contact with a female.

This report deals primarily with a successful artificial mating attempt with *O. aureoargentatus*, the description of its hitherto unknown first stage larva and its comparison with the first stage larva of *Oestrus ovis* Linnaeus (1758).

Materials and Methods

Full-grown oestrid larvae were collected from the nasal cavities of two hartebeest and four blue wildebeest in the Kalahari Gemsbok National Park and the Kruger National Park. The larvae were allowed to pupate in about two inches of dry sand and then kept at room temperature in the laboratory at Onderstepoort to await the emergence of adults. On emergence the flies were transferred to a room kept at ± 80° F and ± 90 per cent R.H. Mating of the flies was then attempted with the methods described by Weintraub (1961) for the artificial mating of *Hypoderma bovis* (L) and *H. lineatum* (De Vill.). This consisted of affixing eight inches of thread to the thorax by means of paraffin wax. One end of the thread was dipped into molten wax of high melting point (60° C) until a small drop of wax had formed. The fly was then anaesthetized with CO_2 and a hot needle used to fuse the drop of wax with its thread onto the mesothorax. Mating of these tethered flies in flight

Received for publication on 28 April, 1966.—Editor

was attempted repeatedly in the laboratory and also outside in sunlight for periods varying from half to two hours. The flight method was always attempted first and usually repeated over several days. If unsuccessful, the second method of decapitating the males was used in the laboratory. Trials with decapitated males took place under a dissecting microscope, the female being held carefully in the left hand, the male in the right. Contact of the abdomens normally brought about a clasping movement of the male genitalia.

The trials resulted in one successful mating. The mated *O. aureoargentatus* female was dissected after 14 days and the almost spherical uterus removed into normal saline. The viable larvae obtained on opening the uterus were counted under a dissecting microscope.

In order to determine the pathogenicity of the larvae to sheep, about 96 were instilled into the eyes of a full grown Merino sheep and approximately 126 intranasally into a second sheep. Normal saline was used as vehicle. At the time of instillation both sheep were free of eye lesions and a nasal discharge which is typical of *O. ovis* infestation.

About half the larvae were retained for study by killing in hot water and preservation in 70 per cent alcohol. They were mounted directly in Berlese's fluid which has the advantage of clearing them without distortion.

The drawings were made with the use of a camera lucida and phase contrast microscope. Some of the finer details such as spines were drawn freehand or from photomicrographs. The larva is described below and compared with the first stage larva of *O. ovis* in Table 2.

Results

Artificial mating attempts

These attempts are summarized in Table 1. The only successful mating was obtained with an *O. aureoargentatus* female and a decapitated male two days after emergence. Mating lasted 12 minutes. Fourteen days later this female was still alive and was dissected to determine if mating had been successful. On dissection the uterus was found to contain 440 living first stage larvae and 23 unhatched ova.

Attempts to infest sheep with the larvae of O. aureoargentatus

The two sheep artificially infested in the manner described were examined daily for five days, but no lesions or symptoms of any kind developed. Observations were terminated after 43 days when they still showed no signs of infestation.

Description of O. aureoargentatus *first stage larva*

The first stage larva is more or less cylindrical, about 1·4 mm long, 0·4 mm broad and has twelve segments. The small first segment bears a pair of cephalic lobes with sensory papillae. The second segment has a rosette of several rows of sharply pointed, curved spines and the number of rows increases postero-ventrally. The cephalo-pharyngeal skeleton consists of the anterior oral hooks (mandibular sclerites), basal sclerites (ectostomal sclerites), hypostomal sclerite with its middle peak and the longer pharyngeal sclerites which project posteriorly from either side

of the hypostomal sclerite. The pair of basal sclerites differs distinctly from that of *O. ovis* as illustrated by Grunin (1957) and Basson (1962), and constitutes the most important feature for differentiating the cephalo-pharyngeal skeletons of these two species (Fig. 3).

All the segments except the first bear several complete and incomplete rows of spines antero-ventrally. Thick, flexible, hair-like structures are found latero-ventrally on all the segments except the first and the last. Very short, incomplete accessory rows of curved spines are situated latero-ventrally and posteriorly to the complete rows on segments 3 to 12. All the lateral spines in the complete ventral rows tend to be more hook-like than the central ones. A short incomplete median row of 8 to 13 spines is present anteriorly to the complete ventral rows on segments 6 to 12 and an incomplete, medially interrupted posterior row is present on segments 6 to 11. The spines in the first complete ventral row on each segment are larger than the more posterior spines.

Dorsal spines are situated on segments 2 to 7 only. The second segment has several complete rows of both large and small, curved spines. Three complete antero-dorsal rows are present on the third segment and two or three medially interrupted (incomplete) rows on segments 4 to 7.

The ventral and dorsal spines, with the exception of some latero-ventral spines and those on the second segment, are three-pointed with a lateral shorter point on either side of a larger and longer medial point (Fig. 1c). In *O. ovis* only the spines on segments 2 to 4 are distinctly three-pointed and the more posterior ones show a marked decrease in this tendency (Grunin, 1957; Basson, 1962). A pair of triplet bristles is found ventrally in a paramedian position on segments 2 to 4 and a pair of very small tufts of delicate hairs just posterior to the ventral spines on segments 5 to 11. One small seta is situated latero-ventrally on either side of segments 5 to 11. About 50 to 54 retractile terminal hooklets are found ventrally on the last segment.

In Table 2 the characteristic features of each segment of *O. aureoargentatus* are summarized and compared with those of *O. ovis* as described by Basson (1962). The most important points of difference are the shape of the larvae, the number of ventral and dorsal rows of spines, the regional distribution of the characteristic three-pointed spines, the cephalo-pharyngeal skeleton and the number of terminal hooklets (Fig. 1 to 3). There appears to be only insignificant points of difference between the posterior spiracles of the two species (Fig. 1).

Discussion

Studies on the Oestridae have long been hampered by the inability to breed them in captivity. This was particularly so with *Gedoelstia* spp. whose first stage larvae cause serious and fatal lesions in abnormal hosts such as sheep (Basson, 1962). The identification of the larvae responsible for this disease was only possible after a prolonged, rather complicated study. The present successful mating attempt with *O. aureoargentatus* has not only led to the study and description of its hitherto unknown first stage larva, but has also paved the way to further trials in artificial breeding, particularly of pathogenic species such as *Gedoelstia* and those whose

first stage larvae are still unknown to diagnosticians and taxonomists. It is further necessary to draw attention to the fact that the only successful mating attempt in the present study was achieved two days after emergence of the adult stages and that the flies appeared to be most active during these first few days. This could indicate that the first few days are the most suitable and critical time for mating.

The negative results obtained with attempts to infest sheep intra-ocularly and intranasally with *O. aureoargentatus* first stage larvae indicate that they are probably host-specific and non-pathogenic to sheep.

Summary

A successful attempt at artificial mating by applying the method of decapitation in *O. aureoargentatus* is recorded. The hitherto unknown first stage larva of this oestrid fly is described and compared with that of *O. ovis*. It proved to be non-pathogenic to sheep. Further unsuccessful trials with the tethering method of Weintraub (1961) are also described.

Acknowledgements

Thanks are due to the National Parks Board for their permission to collect the larvae in the various parks, to the Senior State Veterinarian at Skukuza, Dr. J. W. van Niekerk and other officials for their assistance in procuring the materials needed, to Mr. A. M. du Bruyn, Section of Photography at Onderstepoort for the preparation of the photographs and to Prof. R. M. du Toit, Section of Entomology, for his helpful criticism in the preparation of the manuscript.

References

BASSON, P. A., 1962. Studies on specific oculo-vascular myiasis of domestic animals (uitpeuloog): II. Experimental transmission. *Onderstepoort J. Vet. Res.*, 29 (2), 203–209.

BASSON, P. A., 1962. Studies on specific oculo-vascular myiasis (uitpeuloog). III. Symtomatology, pathology, aetiology and epizootiology. *Onderstepoort J. Vet. Res.*, 29 (2), 211–240.

GRUNIN, K. Y., 1957. Oestridae. *Fauna USSR*, No. 68, pp. 233, 243, 244 (In Russian).

RODHAIN, J. & BEQUAERT, J., 1912. Sur deux oestrides nouveaux parasites du potamochère et de l'antilope chevaline au Congo belge. *Rev. zool. afr.*, 1, 381 (cited by Zumpt).

WEINTRAUB, J., 1961. Induced mating and oviposition of the warble flies *Hypoderma bovis* (L.) and *H. lineatum* (De Vill.) (Diptera: Oestridae) in captivity. *The Canadian Entomologist*, 93, 149–156.

ZUMPT, F., 1965. Myiasis in man and animals in the old world. First ed. pp. 180–181. London: Butterworths & Co.

TABLE 1.—*Summary of artificial mating attempts with Oestrid flies*

Larval collections			Oestrid species	Number and sex	(1) Pupal period (days)	(2) Interval (days) before artificial mating by		Adult longevity (days)
Date	Place	Host				Tethering	Decapitation	
10.9.64	Kalahari Gemsbok Park	2 Hartebeest.....	*Oestrus variolosus*.....	1 ♂	36	—	—	—
				6 ♀♀	38–41	—	—	—
9.3.65 & 10.3.65	Kruger National Park...	2 Blue Wildebeest.....	*O. aureoargentatus*.....	3 ♂♂	25–27	4–6	8–18	—
				4 ♀♀	29–31	0–2	4–14	12–29
			O. variolosus.....	9 ♂♂	31–34	9–12	9–13	—
				8 ♀♀	32–35	8–10	8–11	14–19
10.8.65 & 12.8.65	Kruger National Park...	2 Blue Wildebeest.....	*Gedoelstia hässleri*.....	1 ♂	34	1	5	—
				2 ♀♀	35	0	4	14–15
			G. cristata.....	1 ♂	40	—	—	6
			O. variolosus.....	1 ♂	49	1	6	—
				1 ♀	50	0	5	14
			O. aureoargentatus.....	1 ♂	42	2	2	—
				2 ♀♀	42	2	2 (3)	16

(1) Includes period between collection and emergence
(2) Interval between emergence and first attempt at artificial mating by specific methods mentioned
(3) 1 ♀ mated successfully

TABLE 2.—*Comparative features of first stage larvae*

Structures	Segments																						
	Oestrus aureoargentatus												*Oestrus ovis*										
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Ventral aspect:																								
Spines:																								
Complete rows.....	—	+++	5	5	5	4	4	4	4	4	4	3	—	+++	2	2	2	2	2	2	2	2	2	2
Incomplete anterior median row.....	—	—	—	—	—	1	1	1	1	1	1	1	—	—	—	—	—	1	1	1	1	1	1	1
*Incomplete post. median row.....	—	—	—	—	—	—	—	—	—	—	—	— (1)	—	—	1 (2)	1 (2)	1 (2)	—	— (1)	— (1)	— (1)	— (1)	— (1)	— (1)
Incomplete, medially interrupted post. row.....	—	—	—	—	—	1	1	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
**Accessory curved laterals.....	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	+	+
Hooklets: terminal.....	—	—	—	—	—	—	—	—	—	—	—	50+	—	—	—	—	—	—	—	—	—	—	—	18–22
Triplet bristles.....	—	2	2	2	—	—	—	—	—	—	—	—	—	2	2	2	—	—	—	—	—	—	—	—
Tufts of fine hairs.....	—	—	—	—	2	2	2	2	2	2	2	—	—	—	—	—	2	2	2	2	2	2	2	—
Lateral hairlike structures	—	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	—
Lateral setae.....	—	—	—	—	1	1	1	1	1	1	1	—	—	—	—	—	1	1	1	1	1	1	1	—
Dorsal aspect:																								
***Spines:																								
Rows.....	—	+++	3 (4)	3	2 (3)	2	2	—	—	—	—	—	—	++	1	1	1	—	—	—	—	—	—	—
Setae.....	—	2	—	—	—	—	—	—	—	—	—	10	—	2	—	—	—	—	—	—	—	—	—	10

*Numbers in brackets indicate the presence of such structures in some larvae only
**The accessory lateral spines in *O. ovis* are either straight or curved
***Numbers in brackets indicate the presence of one additional shorter incomplete row
+++Several rows, probably more than 5, but not counted
++More than 2 rows
+Present
—Absent

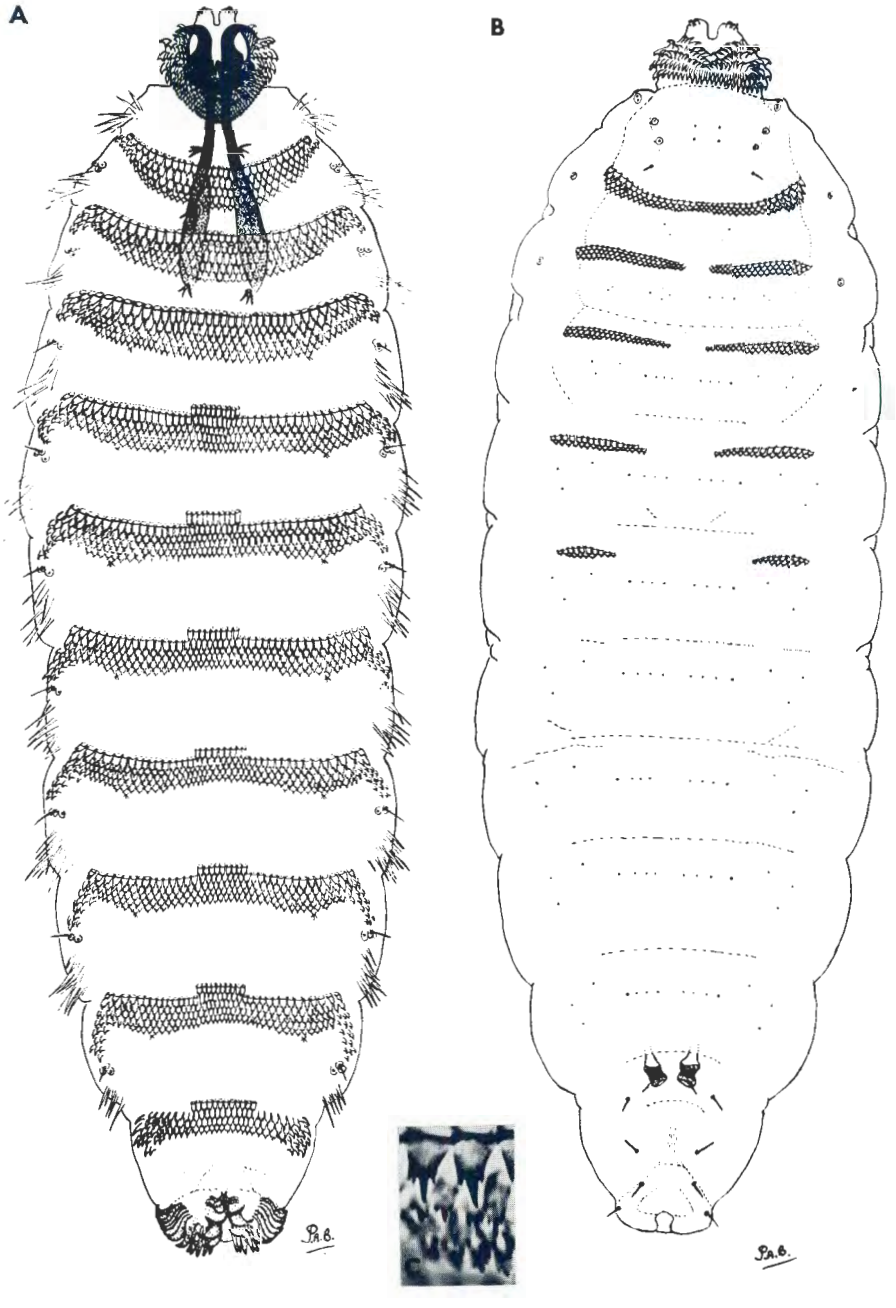

FIG. 1.—*Oestrus aureoargentatus* first stage larva
A. Ventral view. B. Dorsal view. C. Photomicrograph showing the typical three-pointed ventral spines

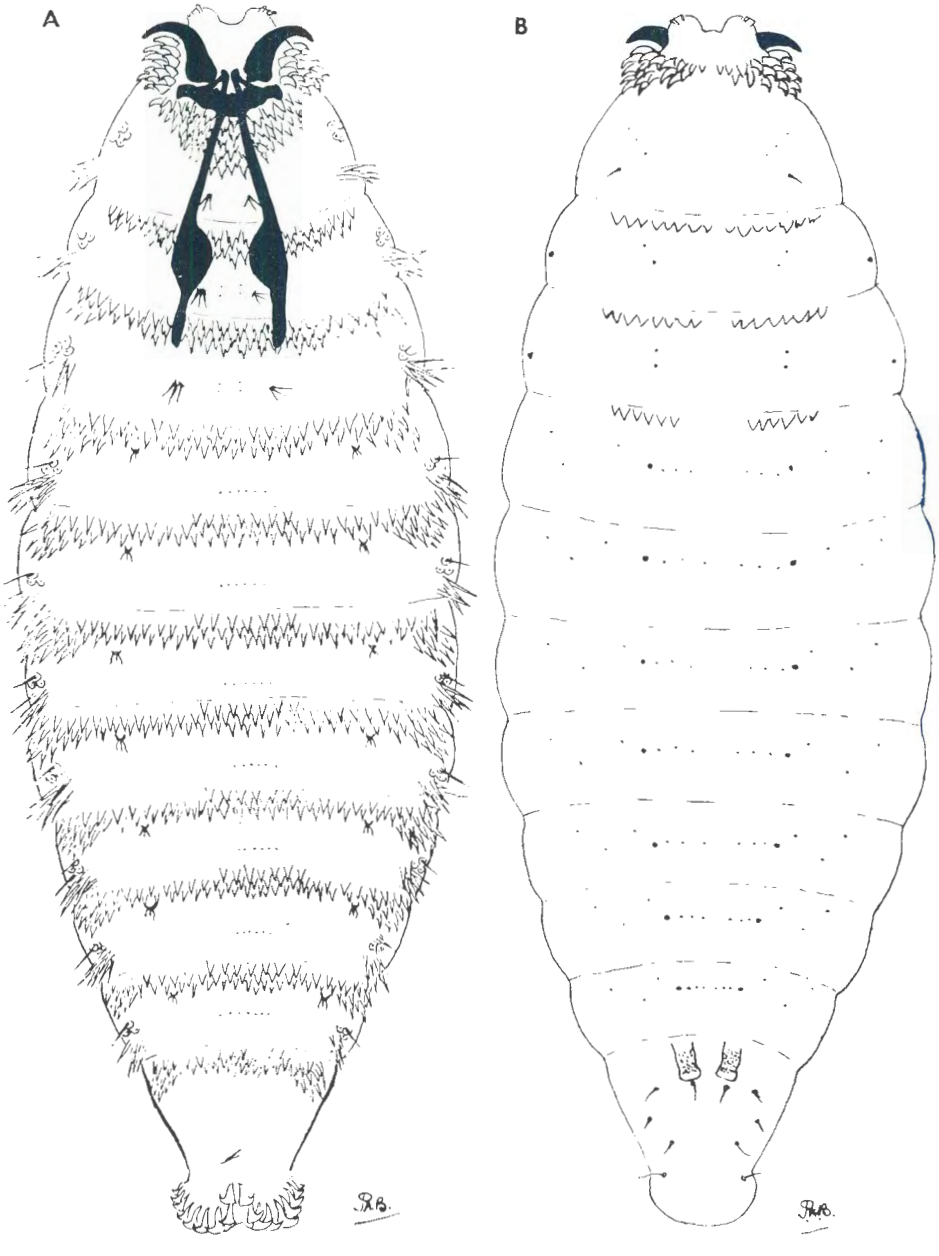

FIG. 2.— *Oestrus ovis* first stage larva (after Basson, 1962)
A. Ventral view. B. Dorsal view

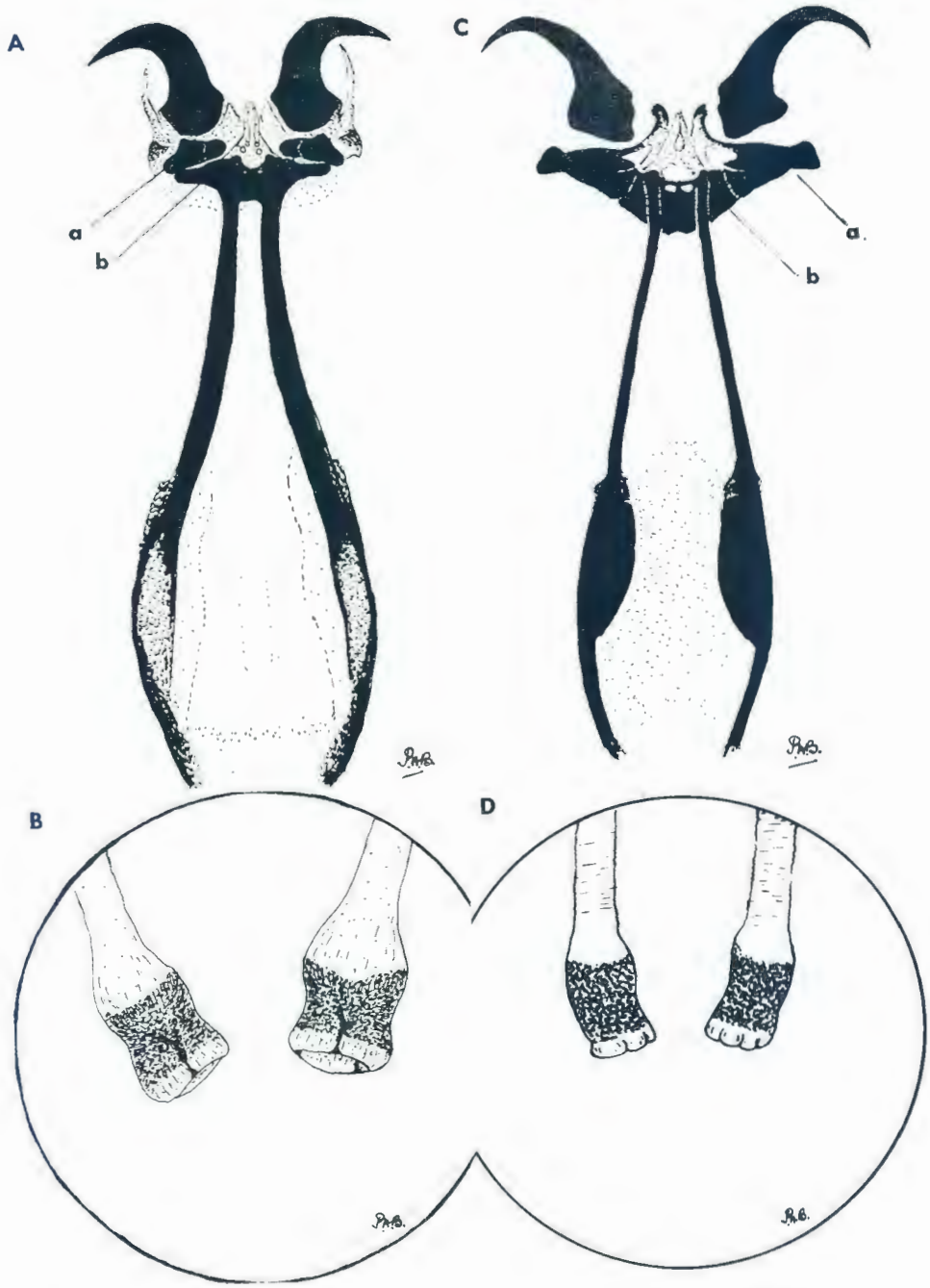

FIG. 3.—A. *O. aureoargentatus:* Cephalopharyngeal skeleton. a. Basal (ectostomal) sclerite. b. Hypostomal sclerite

B. *O. aureoargentatus:* Posterior spiracles

C. *O. ovis:* Cephalopharyngeal skeleton (after Basson, 1962). a. Basal (ectostomal) sclerite b. Hypostomal sclerite

D. *O. ovis:* Posterior spiracles (after Basson, 1962)