Sarconema eurycerca (Wehr) : the heartworm of swans and the role of *Trinoton anserinum* (F) as an intermediate host

by

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ABSTRACT

All filarial nematodes of the family Onchocercidae are parasites of vertebrates and require intermediate insect hosts for completion of their life-cycles. Eighteen genera within this family are known to infect birds but very few of the associated intermediate hosts have been elucidated. *Sarconema eurycerca* (Wehr) is a filarial nematode of swans and geese. A recent study of *S. eurycerca* in American Whistling Swans (*Cygnus c. columbianus*) has demonstrated that the intermediate insect host is a feather louse, *Trinoton anserinum* (Fabricius) (Seegar, 1977). The main aim of the present study was to investigate the relationship between *S. eurycerca* and British swans and to determine whether *T.anserinum* is the intermediate host of the parasite in this country.

A total of 1128 swans (of all species) were examined from sites in Britain, Denmark and Iceland. Infected swans were detected by examining blood samples for larval stages of *S. eurycerca* (microfilariae) using a new sedimentation technique developed in the study. An overall incidence of 15.0% was recorded with a significantly higher proportion of juvenile swans being infected. The microfilariae of *S. eurycerca* exhibited a diurnal sub-periodic rhythm within the host, with maximum counts occurring between 11.00 and 19.00 hours in the peripheral blood supply.

T. anserinum was found to satisfy all the attributes required of an intermediate insect host. As an obligate ectoparasite, *T.anserinum* has a close spatial and temporal relationship with the swan. *T. anserinum* appears to be capable of ingesting microfilariė of *S. eurycerca* whilst feeding on blood. All developing larval stages of *S. eurycerca* were found in *T. anserinum* and the louse was very mobile and capable of transmitting the nematode from one swan to another.

Examinations were made of the nematode, its morphology and pathological effects on both heart tissue and blood components of the swan. Significantly higher lymphocyte percentages and lower eosinophil percentages, haematocrit and red blood corpuscle counts were recorded in swans infected with *S. eurycerca*.

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CHAPTER I

GENERAL INTRODUCTION

Parasitism is a relationship between two species in which one, the parasite, obtains its nutritional requirements from the body of the other, the host (Baer, 1951; Olsen, 1962; Askew, 1971). Within the diversity of insect/vertebrate associations however, there exist a number of interactions which do not conform with the above definition. Insects may feed on hosts without necessarily being dependent upon them (facultative haematophagy) or insects may be dependent on hosts without causing injury (e.g. phoresy) (Waage, 1979). Parasitism is therefore sometimes applied only to haematophagous forms where a negative effect on host fitness is a reasonable assumption (Waage, 1979).

The relationship between parasite and host usually extends over most of the parasite's life-cycle and it may exhibit morphological, physiological and behavioural adaptations to this way of life. For example, the species in which the parasite reaches sexual maturity is known as the definitive host (Wakelin, 1984) although other developmental stages within the life-cycle may take place in one or a number of secondary or intermediate hosts.

For the successful completion of the life-cycle all parasites must be capable of escaping from their hosts and locating new ones to ensure survival of the species. This dispersal can be accomplished in several ways, usually involving the parasite spending some stage of its life-cycle outside the definitive host. To overcome adverse climatic conditions encountered in this transmission process, some parasites incorporate an inactive, protected resting stage in the life-cycle for example, *Ascaris* sp. and *Nematodirus* sp.. Other parasites incorporate vectors or intermediate hosts to achieve dispersal and overcome adverse environmental conditions.

An intermediate host can be differentiated from a vector by providing both a means of dispersal and an environment in which larval or juvenile stages of the parasite develop; a vector simply transmits the parasite from one host to another (Wakelin, 1984). Whichever dispersal strategy is adopted, the parasite is removed to some extent from the dangers of climatic fluctuations, but an intermediate host also assists in the dispersal of the parasite by extending the range in space as a result of the activities of both definitive and intermediate hosts.

When transfer between hosts is accomplished by active free-living larval stages, location of the next host is to a large extent dependent upon the activities of the parasites themselves. For example, eggs of *Nematodirus spathiger* , a sheep nematode, are passed out of sheep in the summer, but are unable to hatch until they have experienced long periods at cold temperatures (Crofton, 1963). The stimulus which allows *N. spathiger* larvae to emerge only under suitable conditions, ensures that the nematode is active and free-living in spring when susceptible lambs are available for infection. When resting stages are free-living but do not produce active larvae, their ingestion by the next host is entirely fortuitous. Thus, eggs of *Ascaris lumbricoides* are released into the soil in faeces and only develop further if ingested by a suitable host (Croll and Matthews, 1977). The eggs are notoriously resistant and long-lived and can remain in soil for years without development or deterioration.

The inclusion of an intermediate host or a vector in the life-cycle of a parasite appears to provide a more reliable method of dispersal. Location of the next host is not dependent on any activity on the part of the parasite but depends upon the relationship between intermediate host or vector and the definitive host.

When myxomatosis swept through the United Kingdom in 1953, the dispersal agent was regarded solely as a vector; the myxoma virus being transmitted on the mouthparts of the rabbit flea, *Spilopsyllus cuniculi* and not developing in any way whilst being conveyed.

In a major family of tissue-invading nematodes of vertebrates, the Onchocercidae, dispersal is achieved by blood-feeding intermediate arthropod hosts. Adult female nematodes liberate live larvae, microfilariae, which circulate in the blood of the definitive host or accumulate in the skin. The intermediate arthropod host takes up microfilariae when it feeds, provides an environment in which development to the infective stage can occur, and then reintroduces the parasite into the definitive host at a subsequent blood meal.

Filarial infections such as these are responsible for some of the most important parasitic diseases of man. Our knowledge of the role of insects as hosts to filarial nematodes began with Manson's observations of the infection of *Culex fatigans* by *Wuchereria bancrofti* which causes elephantiasis (Manson, 1878). Inflammation and blockage of lymph nodes as seen in elephantiasis is also characteristic of *Brugia malayi* , also

transmitted by mosquitoes (*Culex fatigans* and other species) (Laing, 1961; Jordan, 1962; Wharton, 1962).

Another filarial parasite that uses an intermediate host is *Onchocerca volvulus* . The nematodes live in skin nodules in man and sometimes enter the eye and cause blindness. *Onchocerca volvulus* microfilariae develop within and are transmitted by blackflies, *Simulium damnosum* (Blacklock, 1926).

Filarial diseases within the Onchocercidae infect other vertebrates (Nelson, 1964; Grove, 1982). The nematode heartworm *Dirofilaria immitis,* causes a pathogenic disease in dogs (Bradley, 1972). Calandruccio (1892) noted filarial larvae in the gut of a mosquito and presumed these were developing stages of *D. immitis* , but Grassi and Noé (1900) were the first to demonstrate this development experimentally. Since then, workers have shown that *D. immitis* can develop in a number of mosquito species (Kartman, 1953; Otto, 1970). *Dipetalonema reconditum* , another filarioid of dogs is non-pathogenic and is found in sub-cutaneous connective tissues. The life-cycle is similar to that of *D. immitis* except the intermediate hosts are fleas rather than mosquitoes. *D. reconditum* develops in *Ctenocephalides felis, C. canis, Xenopsylla cheopis, Pulex irritans, Echindophaga gallinacae,* and *Orchopeas wickhami* (Newton and Wright, 1956; Nelson, 1962). Stueben (1954) and Breinl (1921) report the development of *D. immitis* in fleas but have probably mis-identified the microfilariae of *D. reconditum* (Newton and Wright, 1956).

In his review of the hosts of the Onchocercidae, Nelson (1964) remarked upon the apparent predominance of mosquitoes as intermediate hosts. The reason, he explained, was undoubtedly due to intensive studies on the mosquito-transmitted human parasite *W. bancrofti* . Nelson (1964) also points out that "all too often fruitless searches have been made for mosquito vectors when the real vectors may have been the more accessible ectoparasites". In making this statement, Nelson may well have been referring to the other intermediate hosts of *D. reconditum* . Nelson (1962) discovered that the louse, *Heterodoxus spiniger* (Amblycera) also acts as an effective intermediate host for the dog filarioid. During his observations, Nelson also confirmed the original findings of Dutton (1905) that the mallophagan *Dennysus hirundis* is the intermediate host of *Filaria cypseli* of the African swift.

Eighteen genera of the family Onchocercidae are known to infect birds (Anderson, 1956) but very few of the intermediate hosts of these nematodes

have been elucidated. Dutton (1905) provided the first information on the life-cycle of an avian filarioid (*Filaria cypseli*). The adults of this nematode are located in sub-cutaneous tissues of the bird and microfilariae are ingested from lymph rather than blood when the lice feed. Developing larvae are found in the fat bodies of lice. A number of attempts have been made to determine the intermediate hosts of other avian filariae. Thomas (1931) observed one microfilaria in the gut contents of a blackfly which he presumed had fed from a domestic duck. Gönnert (1937) made unsuccessful attempts to determine the arthropod host of *Ornithofilaria mavis* in a thrush (*Turdus musicus*). Robinson (1955) fed over 1000 mosquitoes of several species on members of the crow family (*Corvidae*), on white-throated sparrows (*Zonotrichia albicollis*) and cardinals (*Richmondena cardinalis*); all birds were infected with unidentified filarioids but no development was observed in any of the mosquitoes.

The first comprehensive study of the life-cycle of an avian filarioid was performed by Anderson (1956) who demonstrate that *Ornithofilaria fallisensis* is transmitted by simulids in domestic and wild ducks. The study shows that under experimental conditions, *O. fallisensis* develops in several species of simulids. But Anderson emphasized that some caution is required in concluding that each of these species is necessarily a natural intermediate host. Special note had to be taken of the feeding habits of each species of insect and although *O. fallisensis* developed in *Simulium venustum* and *S. parnassum* in the laboratory, they are of little significance as natural hosts because both lack a large basal claw which allows ornithophilic simulids to crawl under feathers to take blood meals.

The only study of avian filarioids to follow the work of Anderson was undertaken by Seegar (1976, 1977) who demonstrated that a feather louse, *Trinoton anserinum* (Amblycera) is the intermediate host of the heartworm nematode *Sarconema eurycerca* (Wehr) of Whistling Swans (*Cygnus columbianus columbianus*) in the U.S.A. Seegar made observations on the development of infective larvae dissected from *T.anserinum* which had then been injected into non-parasitised Mute Swan cygnets (*Cygnus olor*) and reported development of heartworm infections after approximately 14 weeks. No close observations were made of the feeding habits and behavioural characteristics of *T. anserinum* and recommendations were made for further studies of feeding mechanisms, population dynamics and the development of the larval stages of *S.eurycerca* within the intermediate host.

In an attempt to substantiate and verify the role of *Trinoton* found on swans, the present thesis has three main aims:

1) to determine the incidence and infection levels of *Sarconema eurycerca* in resident Mute Swans and wintering flocks of Whooper and Bewick's Swans in Great Britain;

2) to examine the relationships between *Trinoton anserinum* (louse), *Sarconema eurycerca* (nematode) and the swan with specific reference to the role of the insect as an intermediate host;

3) to investigate the pathological effects of *S. eurycerca* on swans.

CHAPTER II

INTRODUCTION

Until the 1950s, relatively little was known about the biology of the Mute Swan. This was partly due to widespread beliefs that swans were not truly wild birds and their semi-domestic status did not justify scientific investigation (Ogilvie, 1967). Another reason for neglecting their study was the absence of a suitable swan ring for marking individuals. Before the introduction of a stronger, longer-lasting ring in 1960, fewer than 1000 Mute Swans had been marked. By 1965 however, there had been a great upsurge of interest amongst ringers and by the end of that year 14,000 swans had been ringed.

Ringing provides information about many aspects of Mute Swan biology including population dynamics, regional and local movements, breeding territories and life-history studies. Ringing recoveries often provide information about causes of death. Eltringham (1963) reports that in a sample of 400 ringed swans death was mostly due to collisions with overhead wires, parasitism (intestinal), collisions with vehicles and buildings and oil pollution. More recently, lead poisoning due to ingestion of anglers' lead weights has been found to be a major mortality factor (Ogilvie, 1981; NCC report, 1981; Birkhead, 1982, 1983).

A national investigation conducted by MAFF (NCC report, 1981) reported that 50% (N = 226) of all swans examined from England died from lead poisoning. Detailed studies highlight particular localities where the percentage of swans dying from lead poisoning is even higher than the national average; on the river Thames (between Oxford and Richmond) 75% (Birkhead, 1982), the river Trent 90% and Stratford-on-Avon area 77% (Hunt in NCC report, 1981).

Ongoing research by the Edward Grey Institute (Oxford University) monitors the blood lead levels of Mute Swans on the Thames. The most recent work by J. Sears (1986) shows the incidence of lead poisoning in this area is decreasing. Interest in lead poisoning has extended to Whooper and Bewick's Swans wintering in Britain, whose lead levels are also determined from blood samples.

Blood collection has therefore formed an integral part of swan catching in recent years. During large swan catches at Caerlaverock, Abbotsbury

and Welney for example, it has been customary to work in a team to obtain a maximum number of blood samples. The blood is then portioned for a variety of analyses including the detection of lead and *S. eurycerca* microfilariae.

In this chapter, the relationship between the nematode *S. eurycerca* and the swan is investigated by studying:

1) the distribution of swans and methods of swan capture;

2) the diagnosis and incidence of *S. eurycerca* in swans;

3) the seasonal and daily periodicity of *S. eurycerca* microfilariae.

1. DISTRIBUTION OF SWANS AND METHODS OF CAPTURE

The British Isles and countries of north-west Europe are the winter location of large numbers of wildfowl. Northern latitudes are suitable breeding sites in the summer but become cold and inhospitable to birds in winter. Consequently, many wildfowl are forced to migrate to warmer climes for the winter months.

Mute Swans have the most southerly breeding range of all the Eurasian swans, and unlike the Bewick's and Whooper Swans, many inhabit areas which are mild enough in winter for them to remain resident. Within Britain, Mute Swans tend to breed in the same general natal area. Indeed, permanent movements of more than 50 km are not common. Seasonal movements of Mute Swans are usually related to food supply, severe weather or searches for new territories.

Whooper Swans, whose breeding territories are in Iceland, migrate to Scotland and northern England for winter. Many are seen each year at the Caerlaverock Wildfowl Trust Nature Reserve near Dumfries.

Bewick's Swans breed around the Kara Sea in northern Russia and fly 2,300 miles (3,700 km) to and from their wintering sites in southern England and Ireland. Some 5000 - 5,500 Bewick's Swans winter in Britain each year; in particular, large numbers (up to 2000) collect on the Ouse Washes near the Wildfowl Trust Refuge at Welney (Birkhead and Perrins, 1986).

MATERIALS AND METHODS

1.1. Methods of Swan Capture

Swans were captured by one of three methods : by baiting, by canoe round-ups or in swan pipes.

Mute Swans on urban lakes and rivers are semi-domesticated and survive to a large extent on food provided by the public. When food is offered, swans usually come close enough to be captured straight from the water. A swan hook is used for those birds which are more cautious.

Each summer, swans moult all their flight feathers and for a few weeks are unable to fly. Ringers and researchers take advantage of this flightless

FIGURE 1 : Capturing and processing swans.

A. Swan pipe where migrant and resident swans are trapped at Wildfowl Trust Reserve, Caerlaverock, Dumfries. The two gaps in the wooden fence usually remain open and swans come to feed within the area (swan pipe). Once a year, the pipe is enclosed and swans feeding inside are trapped.

B. Pens at the end of the swan pipe from where the birds are caught individually with swan hooks.

C. Whooper Swans at the blood collecting field station.

D. Blood sample taken from the tarsal vein (leg).


A


B


C


D

period and use canoes to round-up whole flocks of Mute Swans. Swans were caught in canoe round-ups at Alvecote, Abbotsbury and in Leicester.

Swan pipes have been constructed by the Wildfowl Trust to trap large numbers of migratory and resident swans (Fig.1A). Such pipes are employed at Caerlaverock and Welney to capture Bewick's and Whooper Swans. Swans are encouraged to feed from grain provided in the swan pipe every day. Once or twice a year the gates at either end of the pipe are closed and the swans are channelled into pens from where they are captured and examined (Fig. 1B).

1.2. Captive Swans

Within the Wildfowl Trust collections there are representatives of all eight species of swans. All species were examined at Peakirk and Washington.

Increasing concern over the incidence of lead poisoning in swans has prompted many people in Britain to establish swan rescue centres. Three prominent rescue services are based in Norwich, Cheltenham and Windsor. Veterinary and RSPCA centres also care for swans, and with the cooperation of staff it was possible to obtain samples from many sick and injured Mute Swans.

Five Mute Swans were maintained in captivity at a field station within easy access of the laboratory. The enclosure was an area of 5 m x 15 m secured on three sides by chicken wire. Wooden boards protected the remaining side from prevailing winds. A pool (2.5 m x 1.5 m x 0.7 m) was located at one end of the enclosure. Swans were fed on wheat mixed with a balanced vitamin complex (Vitalin). Bread and fresh green vegetables supplemented the basic foodstuffs.

1.3. Processing captured swans

The ring number of each captured Mute Swan was noted, or if unringed a metal BTO ring was placed on the leg. In addition to a metal ring, a conspicuous plastic Darvic ring was placed on Whooper and Bewick's Swans by the Wildfowl Trust. This enables individual swans to be identified by field inspection.

A timed blood sample (3.0 cm^3) was taken from the tarsal vein in a 5.0 cm^3 heparinised syringe (Sarstedt-Monovette lithium-heparin coated) (Fig.1D). Each swan was weighed by being restrained with bandages and suspended from a spring balance.

As far as possible the sex of each swan was determined. Mute Swans were distinguished when there were obvious differences in the appearance of the head. Usually the male and female of a pair are comparable in this way. Females tend to have smaller heads than males, and the male has a large, black fleshy knob at the base of the bill. Whooper and Bewick's Swans were sexed by cloacal examination.

Swans were aged on their plumage characteristics. Adults are all white with a full coloured bill. Cygnets (first winter birds) were identified by their grey plumage and dull coloured bill. Juveniles were immature birds with white plumage and pale orange bills.*

*For the purpose of this study both cygnets and juvenile swans are referred to collectively as "juveniles".

RESULTS

During the course of this investigation, 1128 swans were processed. From the total swans sampled, 71.5% were adults, 27.0% juveniles and 1.5% were of unknown age. (Fig.2B). The proportion of female to male swans caught was almost equal : 40.3% were female, 42.5% male and for 17.2% was not possible to determine the sex (Fig.2C).

Three main species were examined: Mute Swans, constituting 63.6% of the total number caught. Whooper Swans (31.1%) and Bewick's Swans (3.1%). The remaining 2.2% were other species processed from Wildfowl Trust collections. (Fig.2A).

Mute Swans (N = 718) were captured at 50 sites in Great Britain, including four Wildfowl Trust reserves and six rescue centres (N = 4) (Fig. 3).

Mute Swans (N = 149) were also captured in Denmark (1985).

A total of 351 Whooper Swans were caught; they were sampled in Caerlaverock (N = 219), in Iceland (N = 123) and from the Wildfowl Trust collections at Peakirk (N = 5) and Washington (N = 4) (Fig. 3).

Thirty-five Bewick's Swans were trapped, mostly from Welney (N = 25), and others from Caerlaverock (N = 6), Washington (N = 2), Peakirk (N = 1) and Iceland (N = 1).

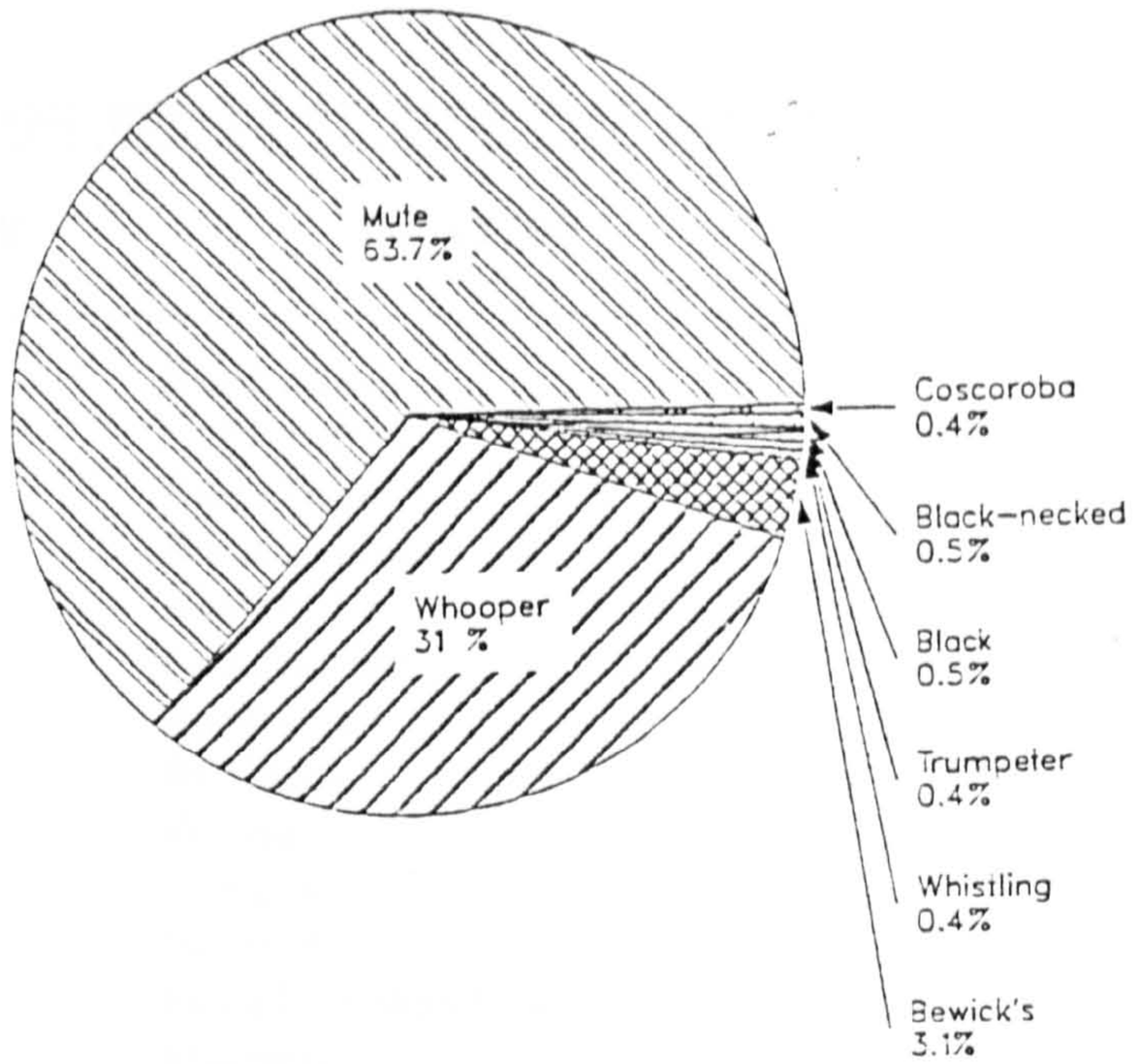
Table 1 summarises the locations and numbers of each species of swan examined in this study.

FIGURE 2 : Proportions of each species, age and sex of swans out of the total number trapped.

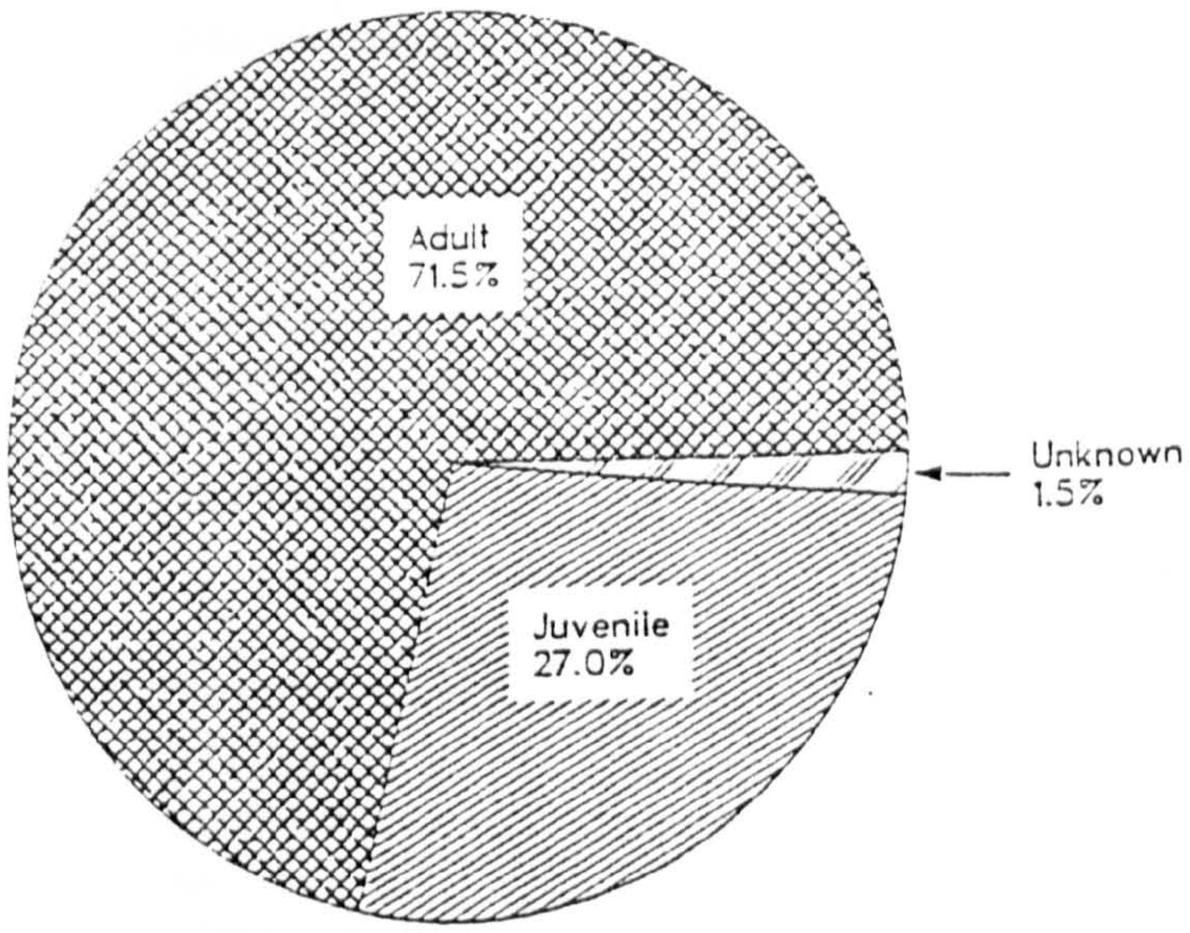
A. Proportion of each swan species trapped during the course of the investigation (Total number of swans trapped = 1128).

B. Proportion of adult and juvenile swans trapped (Percentage of juvenile swans includes cygnets)

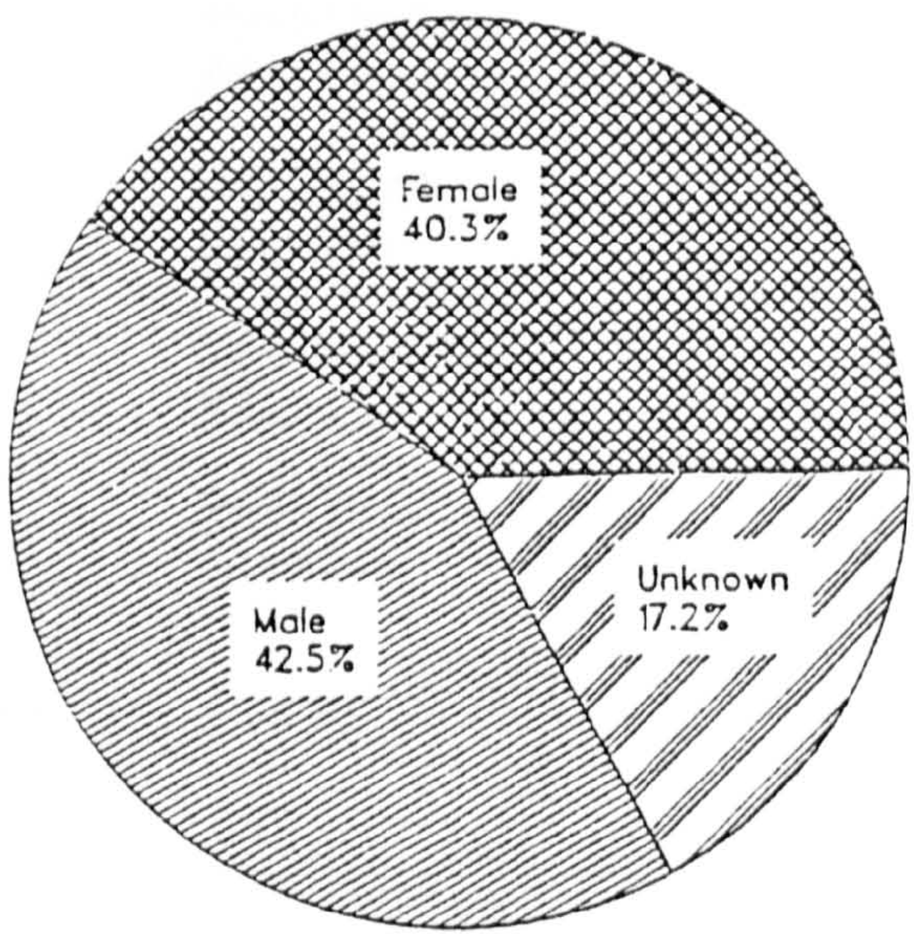
C. Proportion of male and female swans trapped.


Mute
63.7%
Whooper
31 %
Coscoroba
0.4%
Black–necked
0.5%
Black
0.5%
Trumpeter
0.4%
Whistling
0.4%
Bewick's
3.1%

A


Adult
71.5%
Juvenile
27.0%
Unknown
1.5%

B


Female
40.3%
Male
42.5%
Unknown
17.2%

C

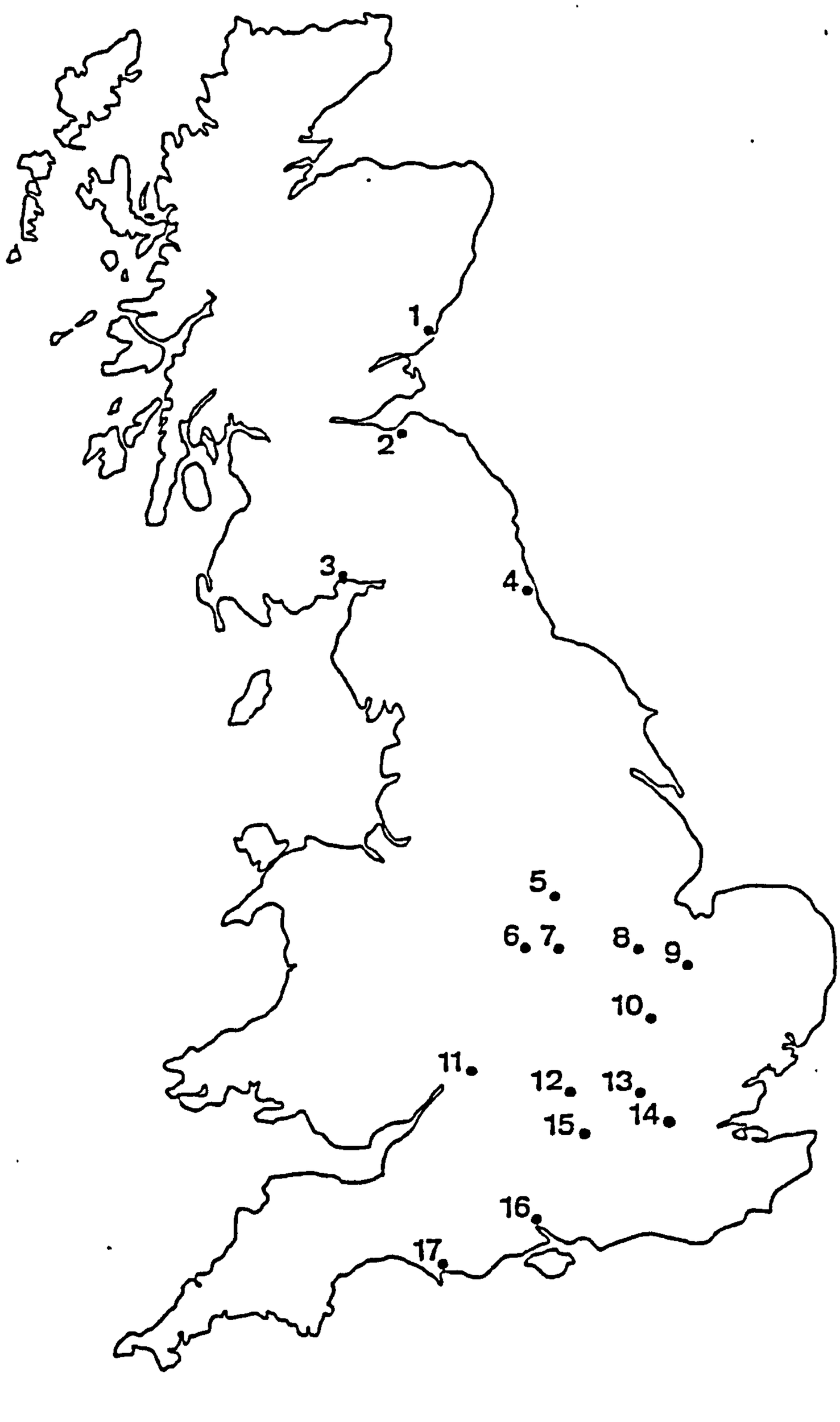
TABLE 1: LOCATION AND NUMBER OF EACH SWAN SPECIES CAPTURED

SPECIES	LOCATION CODE	LOCATION	NUMBER
MUTE	1.	Montrose	81
	2.	Lothians	18
	3.	Caerlaverock	77
	5.	Nottingham	5
	6.	Alvecote	149
	7.	Leicester	51
	8.	Peakirk	2
	9.	St Neots	1
	10.	Welney	31
	11.	Cheltenham	20
	12.	Oxford	41
	13.	Hemel Hempstead	1
	14.	Thames	25
	15.	Reading	28
	16.	Lymington	1
	17.	Abbotsbury	38
		Denmark	149
			718
WHOOPERS	3.	Caerlaverock	219
	4.	Washington	4
	8.	Peakirk	5
		Iceland	123
			351
BEWICK'S	3.	Caerlaverock	6
	4.	Washington	2
	8.	Peakirk	1
	10.	Welney	25
		Iceland	1
			35
WHISTLING	4.	Washington	2
	8.	Peakirk	2
TRUMPETER	4.	Washington	2
	8.	Peakirk	2
BLACK	4.	Washington	4
	8.	Peakirk	2
BLACK-NECKED	4.	Washington	4
	8.	Peakirk	2
COSCOROBA	4.	Washington	2
	8.	Peakirk	2
			24
GRAND TOTAL			1128

FIGURE 3 : KEY TO LOCATIONS OF SWAN CAPTURE IN GREAT BRITAIN

1. Montrose
2. Lothians region*
3. Caerlaverock (Wildfowl Trust reserve), Dumfries
4. Washington (Wildfowl Trust collection), Tyne and Wear
5. Nottingham region*
6. Alvecote, Staffs.
7. Leicester region*
8. Peakirk (Wildfowl Trust collection), Cambs.
9. St Neots, Cambs.
10. Welney (Wildfowl Trust reserve), Cambs.
11. Wildlife Hospital, Cheltenham, Glos.
12. Oxford region*
13. Hemel Hempstead
14. Thames region*
15. Reading region*
16. Lymington, Southampton
17. Abbotsbury, Dorset

(* swans were caught at a number of sites within the region)


1
2
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17

2. DIAGNOSIS, DISTRIBUTION AND INCIDENCE OF *S. eurycerca* IN SWANS

To date, most studies of *S. eurycerca* have presented results of post-mortem examinations by reporting the presence of adult nematodes in Whistling Swans. The most comprehensive study of *S. eurycerca* in live swans was performed by Seegar (1977) who surveyed 795 Whistling Swans in USA and Alaska to determine the incidence of microfilariae in blood samples.

There is no national record of the distribution and incidence of either adults or microfilariae of *S. eurycerca* in swans in Britain. Until the present study, Mute Swans have been surveyed at only three sites in Britain, where the overall incidence of the nematode was 17.1% (Seegar, 1979a). There is one report in the literature of a Mute Swan from the Thames with adult nematodes in the heart (Boughton, 1965).

In this section, the techniques available for detecting filariae and their applicability in this type of study are examined. One technique is used to determine the incidence of *S. eurycerca* in British Mute Swans and in migrant Whooper and Bewick's Swans wintering in Britain.

MATERIALS AND METHODS

2.1. Laboratory diagnosis of filariasis

Filariasis is diagnosed by identifying microfilariae in blood samples. Many methods have been developed for this purpose. There are three essential requirements of a method for diagnosing *S. eurycerca* in swan blood.

The method has to be:

1) quantitative;
2) applicable to both fresh and preserved blood;
3) inexpensive for routine use.

Eight widely used tests and one new technique were assessed as to their suitability for diagnosing the presence of *S. eurycerca*.

2.1.1. Established diagnostic techniques for filariasis

At the simplest level, a direct wet blood smear can detect microfilariae. A thick mount of fresh whole blood was placed on a glass slide and examined under the low power (x10) magnification. Microfilariae were detected by their active wriggling motion (Bradley, 1972).

A thin smear was prepared by placing a drop of blood (0.02 cm^3) at one end of a slide and by using another slide, the blood is spread out in a thin layer. Microfilariae were stained using May-Grünwald and Giemsa (Appendix 1).

Counting chambers have been employed to diagnose infected samples. Blood (0.02 cm^3) was placed into the chamber of a Sedgwick-Rafter counting cell. The cell was designed for enumerating organisms in water and consists of a slide with a depression 0.1 cm deep and 2 x 5 cm in area; it holds 1.0 cm^3 of fluid. Hydrochloric acid (1.0 cm^3 0.1 M) was added, the suspension stirred with a dissecting needle and a coverslip applied. Microfilariae settled rapidly to the bottom of the chamber (Brady and Lawton, 1944). Denham (1971) modified this method for use with live microfilariae by excluding the addition of acid to the counting cell.

The most accurate techniques for detecting microfilariae are those which concentrate the larvae into small volumes. One method which uses this principle is the microcapillary tube technique (Bennett, 1962). Anticoagulated blood collected in a heparinised syringe was centrifuged (1500 rpm) for 5 minutes in a microcapillary tube (0.075 cm^3) sealed at one end with putty. The plasma was examined with the microscope (x10). Live microfilariae were visible just above the white blood cell fraction. Five or six capillary tubes were examined together by fixing them at one end of a glass slide in a piece of plasticine (Schalm and Jain, 1966).

The Knott test also concentrates larvae in a small volume of stained blood. A blood sample (1.0 cm^3) was placed in a tube with 2% formalin (9.0 cm^3) and thoroughly mixed. The tube was centrifuged for 5 minutes at 1500 rpm. The supernatant was discarded and the sediment resuspended with several drops of 0.1% methylene blue. The stained sediment was placed on several glass slides and examined (Knott, 1939).

In the petri-dish technique (Obeck, 1973), blood (3.0 cm^3) was collected and deposited in the bottom of a sterile plastic petri dish (15 x 150 mm diameter). The blood was spread over two-thirds of the dish. After the initial clot had formed, the plate was stored at a slight angle overnight, with the bloodless third of the plate in the lowest position. Twelve hours later the serum which had collected in the clear portion of the dish, was drawn off with a syringe and needle. Pooled serum samples were then placed in tubes (50.0 cm^3) and centrifuged at 200 g for 10 minutes. After discarding the supernatant, the sediment containing microfilariae was thoroughly mixed and washed twice with distilled water. The final sediment was washed twice with saline. Obeck (1973) found it was possible to recover 50-85% of microfilariae from blood by this method.

The Millipore membrane filter technique, based on the method described by Bell (1967), has been further developed by Desowitz and Southgate (1973). A blood sample (1.0 cm^3) was collected in a syringe containing sodium citrate solution (2.0 cm^3). Teepol (10%, 9.0 cm^3) in normal saline was drawn into the same syringe and rotated or shaken until the blood was completely haemolysed. The needle was removed and replaced by a Swinnex filter holder containing Millipore membrane (25 mm diam., 5 μm porosity). Haemolysed blood was passed through the filter by a steady pressure on the syringe plunger. Normal saline (5.0 cm^3) was passed through the syringe to wash the membrane, followed by formal saline (10.0 cm^3) to fix any microfilariae collected on the filter. The membrane was washed twice with distilled water (10.0 cm^3). The Millipore membrane was removed and stained with Giemsa or Haemalum. It was rinsed and allowed to dry before being placed on a microscope slide and scanned under a low power objective. This technique requires considerable technical time and expensive, expendable supplies.

The anion-exchange centrifugation technique was originally devised by Lanham (1968) to isolate trypanosomes from the blood of rats and mice and was later applied to trypanosomes in man and other mammals (Lanham and Godfrey, 1970). The separation of parasites from blood cells and platelets depends fundamentally on differences in surface charge. The anion-exchanger consisting of a DEAE-cellulose column absorbs more negatively charged blood components whilst less negatively charged flagellates are eluted. The optimal ionic strength of the phosphate-saline-glucose (PSG) buffer used to elute the parasites, varies for different species (Lanham,

1985). The technique was modified and the apparatus miniaturised for use in the field to detect low trypanosomaemias in man (Lumsden *et al*, 1979). During these field surveys it was found that microfilariae of *Dipetalonema perstans* also passed through the anion-exchange column (Lumsden *et al*, 1980). An attempt was made to isolate *S, eurycerca* from swan blood using this technique. A range of buffer concentrations was used to find the optimal ionic strength for eluting *S. eurycerca* (ratio PO4 : glucose; 5:5, 4:6, 3:7, 2:8, 1:9). The eluate from each buffer concentration was centrifuged at 700 g for 10 minutes and examined for microfilariae. However, no microfilariae were extracted by this method.

2.1.2. Sedimentation technique for the diagnosis and enumeration of *S. eurycerca* in swan blood

This technique was originally devised by Lund *et al* (1958) for counting freshwater algae. The apparatus consisted of a cylindrical chamber (50.0 cm^3) and a separate base into which material sedimented. The chamber was placed onto the base and filled with hydrochloric acid (50.0 cm^3, 0.1 M) to lyse the red blood cells. Blood (*) was mixed into the acid using pre-calibrated disposable glass pipettes. The chamber was then filled with acid and sealed with a cover slip. The chamber was left for 24 hours to ensure sedimentation of all microfilariae.

In an infected sample, microfilariae were deposited on the bottom of the base. To examine sedimented material, the chamber was removed from the base releasing extraneous liquid. Microfilariae were detected and counted on an invertescope at x100 (IM35 Zeiss).

(* Trial and error suggested an optimal volume of blood to sediment was 2.5 cm^3 of preserved blood and 0.1 cm^3 of fresh blood. These dilutions ensured that whether preserved or fresh blood was sedimented, all microfilariae were visible amongst the sedimented debris. Preserved blood was collected as 1.0 cm^3 fresh blood in 9.0 cm^3. 2% formalin solution (a ratio of 1:9). Therefore, 2.5 cm^3 of preserved blood would contain 0.25 cm^3 fresh blood and 2.25 cm^3 2% formalin according to the original ratio of 1:9. It was necessary when counting microfilariae from 0.1 cm^3 of fresh sedimented blood that a correction factor of 2.5 was applied to make the count up to 0.25cm^3 and therefore comparable with counts made from preserved blood.)

2.1.2.1 Efficiency of sedimentation technique when compared with three established methods for diagnosing filariasis

As a new technique for estimating the number of microfilariae in blood, the efficiency of the sedimentation method was compared with three established methods. The capillary tube, the wet mount and thin smear tests were chosen because they were quick and easy to perform on large numbers of blood samples.

In February 1985, 88 Whooper Swans and 6 Bewick's Swans were captured at the Wildfowl Trust reserve at Caerlaverock, Dumfries. Between 08.00 and 13.00 blood samples were taken from the tarsal vein in heparinised syringes and kept in a cool box until they were analysed. Capillary tube, wet mount and thin smear tests were all completed within 12 hours of blood collection. Blood (1.0 cm^3) was removed from each sample tube and preserved in 2% formalin (9.0 cm^3) until examination by the sedimentation method.

2.1.2.2 Preservation of blood

When more than 20 blood samples were collected at one time, it was impossible to analyse them all as fresh samples. Therefore, some samples had to be preserved in formalin for periods up to three months. It was essential to determine the preserving effects of formalin on microfilariae and to ensure that the levels of infection in each sample were not significantly altered by preservation.

Eight samples were quantified as fresh blood by sedimentation. Each sample was then preserved by diluting fresh blood (1.0 cm^3) in formalin 2% (9.0 cm^3). Samples were preserved for 20 and 90 days at 7 - 12°C. After each period of preservation the level of infection was determined.

2.1.2.3 Estimation of sample variation in sedimentation technique

All methods of estimating the abundance of organisms are subject to chance sampling errors. The errors involved in estimating microfilariae by sedimentation were investigated.

Two potential sources of variation exist: errors arising from counting microfilariae by the operator (operator error) and, errors due to variations in sub-samples counts (that is, different volumes of blood taken from the original sample [sub-sample error].

Ten blood samples were examined and the errors determined by:

1) operator error - five counts of the same sub-sample;

2) sub-sample error - five sub-samples were counted from one sample.

2.2 Distribution and incidence of *S. eurycerca* in swans

Blood samples were taken from eight species of swans in Britain, Iceland and Denmark to determine the distribution of *S. eurycerca*. Geographic locations of capture sites are described in Section 1. Each blood sample was examined and the incidence of *S. eurycerca* assessed using the sedimentation technique (Section 2.1.2).

RESULTS

2.3 Assessment of tests for diagnosis of *S. eurycerca*

Three attributes were required of a technique for detecting *S. eurycerca* microfilariae (p.15). Each test was assessed in terms of these attributes (Table 2).

TABLE 2 : SUITABILITY OF TESTS FOR DETECTING *S. eurycerca* MICROFILARIAE

TEST	ATTRIBUTES OF TEST		
	Quantitative	Applicable to fresh and preserved blood	Inexpensive
Wet mount	✓	X	✓
Thin smear	✓	X	✓
Sedgwick Rafter	✓	X	✓
Capillary tube	✓	X	✓
Knott test	X	✓	✓
Petri dish	✓	X	✓
Millipore membrane	✓	✓	X
Anion exchange	✓	X	X
Sedimentation	✓	✓	✓

None of the eight established techniques had all the attributes required of a test. The sedimentation technique, however, did exhibit all the requirements and was therefore adopted for use in this study.

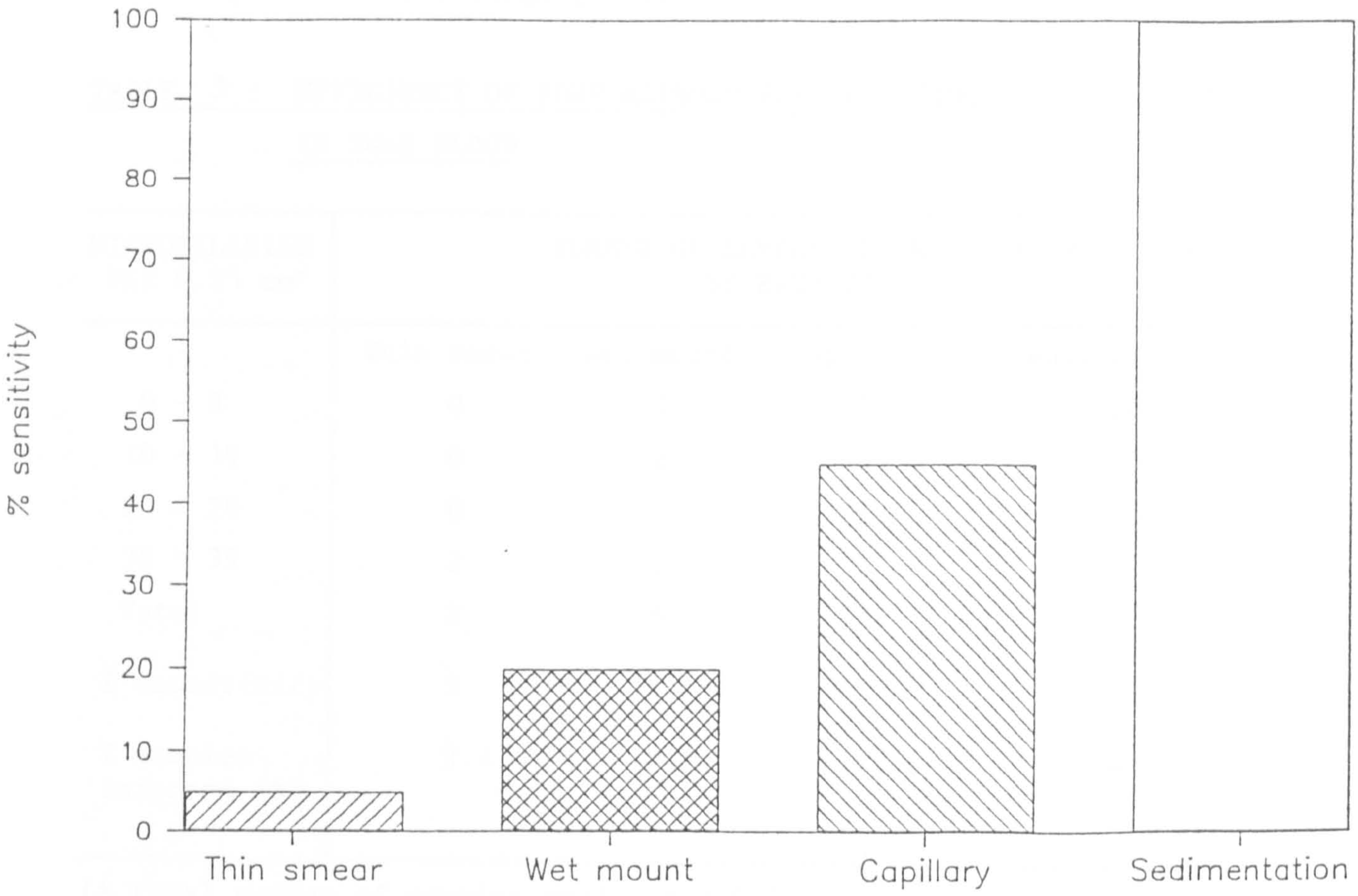
2.4 Sedimentation technique - efficiency compared with three established methods

Table 3 is presented in the same format as that adopted by Seegar (1979b). A comparison of the results of all four methods shows that the sedimentation technique is the most sensitive in detecting microfilariae in swan blood.

The thin smear test detected 5.0% of samples determined to be positive for microfilariae by sedimentation. The wet mount test detected 20.0% and the capillary tube test 45.0%. The sedimentation technique was taken as detecting 100%. (Fig.4)

FIGURE 4 : Comparison of the efficiency of the sedimentation technique with three established methods of detecting *S. eurycerca* microfilariae.

(The sedimentation technique is taken as detecting 100% of infected samples)


% sensitivity
100
90
80
70
60
50
40
30
20
10
0
Thin smear
Wet mount
Capillary
Sedimentation

The sedimentation technique estimated microfilariae per 0.25 cm^3 of preserved blood. The sensitivity of the capillary tube and wet mount tests decreased when the level of microfilarial infection was less than 30 per 0.25 cm^3. Below this level, 27 known positive samples, as detected by sedimentation, were recorded as negative by the capillary test and 37 were recorded as negative by the wet mount test. Overall the new technique detected 60% more infected swans of which 72% had microfilaremias below 10 per 0.25 cm^3.

The mean microfilarial infection in the swans that were sampled (N = 94) was 8.0 per 0.25 cm^3, ranging from 1 to 46 per 0.25 cm^3.

TABLE 3 : EFFICIENCY OF FOUR METHODS FOR DETECTING *S.eurycerca* MICROFILARIAE IN SWAN BLOOD

MICROFILARIAE PER 0.25 cm^3	NUMBER OF SAMPLES DETECTED AS POSITIVE BY EACH TEST			
	Thin smear	Wet mount	Capillary	Sedimentation
0 - 9	0	1	7	26
10 - 19	0	2	6	8
20 - 29	0	2	2	3
30 - 39	2	3	3	3
Total	2	8	18	40
% sensitivity	5	20	45	100
% samples infected (*)	2.1	8.5	19.1	42.5

(* total number of samples analysed = 94)

2.4.1 Effects of preservation of blood

Three counts of microfilariae were obtained for each of eight samples (Table 4) :

1) number in fresh blood (per 0.1 cm^3 x 2.5 correction factor);
2) number in blood preserved for 20 days (per 0.25 cm^3);
3) number in blood preserved for 90 days (per 0.25 cm^3).

For each of the samples, counts from fresh and preserved blood were compared statistically to test for any effects of the preservation process (Table 4).

One of the samples (YSH) had significantly more microfilariae in fresh blood than in preserved blood. The other seven samples showed no significant differences.

2.4.2 Estimation of sample variation in sedimentation technique

1. Operator error

   For each of ten samples, five counts were made of one sedimented sub-sample. Counts of microfilariae were exactly the same for each of five counts in each sample (Table 5). There was no error due to the operator during counting and the risk of this is therefore ignored in further work.

2. Sub-sample error

   Variations in sub-sample counts were observed and the chi-square test was applied to determine if the differences in counts were significant (Table 5). None of the sets of counts was significantly different. There was no significant error due to sub-sampling.

TABLE 4 : Counts of microfilariae to determine the effects of preservation in formalin (2%)

SAMPLE	COUNTS OF MICROFILARIAE				
	Fresh blood	Preserved for 20 days	Preserved for 90 days	X^2	
229	13	12	14	0.15	ns
YSH	23	5	5	19.64	p <0.01
YRL	30	24	28	0.68	ns
SID	33	34	41	1.05	ns
707	25	20	20	0.77	ns
707	38	35	33	0.36	ns
COL	30	39	34	1.19	ns
707	38	39	43	0.35	ns

TABLE 5 : Variation in microfilariae counts using the sedimentation method

BLOOD SAMPLE	OPERATOR ERROR (N* = 5)	SUB-SAMPLE ERROR (N = 5)						
1	0	57	37	49	60	43	7.41	ns**
2	0	5	6	3	4	4	1.1	ns
3	0	23	16	27	17	16	4.99	ns
4	0	20	22	13	7	17	9.03	ns
5	0	3	6	6	1	4	4.5	ns
6	0	13	16	25	20	19	4.36	ns
7	0	29	18	17	17	-	5.07	ns
8	0	22	32	33	23	33	4.38	ns
9	0	4	8	4	3	-	3.10	ns
10	0	12	12	15	13	-	0.46	ns

(*N = number of counts made; ns** not significant)

2.5 Distribution and incidence of *S. eurycerca* in swans

A total of 1128 blood samples were examined for *S.eurycerca* of which 169 (15.0%) were infected (Table 6).

The geographical distribution of infected swans is shown in Fig. 5. No infection was found in swans from the Thames, Oxford, Southampton, Hemel Hempstead and St. Neots. Collections of swans from the Wildfowl Trust Centre in Washington were also not infected. None of the 'collection' swans at Peakirk were infected, but one wild Mute Swan from that area was infected and is therefore indicated on the map.

Incidences of *S. eurycerca* are higher than the overall incidence (15.0%) in Abbotsbury (42%), Cheltenham (33.3%), Nottingham (40%), Lothians (39%), Caerlaverock (26%), and Iceland (24%). The incidence of *S.eurycerca* in the Nottingham region of 40% is perhaps slightly misleading since only five swans were sampled from this area (Table 6).

Of all the swans with *S. eurycerca*, 77 were Mute Swans, 88 were Whoopers and 4 were Bewick's. None of the five other species kept in collections were infected (Table 7).

TABLE 6 : Location and incidence of *S. eurycerca* in swans

LOCATION	STATUS*	No.SWANS SAMPLED	SPECIES**	No.SWANS WITH *S. eurycerca*	% SWANS WITH *S.eurycerca*
1. Montrose	MF	81	M	2	2.5
2. Lothians		18	M	7	39.0
3. Caerlaverock (Total)	WF	302	MWB	79	26.0
		77	M	18	23.4
		219	W	58	26.5
		6	B	1	16.6
4. Washington	COLL.	20	ALL SP.	0	0.0
5. Nottingham	P	5	M	2	40.0
6. Alvecote	MF	149	M	15	10.0
7. Leicester	P,IF	51	M	3	5.9
8. Peakirk	COLL	18	ALL SP.	1	5.5
9. St. Neots	P	1	M	0	0.0
10. Welney (Total)	WF	56	M B	3	5.3
		31	M	2	6.4
		25	B	1	4.0
11. Cheltenham	COLL	20	M	8	33.3
12. Oxford		41	M	0	0.0
13. Hemel H.	P	1	M	0	0.0
14. Thames		25	M	0	0.0
15. Reading		28	M	1	3.6
16. Lymington	P	1	M	0	0.0
17. Abbotsbury	C	38	M	16	42.0
Iceland		124	W	30	24.0
Denmark		149	M	2	1.3
Total		1128		169	15.0%

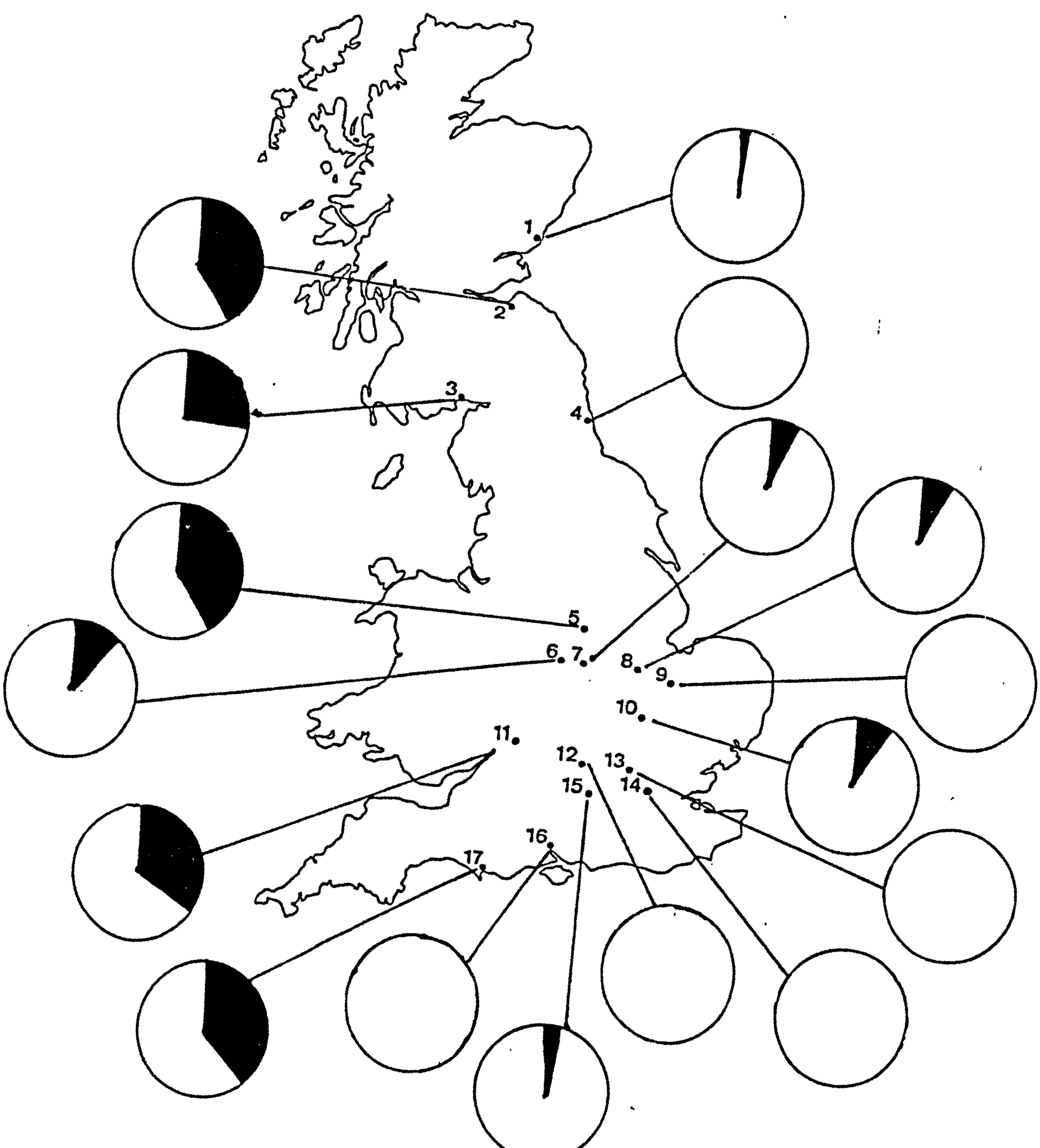
KEY:

*STATUS
- MF Moulting flock
- WF Wintering flock
- COLL Collection
- P Pair
- IF Immature flock
- C Colony

**SPECIES
- M Mute Swan
- W Whooper Swan
- B Bewick's Swan
- ALL SP. All species of swans

FIGURE 5 : Geographical distribution and incidence of *S. eurycerca* microfilaria in swans.

Information is given in Table 6 (p.26) about swans trapped at each location, the species, status and incidence of *S. eurycerca*.


1
2
3
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17

A significantly higher proportion of juvenile swans (20.3%) were infected than adults (12.9%) (X^2 = 9.59 $p<0.01$) Table 7. Almost equal proportions of female (15.8%) and male (15.9%) swans were infected. Overall, the mean number of microfilariae per 0.25 cm^3 was 8.44 (N = 147) with a range of 1-46 mf per 0.25 cm^3. (Table 8)

2.5.1 Mute Swans

In a sample of 718 Mute Swans, 77 (10.7%) were infected. A higher proportion of juveniles (15.8%) were infected than adults (8.4%). Almost equal proportions of females (10.6%) were infected as males (10.8%). The mean infection level per bird was 9.52 mf per 0.25 cm^3 with a range from 1-42 (N = 67). (Tables 8 & 9)

2.5.2 Whooper Swans

Of 351 Whooper Swans, 88 (25.1%) were infected. A higher proportion of juveniles (36.7%) were infected than adults (22.1%). Slightly more females (26.5%) were infected than males (24.4%). The mean infection level per swan was 7.59 mf per 0.25 cm^3 with a range from 1-46 (N = 77). (Tables 8 & 10)

2.5.3 Bewick's Swans

Of 35 Bewick's Swans, 4 (11.4%) were infected. One swan was an adult and two were juveniles; one was of unknown age. One infected swan was a female and two were males; one was of unknown sex. (Tables 8 & 11)

Even though the sample size for this species was relatively small, proportionally more juveniles (4.0%) were infected than adults (3.4%). The mean level of infection was 6.00 mf per 0.25 cm^3 and the range was from 3-10 mf per bird.

2.6 Level of infection of *S. eurycerca*

The levels of infection of microfilariae of *S. eurycerca* were analysed in groups of swans. The groups were divided according to age, sex and species. The sample of Bewick's Swans was too small to analyse statistically (Table 8). The mean number of microfilariae from each group were analysed statistically (t-test) to determine if there were significant differences between the levels of infection in each of the groups. No significant differences were found. The highest infection levels were 46 mf per 0.25 cm^3.

TABLE 7 : Incidence of *S. eurycerca* in swans of different species, age and sex

	SPECIES			AGE		SEX	
	Mute	Whooper	Bewick's	Adults	Juveniles	Male	Female
Number infected	77	88	4	104	62	72	76
% *	10.7	25.1	11.4	12.9	20.3	15.8	15.9

(* Percentages calculated in relation to total number of swans caught in each category)

TABLE 8 : Levels of infection of *S. eurycerca* in swan species

SWAN SPECIES	N	N*	NO. OF MICROFILARIAE PER 0.25 cm^3 BLOOD		
			Mean	SD**	Range
Mute	67	10	9.52	10.85	1 - 42
Whooper	77	11	7.6	8.7	1 - 46
Bewick's	3	1	6.0	3.6	3 - 10
All Species	147	22	8.44	9.69	1 - 46

(N* = missing data; SD** = standard deviation)

TABLE 9 : Incidence of *S. eurycerca* in Mute Swans

	AGE		SEX	
	ADULT	JUVENILE	FEMALE	MALE
NO. OF INFECTED SWANS	39	38	28	29
% OF INFECTED SWANS	8.4%	15.8%	10.6%	10.8%

TABLE 10 : Incidence of *S. eurycerca* in Whooper Swans

	AGE		SEX	
	ADULT	JUVENILE	FEMALE	MALE
NO. OF INFECTED SWANS	64	22	43	45
% OF INFECTED SWANS	22.14%	36.7%	26.5%	24.4%

TABLE 11: Incidence of *S. eurycerca* in Bewick's Swans

	AGE		SEX	
	ADULT	JUVENILE	FEMALE	MALE
NO. OF INFECTED SWANS	1	2	1	2
% OF INFECTED SWANS	3.4%	4.0%	5.5%	1.3%

2.7 Longterm changes in incidence of *S. eurycerca*

Annual catches of swans at Caerlaverock and Alvecote provided data over 2 - 3 years.

Caerlaverock

Whooper and Mute Swans were trapped at Caerlaverock (Dumfries) in February of 1984, 1985 and 1986 (Table 12). For Whooper Swans the proportion of infected birds in each consecutive year was 20.0%, 43.3% and 10.8%. A similar pattern was observed for Mute Swans. Over the three years, the percentage of infected swans was 29.0%, 35.3% and 10.3%.

Alvecote

Mute Swans were trapped at Alvecote (Staffs.) in July of 1984 and 1985. The proportion of swans infected decreased from 25.0% to 3.8% in those two years.

2.7.1 Changes in infection in individual swans

During annual catches, some swans caught in the previous year were recaptured and therefore re-tested for the presence of microfilariae of *S. eurycerca*. Examples of swans which exhibited a change in the presence of infection are shown in Table 13. Four swans were positive for *S. eurycerca* when first tested but subsequently lost the infection (HPH, HLU, HZB, HUB). Another four swans did not have microfilariae when first caught but were positive the following year (HUA, HTJ, HTZ, IBC).

TABLE 12: Long-term changes in incidence of *S. eurycerca*

LOCATION AND SPECIES	DATE	No. SAMPLED	No. INFECTED	% INFECTED
CAERLAVEROCK WHOOPER SWANS	1984	55	11	20.0
	1985	90	39	43.3
	1986	74	8	10.8
CAERLAVEROCK MUTE SWANS	1984	31	9	29.0
	1985	17	6	35.3
	1986	29	3	10.3
ALVECOTE	1984	44	11	25.0
MUTE SWANS	1985	105	4	3.8

TABLE 13: Changes in infection in individual Whooper Swans

	*PRESENCE OR ABSENCE OF INFECTION OVER YEARS		
RING NO.	1984	1985	1986
HPH	+	+	-
HLU		+	-
HZB		+	-
HUB		+	-
HUA	-	+	
HTJ	-	+	
HTZ		-	+
IBC		-	+

(*NOTE: Presence of infection indicated by + (detected by sedimentation technique); Absence of infection indicated by -).

2.8 Effect of *S. eurycerca* on swan weights

Weight is a simple and informative measure to take of birds in the field and many studies have shown that weights can be usually used as indices of a bird's general health (Bacon and Coleman, 1986). Swans were categorised into groups of the same species, age and sex. In each category, the weights of infected swans were compared with the weights of non-infected swans. A Z-test was used to analyse the weights statistically. The Z scores are tabulated (Appendix 2).

Only one group of swan weights was found to be significantly different in infected and non-infected swans. The weights of infected adult Whooper Swans were significantly lower than non-infected adult Whoopers. Bewick's Swans were too small in number to be analysed statistically.

3. SEASONAL AND DAILY PERIODICITY OF *S. eurycerca* MICROFILARIAE

The biology of parasitic nematodes is inextricably linked to that of their hosts. Microfilariae are released from adult female nematodes in the heart and their circulation in the peripheral blood exhibits a marked circadian periodicity (Hawking, 1967; Worms, 1972). For many nematode species, the maximum microfilaraemia in peripheral vessels correlates with the maximum biting activity of the particular intermediate host. Microfilariae of *W. bancrofti* for example, exhibit a nocturnal periodicity and are transmitted by a night-biting mosquito, *Culex fatigans*. *Loa loa* microfilariae are diurnally periodic with maximum numbers occurring at midday when the vector, a day-biting fly (*Chrysops*) is feeding.

The number of microfilariae present in peripheral blood also varies over a longer period which broadly correlates with a yearly cycle. Seasonal periodicities have been observed in the dog heartworm, *D. immitis* and *D. repens* where the peak of the cycle occurs in the summer months when mosquito vectors are most numerous (Hawking, 1967; Newton, 1968).

The daily and seasonal periodicities of *S. eurycerca* of Mute Swans were examined by:

1) determining if a 24 hour periodicity does exist;

2) observing if a seasonal periodicity exists;

3) examining how the 24 hour periodicity may affect data obtained from other swans collected at different times of day.

MATERIALS AND METHODS

3.1 Daily periodicity

Blood samples (3.0 cm^3) (Lithium-heparin coated syringes) were taken from five microfilaeremic Mute Swans at four hour intervals for a period of 24 hours. For each 3.0 cm^3 sample, blood (1.0 cm^3) was preserved in 2% formalin (9.0 cm^3) for enumeration by the sedimentation technique (p. 18). Each sample was sedimented five times and a mean microfilariae density was derived for each time of day.

For three swans, blood-sampling began at 07.00 hours and continued until the same time the following morning. To identify any effects that handling might have had on the microfilariae numbers, samples were taken from 19.00 hours one day until 19.00 hours the next day, for the other two swans.

3.2 Seasonal periodicity

Two microfilaraemic Mute Swans were maintained in an outdoor pen (p.10). Blood samples were taken from each swan at two-week intervals for periods of 12 months. Blood samples were collected between 13.00 hours and 15.00 hours and the number of microfilariae per 0.25 cm^3 was determined by sedimentation.

RESULTS

3.3 Daily periodicity

Variations in the numbers of microfilariae over 24 hours were observed in all swans examined (Fig. 6). The maximum number of microfilariae occurred between 11.00 hours and 19.00 hours and the minimum number between 19.00 hours and 07.00 hours. A similar pattern was observed for all the swans. In Fig. 7, each point represents the percentage of the maximum microfilarial density observed for each swan over 24 hours. The maxima were 42, 25, 47, 69 and 41 microfilariae per 0.25 cm^3 blood.(Table 14)

Throughout each 24 hour period of observation, there were always some microfilariae present in the peripheral vessels. Even at the points where minimal densities were recorded, between 12-26% of the maximum number were present (Table 15).

3.4 Seasonal periodicity

The first swan to be examined was an adult bird when first captured in December 1984. It was monitored for 12 months and showed an increase in the level of infection in the first 3 months. Over one month (March to April) the infection level decreased from 41 to 12 microfilariae per 0.25 cm^3. It then gradually decreased to an indetectable level over the following 5 months. After October 1986, no microfilariae were detected.

The second swan was monitored for 12 months. It was a first year cygnet when first captured in March 1985. The infection level gradually increased over the first six months from 2-3 to 15 microfilariae per 0.25 cm^3. From September to October, the infection level increased from 9 to 38 microfilariae per 0.25 cm^3. (Table 16; Fig.8)

TABLE 14: Variations in microfilariae counts over 24 hours

	NUMBER OF MICROFILARIAE PER 0.25 cm³ BLOOD					
TIME	SWAN 1	95% C.L.	SWAN 2	95% C.L.	SWAN 3	95% C.L.
0700	13.0	8.8	7.0	6.0	16.0	3.3
1100	32.0	8.0	25.0	9.1	31.0	3.8
1500	42.0	4.3	3.0	0.9	47.0	6.7
1900	11.0	0.6	5.0	1.0	30.0	1.75
2300	13.0	2.45	8.0	0.5	9.0	1.0
0300	17.0	1.8	13.0	4.3	10.0	3.3
0700	33.00	6.2	8.0	5.1	20.0	6.1
	SWAN 4		SWAN 5			
1900	69.0	12.45	41.0	20.2		
2300	37.0	6.7	15.0	13.1		
0300	28.0	8.6	12.0	5.3		
0700	17.0	9.9	7.0	3.8		
1100	20.0	3.5	12.0	7.2		
1500	43.0	3.3	25.0	4.3		
1900	60.0	10.1	38.0	4.2		

TABLE 15: Percentage of maximum number of microfilariae during 24 hours

TIME	% OF MAXIMUM NUMBER SWAN 1	SWAN 2	SWAN 3
0700	31	28	34
1100	76	100*	66
1500	100*	12**	100*
1900	26**	20	64
2300	31	32	19**
0300	40	52	21
0700	78	32	42

	SWAN 4	SWAN 5
1900	100*	100*
2300	54	36
0300	40	29
0700	25**	17**
1100	29	29
1500	62	61
1900	87	93

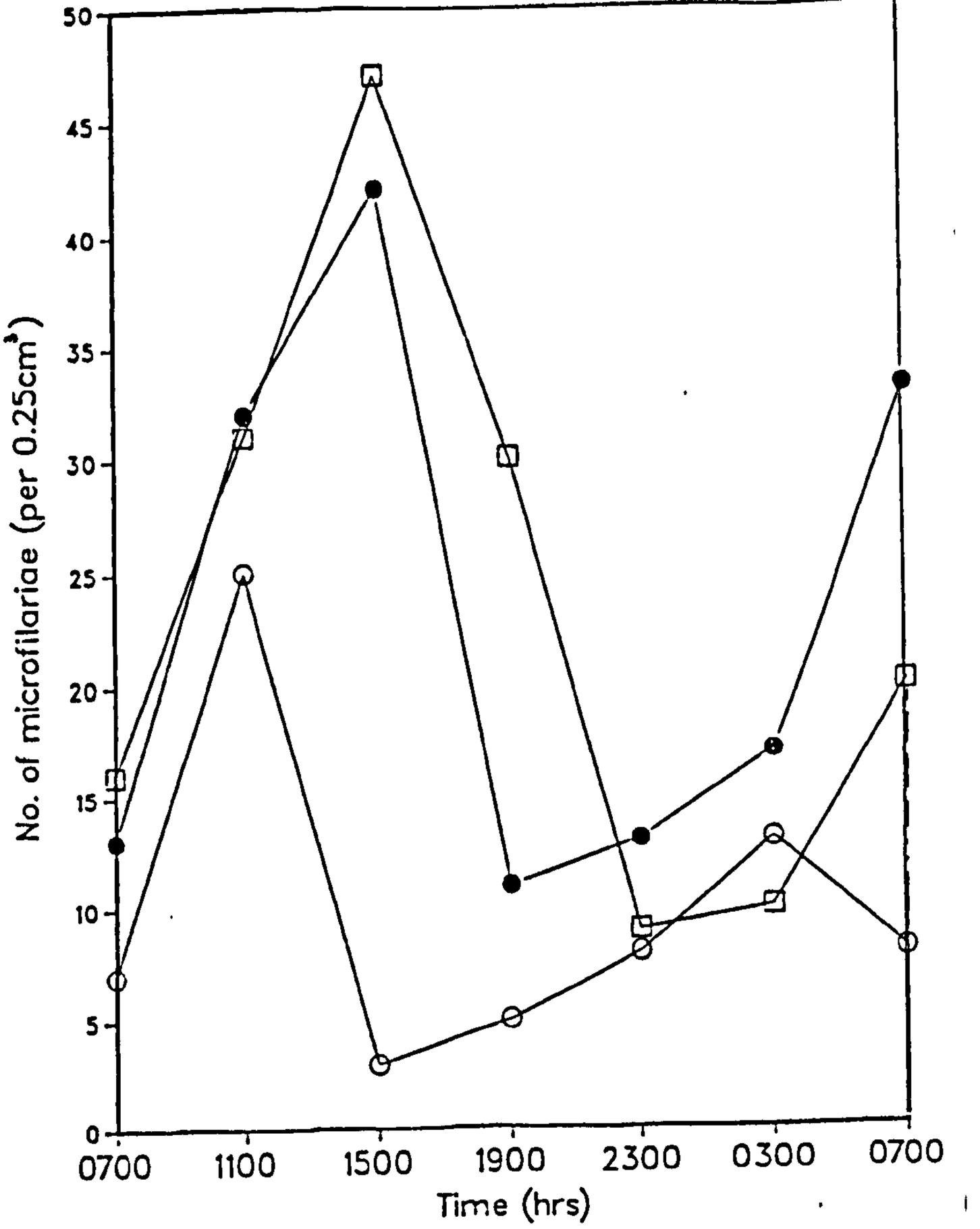
* maximum no. of microfilariae

** minimum no. of microfilariae

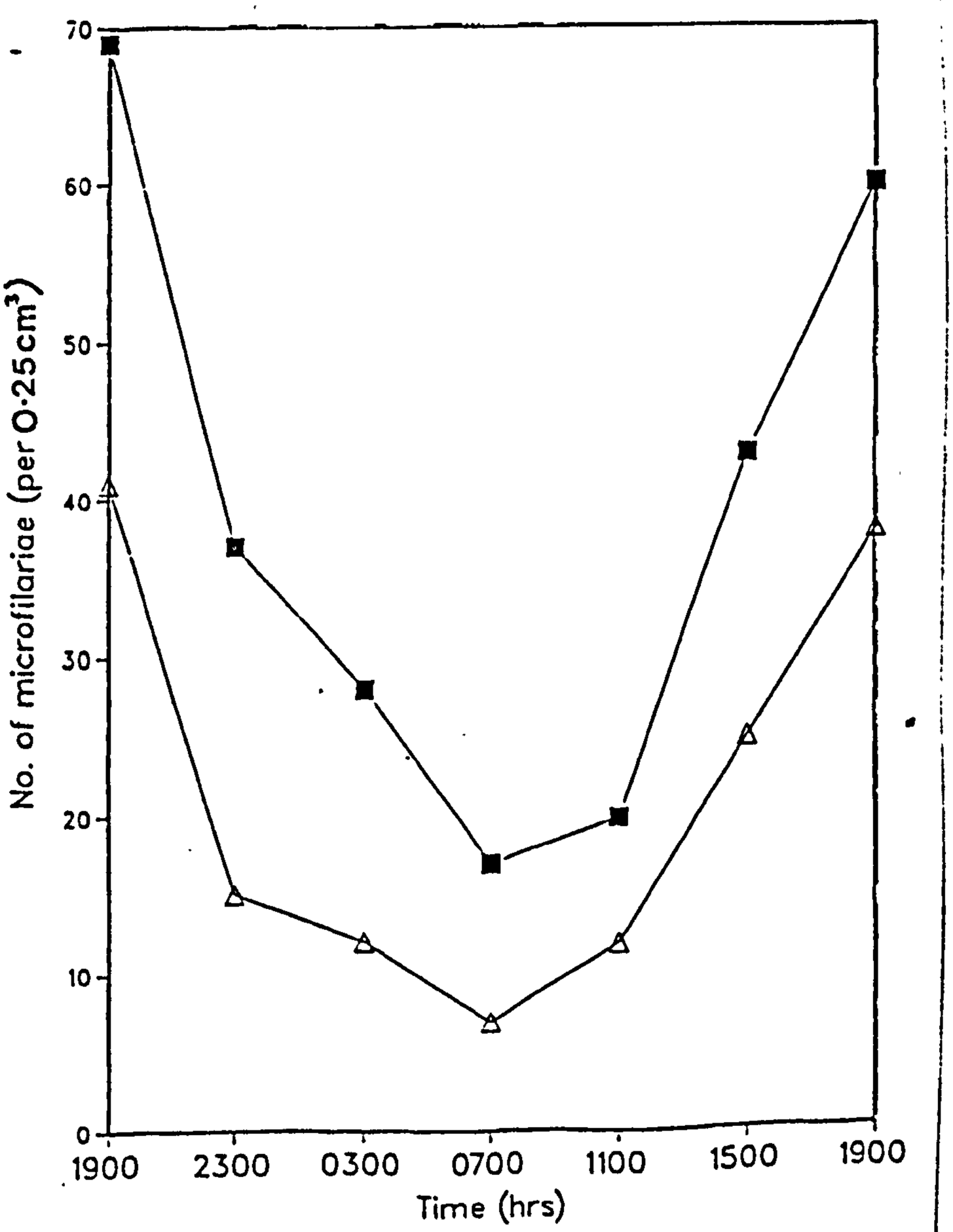
FIGURE 6 : Daily periodicity of *S. eurycerca* microfilariae circulating in peripheral vessels of Mute Swans.

A. Observations made of three swans from 0700 until 0700 the following morning.

B. Observations made of two swans from 1900 until 1900 the following evening.


50
45
40
35
30
25
20
15
10
5
0
No. of microfilariae (per 0.25cm³)
0700
1100
1500
1900
2300
0300
0700
Time (hrs)

A

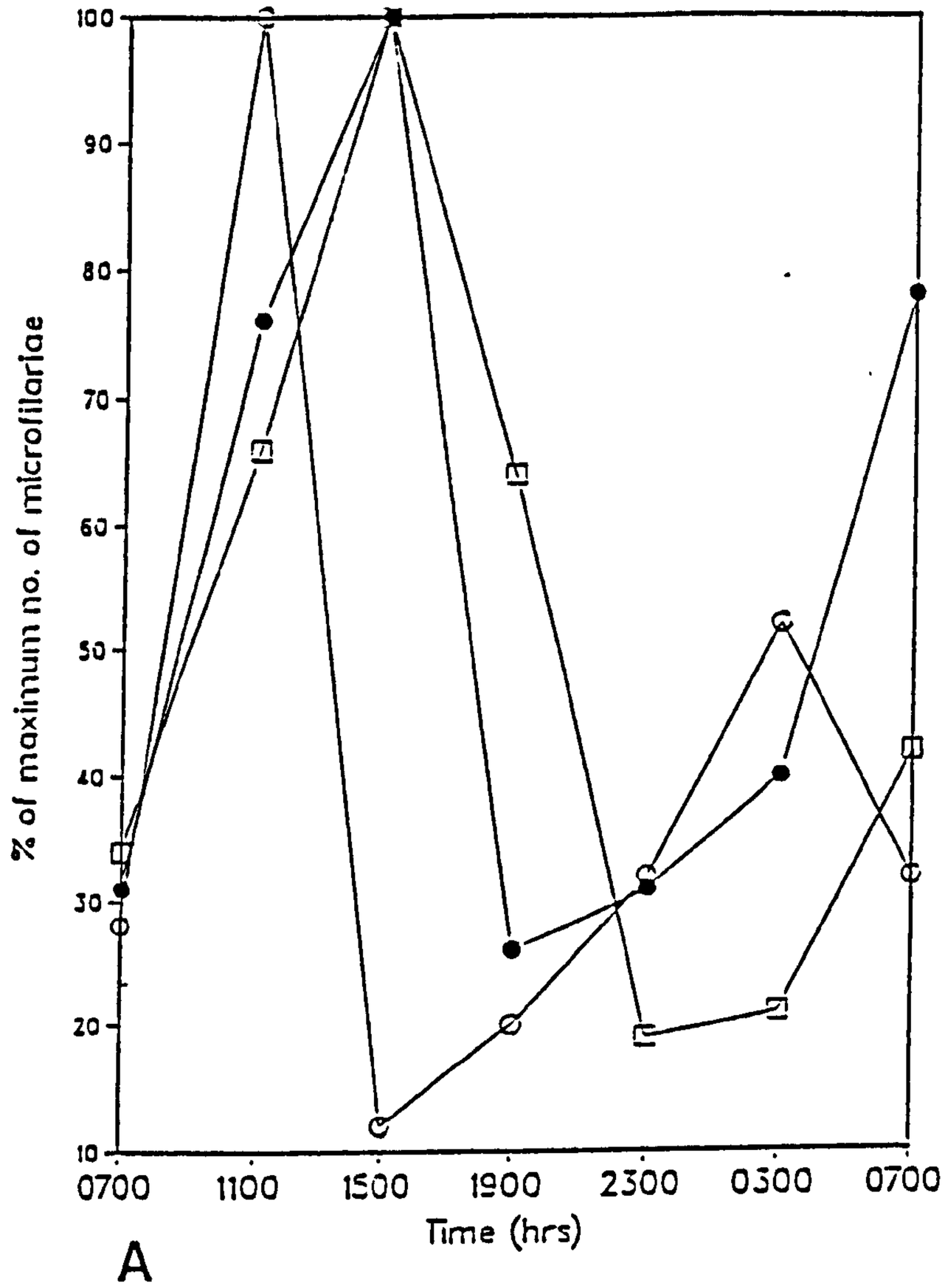

70
60
50
40
30
20
10
0
No. of microfilariae (per 0·25cm³)
1900
2300
0300
0700
1100
1500
1900
Time (hrs)

B

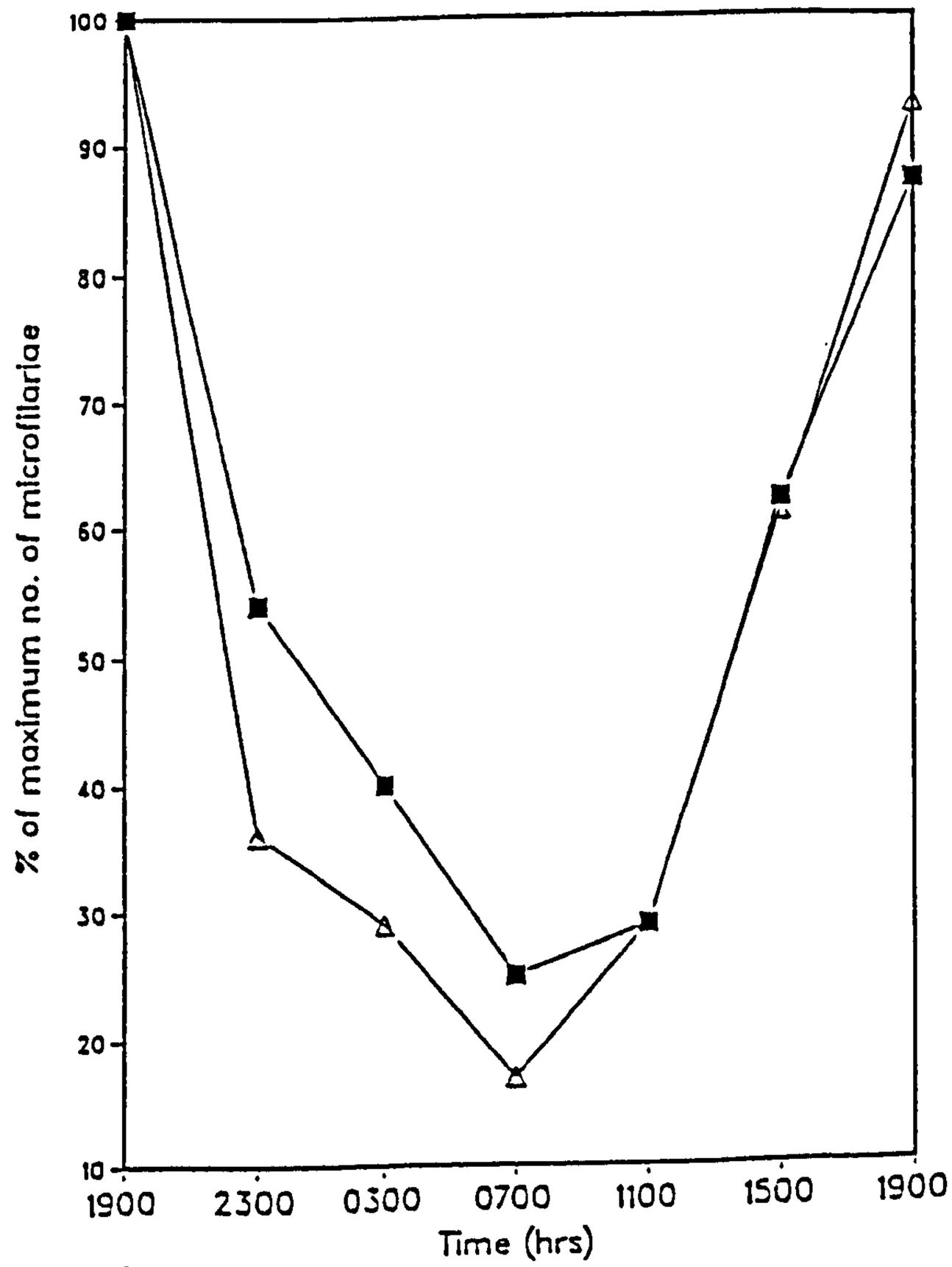
FIGURE 7 : Daily periodicity of *S. eurycerca* microfilariae in Mute Swans, represented as percentages of the maximum microfilariae occurring during 24 hours for each swan.

A. Values for three swans samples from 0700 until 0700 the following morning.

B. Values for two swans sampled from 1900 until 1900 the following evening.


100
90
80
70
60
50
40
30
20
10
% of maximum no. of microfilariae
0700
1100
1500
1900
2300
0300
0700
Time (hrs)

A


100
90
80
70
60
50
40
30
20
10
% of maximum no. of microfilariae
1900
2300
0300
0700
1100
1500
1900
Time (hrs)

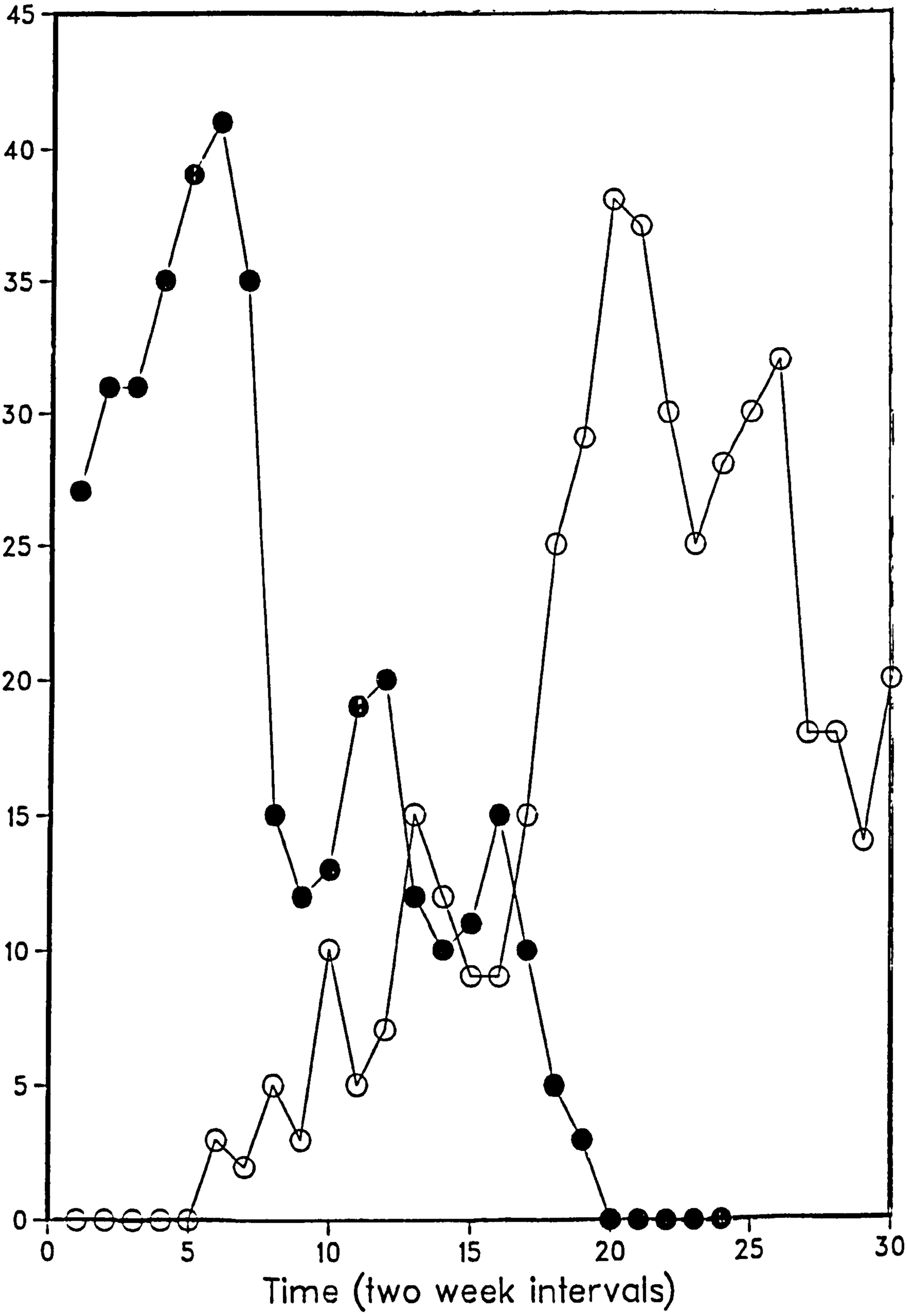
B

TABLE 16: Levels of infection of *S.eurycerca* in two Mute Swans over a 30-week period

TWO-WEEK INTERVALS	NUMBER OF MICROFILARIAE PER 0.25 cm^3 BLOOD	
	SWAN 1	SWAN 2
1	27	-
2	31	-
3	31	-
4	35	-
5	39	-
6	41	3
7	35	2
8	15	5
9	12	3
10	13	10
11	19	5
12	20	7
13	12	15
14	10	12
15	11	9
16	15	9
17	10	15
18	5	25
19	3	29
20	0	38
21	0	37
22	0	30
23	0	25
24	0	28
25	-	30
26	-	32
27	-	18
28	-	18
29	-	14
30	-	20

FIGURE 8 : Seasonal periodicity of *S. eurycerca* microfilariae in two Mute Swans.

Samples were taken from each swan at two-week intervals over a period of 30 weeks.


45
40
35
30
25
20
15
10
5
0
No. of microfilariae (per 0.25cmi3)
0
5
10
15
20
25
30
Time (two week intervals)

DISCUSSION

Detection methods

The sensitivity of a detection technique is of particular importance in situations where the level of infection is low. In this study, levels of infection range from 1 - 42 microfilariae per 0.25 cm^3 in Mute Swans, from 1 - 46 microfilariae per 0.25 cm^3 in Whooper Swans, and from 3 - 10 microfilariae per 0.25 cm^3 in Bewick's Swans (figures determined by a sedimentation procedure).

For any detection technique to be effective, consideration must be given to those factors which may underestimate the incidence of *S. eurycerca* in a swan population. *S. eurycerca* would not be detected in a bird if:

1) the microfilaremia is below the sensitivity of the test;

2) the blood samples are taken during the active phase of periodicity (that is, when minimum numbers are present in the peripheral vessels);

3) in individual birds, the adult heartworms are of one sex;

4) the worms are in a prepatent stage.

Any one of these situations could produce a 'negative' result in a blood sample which was actually infected. The sensitivities of various techniques in detecting filariae have been compared in swans by Seegar (1979), in man by Southgate (1974), McMahon (1979), Denham (1971) and Shibuya (1980) and in dogs by Wylie (1970) and Altman (1972). For human filariae, McMahon (1979) and Denham (1971) found both the wet mount and thin smear tests to be inefficient. However, the wet mount test was more sensitive than the capillary tube method when applied to *Dirofilaria immitis* in dogs (Ohish and Kobayashi, 1961; Wylie, 1970). Altman (1972) also found the capillary tube test to be less sensitive when compared with other concentration techniques such as the Millipore filter used to detect *D. immitis*.

Seegar (1979b) compared the relative sensitivities of detection techniques for *S. eurycerca* in Whistling Swans. He found the capillary tube test was the most sensitive method (100% detection) compared to the wet mount (93%) and the thin smear test (25%). The sedimentation technique, adapted for use in this study with avian microfilariae, detected 55% more samples as positive than the capillary tube test, 80% more than the wet mount and 95% more than the thin smear test. The advantages of the sedimentation

technique over other methods are twofold: first, it detects 55% more samples as positive than Seegar's most sensitive test (capillary tube) and second, it detects almost four times more samples as positive with low microfilariae (1 - 9 microfilariae per 0.25 cm^3 blood) than the capillary tube test. Since most microfilaremias were within this low infection range (Table 3), the sedimentation technique was a very suitable diagnostic method for this type of filarial infection.

Detecting microfilariae may also depend on the periodicity of microfilariae circulating in the blood supply and the time when samples were taken.

Periodicity is known to be characteristic of a number of filarial species parasitising a range of vertebrate hosts (Hawking, 1962). The mechanism of periodicity has been widely researched and Hawking (1967) proposed an explanation for this phenomenon. There are believed to be two phases in a periodic cycle, the passive and the active. During the passive phase, microfilariae are evenly distributed throughout the blood and appear to be more numerous in the circulation. During the active phase, microfilariae are concentrated in the lungs where there is thought to be a more favourable physiological environment for the larvae. For some nematode infections, (e.g. *W. bancrofti*) there are periods during the 24 hour cycle when no microfilariae are circulating in the peripheral vessels and the timing of blood sampling is critical for detection of infected hosts. *S. eurycerca* also exhibits a maximum and minimum number of microfilariae over 24 hours in Mute Swans. This was also shown to occur in Whistling Swans over 24 hour and 88 hour periods (Seegar, 1977). But during the 24 hour cycle in Mute Swans there were always some microfilariae present and for this reason it is referred to as a sub-periodic rhythm (Worms, 1972). In this study, it was noted that some variations occurred in the timing of maximum densities of *S. eurycerca* . Maxima occurred between 11.00 hours and 19.00 hours. Gubler (1966) found similar variation in *D.immitis* , which also exhibits a sub-periodic cycle. Maxima for this nematode occurred between 20.00 hours and 22.00 hours. Seegar (1977) recorded the maxima for *S. eurycerca* in Whistling Swans to occur between 01.00 hours and 04.00 hours. Variation within individual hosts is therefore not uncommon and may be of benefit to vectors with variable feeding times. The results presented in this study as such appear at variance with those of Seegar (1977). All blood samples which were collected in this study, to determine the incidence of *S. eurycerca* in

swans were obtained between 08.00 hours and 17.00 hours. This period coincides with the maximum number of microfilariae occurring between 11.00 hours and 19.00 hours. The timing of blood sampling was not as critical as for *W. bancrofti* , since at least some microfilariae were always present throughout 24 hours. It is likely that the combination of a sensitive detection technique and a sub-periodic cycle of microfilariae results in a positive infection test even if microfilariae are present in low numbers.

The final two situations which could result in failure to detect infection could be overcome if a post-mortem examination was made of each swan and the heart and blood examined. Opportunities were taken to examine dead swans and the results are reported on p.153.

Differential incidence and infection levels of *S. eurycerca* related to host age, sex and weight

It is evident from this survey of 1128 swans that a larger proportion of juveniles (20.3%) were infected than were adult birds (12.9%). Seegar (1977) also found the incidence of *S. eurycerca* to be greater in juvenile Whistling Swans than in adults. Similarly, a greater incidence of *S. eurycerca* was recorded in juvenile Canada geese when compared with adults (Hanson, *et al.*, 1956). Seegar (1977) suggested that a reason for these observations might be an acquired immunity to the nematode in adult birds. Indeed, Anderson (1956) working with *Ornithofilaria fallisensis* (in the same family as *S. eurycerca*), found evidence of acquired immunity in captive ducks . Parasitized birds kept throughout the winter and re-exposed to infection in the spring did not exhibit increases in microfilaremias, and in many ducks microfilariae disappeared.

Results from recapturing individual Whooper Swans at Caerlaverock (Dumfries) over 2-3 years shows the signs of infection can disappear in one year. Also one Mute Swan held in captivity for 12 months exhibited a gradual decrease in microfilaremia until the infection disappeared (p.36). A captive juvenile Mute Swan also showed a decrease in the infection level as it approached 12 months old.

It is therefore possible that swans acquire an immunity to *S. eurycerca* with increasing age. Low density infections as recorded in this study

for juveniles ($\bar{x}$ = 8.0 microfilariae per 0.25 cm^3), could act as a live "vaccine" for which the immune system would produce antibodies ready to react against subsequent infections. Otto (1970) reviews the evidence for acquired immunity to insect-borne nematodes. The available data on the natural occurrence of *W. bancrofti* and *Brugia malayi* in man and *D. immitis* in dogs suggests that there is an intrinsic host factor which adversely affects the worms on continued reinfection. Acquired immunity in man is reflected by declining microfilariae incidences and declining numbers of microfilariae in older age groups. A further explanation for the differential infection levels of juvenile and adult swans might be that there is a difference in susceptibility of the bird to the insect host (p.77-80).

There were no observable differences in the incidence of *S. eurycerca* in male and female swans. The overall incidences were remarkably similar : in males (15.9%) and in females (15.8%). Differences in incidences of parasites between animals of different sexes is uncommon and not well understood (Kennedy, 1975). If any differences do occur they are not usually due to any single cause and involve differences in host diet and physiology.

Overall there were no apparent differences between the weights of infected and non-infected swans (p. 34). In his study of Whistling Swans, Seegar (1977) found that parasitised swans were significantly lighter than non-parasitised swans. If weight is any indication of the health of a swan, it might be that parasitisation with *S. eurycerca* had a deleterious effect on the Whistling Swan population. However, there seems to be no effect on the swans sampled in this study.

The intensity of infection in Whistling Swans was much greater than in swans in the present study. The mean microfilaremia for 138 Whistling Swans was 82 per 0.25 cm^3 with a maximum level of 575 per 0.25 cm^3 (Seegar, 1977). For swans in this study, the mean microfilaremia was 8 per 0.25 cm^3 with a maximum of 46 per 0.25 cm^3. The levels of infection were not significantly different for different species, but were closer to those reported by Seegar who surveyed some Mute Swans in England and recorded a mean microfilaremia of 17 per 0.25 cm^3 and a maximum of 125 per 0.25 cm^3.

The higher microfilaremias in Whistling Swans could have resulted from a range of factors operating in the host population, some of which relate to

the infectivity of the parasites/intermediate host or susceptibility of the host. Whatever the cause, *S. eurycerca* seems to be having a greater adverse effect on the health and weight of these swans compared to Mute and Whooper Swans in this country.

Differential incidence of *S. eurycerca* related to host status and health

Since the transmission of *S. eurycerca* is dependent upon an intermediate host, the incidence of nematodes will be dependent upon the relationship between the intermediate host and the swan. The status of the host, that is, whether it is aggregated, single or in a pair, will directly influence the opportunity for louse transfer and therefore indirectly influence the filarial incidence (p.26).

There are a number of direct relationships between *S. eurycerca* and the swan which would influence the incidence of the nematode. McDonald (1974), in a review of nematode parasites of Canadian waterfowl, states that the nematode infections reflect, amongst other things, the health and physical condition of the birds. One-third of the sick or injured swans in the British Wildlife Hospital (Cheltenham) were infected with *S. eurycerca* but the captive swans in Wildfowl Trust collections (Peakirk and Washington) known to be in excellent health were all free from infection. For swans with lead poisoning, however, the infection levels are reduced (McDonald, 1974). McDonald compared eleven lead-poisoned Whistling Swans with fifteen other "sick" swans. The lead poisoned swans had "between 40.8 - 46.0 fewer *S. eurycerca* " than swans with other diseases. In the present study, no infections were recorded in swans from the Thames and its tributaries (Oxford) where concern is being shown for lead levels which are known to be high (Birkhead, 1983; Sears, 1986).

CHAPTER III

INTRODUCTION

In an attempt to determine experimentally whether or not *T. anserinum* is an intermediate host of *S. eurycerca*, the principle experiments would seek to observe the cyclical process of larval development in the insect and their eventual transmission to another host, in conditions which exclude all other possible vectors.

The basic design of these experiments would require that three swans were housed in an area completely free of and secure from any possible vectors of *S. eurycerca*. The enclosed chamber would be divided into two compartments : one area would house two experimental swans, the other, a 'control' swan. Construction of the enclosure would ensure that insects could not move between the compartments. Of the two experimental swans, one would be infected with *S. eurycerca* and the other completely free of nematode infection (that is, a hand-reared cygnet hatched and maintained in vector-free conditions). The lice used in the experiments would also have to be reared from eggs either *in vitro* or on an uninfected swan, to ensure they too, were free from nematodes.

The experiment would begin by placing a number of *T. anserinum* onto the infected swan. If *T. anserinum* is an intermediate host, the nematode-free swan would eventually become infected and the control swan would remain free from infection in the separated compartment.

Resources were unavailable to conduct such an experiment and an alternative, yet robust methodology was adopted. *In vitro* studies using Amblyceran lice have also failed to rear the life-cycle from egg to adult, a criteria thought necessary for successful completion of the experiment.

The alternative approach considers morphological and behavioural attributes specific to an intermediate insect host of a filarial parasite. Essentially, there are four attributes which such an insect must exhibit:

1) a spatial and temporal relationship between insect and definitive host which assures an opportunity to contact the filarial parasite;

2) a capability of acquiring microfilariae from its host by feeding on blood;

3) a suitable environment within which developmental stages of the larval parasite (L_1 - L_3) can develop and from which, the infective third stage larvae are able to leave and innoculate another swan;

4) a degree of mobility to ensure the transmission of the parasite from host to host.

In this chapter, each attribute listed is considered in the light of experimental observations of *T. anserinum*. The evidence is then examined to determine whether *T. anserinum* is able to acquire, develop and transmit *S. eurycerca* in swans and to see to what degree *T. anserinum* exhibits these features.

1. *T. anserinum* : ITS TAXONOMIC STATUS, COLLECTION AND ADAPTATION TO A PARASITIC MODE OF LIFE

Mallophaga are thought to have evolved from a Psocopteran-like ancestor around the time of the origin of warm-blooded vertebrates, although they may initially have been parasites of nest-dwelling reptiles (Hopkins, 1949; Rothschild and Clay, 1952).

A hypothesis for the evolution of the Mallophaga, proposed by Waage (1979) outlines the adaptations which evolved in these insects for a parasitic mode of life. The process is summarised in three stages: 1) a pre-adaptive nest association in early psocid-like forms leading to phoresis and casual feeding on host epidermis; 2) morphological and behavioural modifications permitting continued existence on the host, efficient feeding on skin and, in some cases, facultative blood-feeding; 3) further modifications in physiology and mouth part morphology permitting piercing of host tissue and a wholly haematophagous diet.

The first stage in the evolution of a vertebrate-insect association can be illustrated by present-day psocids which occasionally inhabit the nests of vertebrates and some species have been collected from the feathers of birds (Mockford, 1967). It is thought that the ancient, nest-dwelling Psocopterans could have started to feed on shed integument in the nest which gradually led to the adoption of the same diet whilst on the host.

The second stage may have involved specific morphological and behavioural adaptations associated with a continued existence on the host. One of the major hazards to ectoparasites is grooming by the host and as a consequence, lice have adopted a number of morphological and behavioural characteristics to facilitate survival. They have highly modified grasping appendages, an extreme dorso-ventral flattening of the body and a preferential aggregation in regions less accessible to grooming (Rothschild and Clay, 1952). Feeding from the host epidermis by psocid-ancestors probably led to a diet of feathers and secretions of sebaceous glands (as seen in present-day Ischnocera). Further modifications of the mouthparts produced the first facultative blood-feeders which could pierce and chew skin and therefore ingest blood.

The final stage in the evolutionary process involved adaptations associated with a wholly haematophagous diet and led to the present day Anoplura and some haematophagous Amblycera.

The evolution of free-living ancient-Psocopterans towards a parasitic association has possibly led to the present day Mallophaga which have an array of morphological features specifically adapted for parasitism. In this section, some of the adaptive features of *T. anserinum* are considered in association with:

1.1) its taxonomic status;
1.2) its collection from avian hosts;
1.3) its adaptive features associated with a parasitic mode of life.

1.1 TAXONOMIC STATUS OF *T. anserinum*

Within the sub-order Amblycera, *T. anserinum* belongs to the family Menoponidiae. This family of lice was classified by Clay (1949) and the genus *Trinoton* is distinguished by the presence of two large sternal plates bearing many setae. All available literature on the genus is reviewed by Eichler (1981), where *Trinoton* is described as a unique genus with few close relatives and because of its large size (4-5 mm long) it is readily distinguishable from other Mallophaga. In his report, Eichler describes 23 species of *Trinoton* taken from 32 species of ducks, geese and swans.

Taxonomic characteristics of *Trinoton* species

Using descriptions given by Eichler (1981) the species collected in the present study were clearly either *T. anserinum* or *T. cygni* .

The description of *T. anserinum anserinum* is based upon 7 specimens collected from domestic geese (*Anser anser*) and outlines three main characteristics which distinguish this species from *T. cygni* :

1. the gular region is very wide and absolutely characteristic. Eichler illustrated the differences between the two species with two diagrams (Fig. 9a);

2. the sternum has a unique shape and arrangement of bristles, also illustrated by Eichler (Fig. 10a). These diagrams show that the sclerotised outer edge around the sternum is wider in *T. anserinum* than in *T. cygni*. Also, Eichler's drawings show how the outer edge of the sternum curves around the top of the plate in *T. cygni*, a feature not displayed by *T. anserinum* ;

3. the bristled region of the fourth and fifth sternites of the abdomen have many additional small bristles which are absent in other *Trinoton* species.

T. cygni cygni was first described as a new sub-species of *T. anserinum*, named *T. anserinum cygni* by Eichler in 1943. At the time, he made this identification from one male specimen taken from a Mute Swan. In his 1981 review, he upgrades *T. anserinum cygni* to *T. cygni cygni* believing it to be "too independent" to be a sub-species, but apparently made this decision with no additional material for comparison. A further difference between the species was in the body length: the lengths of *T. anserinum* males were 5.8 mm and of *T. cygni* males, 5.1 mm (Eichler, 1943).

1.1.1 MATERIALS AND METHODS

To identify the species of *Trinoton* collected in this study, specimens were compared with the British Museum (Natural History) collection of *T. anserinum* and *T. cygni* and with Eichler's descriptions of the two species.

Three characteristics were analysed and illustrated:

1) the arrangement of bristles on the gular region;
2) the shape of the sternum and bristle pattern;
3) the shape of the male genitalia. This characteristic was suggested as a distinguishing feature between males of *T. cygni* , where the shape of the posterior plate is more angular and *T.anserinum* where this structure appears more rounded (C. Lyal, pers. comm.).

The other characteristic that Eichler noted, namely sternite bristles on the abdomen, were not sufficiently well illustrated by Eichler for use in identification.

1.1.2 RESULTS

1.1.2.1 Arrangement of bristles on the gular region

It appears from Eichler's illustrations that the bristles on the gular region of *T. cygni* (Fig. 9b) are more numerous and join together at the posterior end, when compared with *T. anserinum* (Fig. 9a).

Reference specimens from the British Museum do not show the differences in this feature illustrated by Eichler (Fig. 9c-i) and there appear to

FIGURE 9 : Arrangements of bristles on gular region of *Trinoton* sp.

(Gular or "throat" region is situated ventrally on the head of the insect)

a. Copy of drawing by Eichler (1981) of gular region of *Trinoton anserinum*.

b. Copy of drawing by Eichler (1981) of gular region of *Trinoton cygni*.

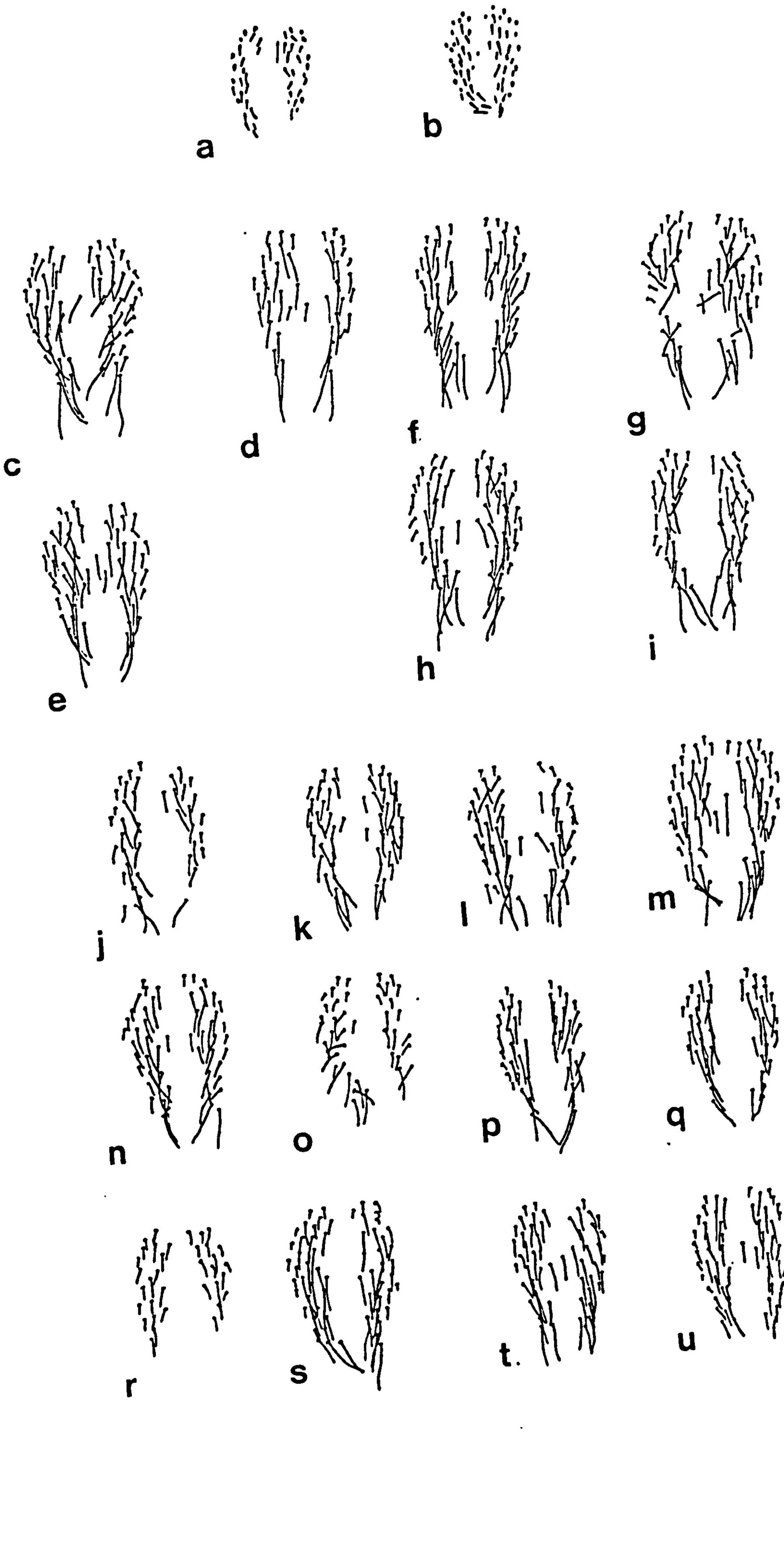
c-e. Arrangements of bristles on gular region of three reference specimens of *T. anserinum* from British Museum of Natural History collection.

f-i. Arrangements of bristles on gular region of four reference speciments of *T. cygni* from British Museum of Natural History collection.

j-u. Arrangements of bristles on gular region of *Trinoton* sp. collected in the present study.

Specimen r most closely resembles Eichler's representation of *T. anserinum*; specimen s best resembles *T. cygni* illustrated by Eichler. However, the remaining specimens do not resemble either of Eichler's representations of the two species.

[Note: Figs a and b, Eichler distinguished the two species of *Trinoton* from differences in bristle arrangements on the gular region]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be no differences in bristle arrangements which distinguish *T. anserinum* (Fig. 9a) from *T. cygni* (Fig. 9b).

The specimens collected in this study do not conform to Eichler's descriptions. The closest bristle arrangement to that of *T. cygni* being shown in Fig. 9s . Fig. 9r best resembles the illustration of *T. anserinum*. The remaining specimens do not resemble either of Eichler's representations of the two species.

1.1.2.2 Sternum shape and bristle arrangement

Eichler (1981) illustrates the differences between the sternum of *T. cygni* and *T. anserinum* (Fig.10a,10b) and describes *T. anserinum* as having a much broader sternum than *T. cygni*. The outer "rim" is curled around the top of the sternum in *T. cygni* but is straight in *T. anserinum* . Eichler also describes the narrowing end of the sternum as pointed in *T. cygni* and less pointed in *T. anserinum*(Fig.10c , 10d).

The seven reference specimens from the British Museum exhibit the differences described by Eichler (Fig. 10 *T. anserinum* e-g *T. cygni* h-k). Amongst the specimens from the present study, some conform to Eichler's specifications. Figs. 11h,i,m,n,o,q for example, show the straight outer "rim" characteristic of *T. anserinum* . Figs. 11a, d, g, k exhibit the curled outer "rim" of *T. cygni* . However, the remaining specimens show variations between these two shapes (Figs. 11b, c, e, f, l, p). One specimen (Fig.11m) was taken from a Black Swan (*Cygnus atratus*) and is probably not *T. anserinum* or *T. cygni* (C. Lyal, pers.comm.).

1.1.2.3 Shape of male genitalia

There was considerable variation in the shape of the posterior plate of the male genitalia (Fig. 12). None of the specimens collected in this study conform to either an "angular" (for *T. cygni*) or a "rounded" (for *T. anserinum*) shape.

1.1.2.4 Species differentiation

Eichler (1981) distinguished *T. cygni* from *T. anserinum* by comparing one specimen with seven specimens respectively.

FIGURE 10 : Shape of sternum and arrangements of bristles of *Trinoton* sp.

(The sternum is positioned on the ventral side of the first thoracic segment.

a. Copy of drawing by Eichler (1981) of sternum of *T.anserinum*.
(note, sternum is slightly broader than *T.cygni* (Fig.b)

b. Copy of drawing by Eichler (1981) of sternum of *T. cygni*.
(note, outer "rim" is curled around the top of the sternum)

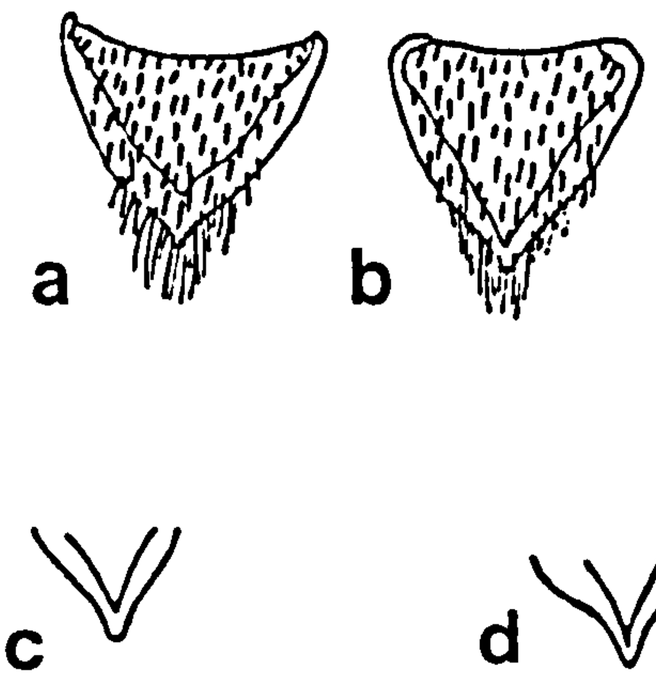
c. Shape of posterior of sternum in *T. anserinum* (after Eichler, 1981)

d. Shape of posterior of sternum in *T. cygni* (after Eichler, 1981)

e-g. Sternum of three reference specimens of *T. anserinum* from British Museum of Natural History collection.

h-k. Sternum of four reference specimens of *T. cygni* from British Museum of Natural History collection.

[note, the shape of the sternum in British Museum specimens appear to conform to Eichler's illustrations]


a
b
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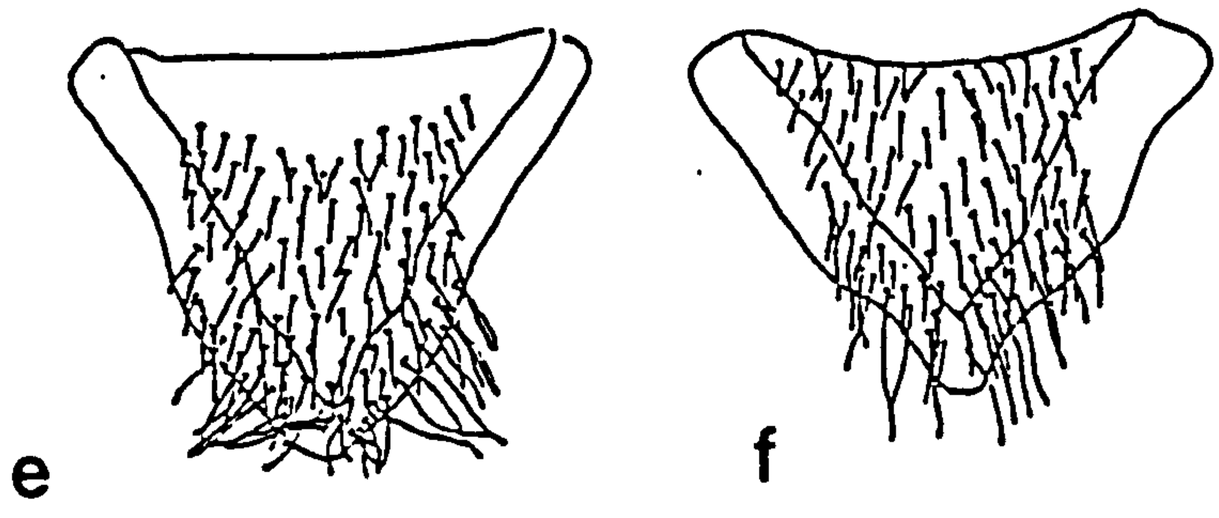
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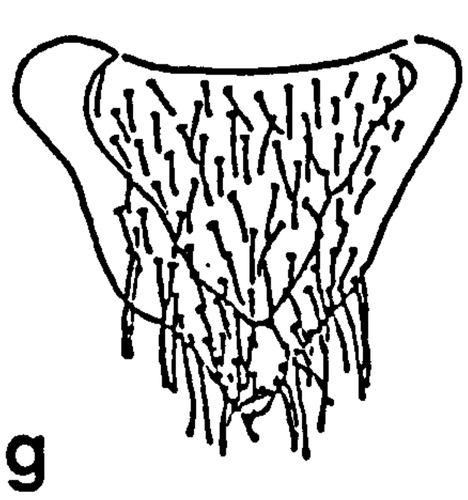
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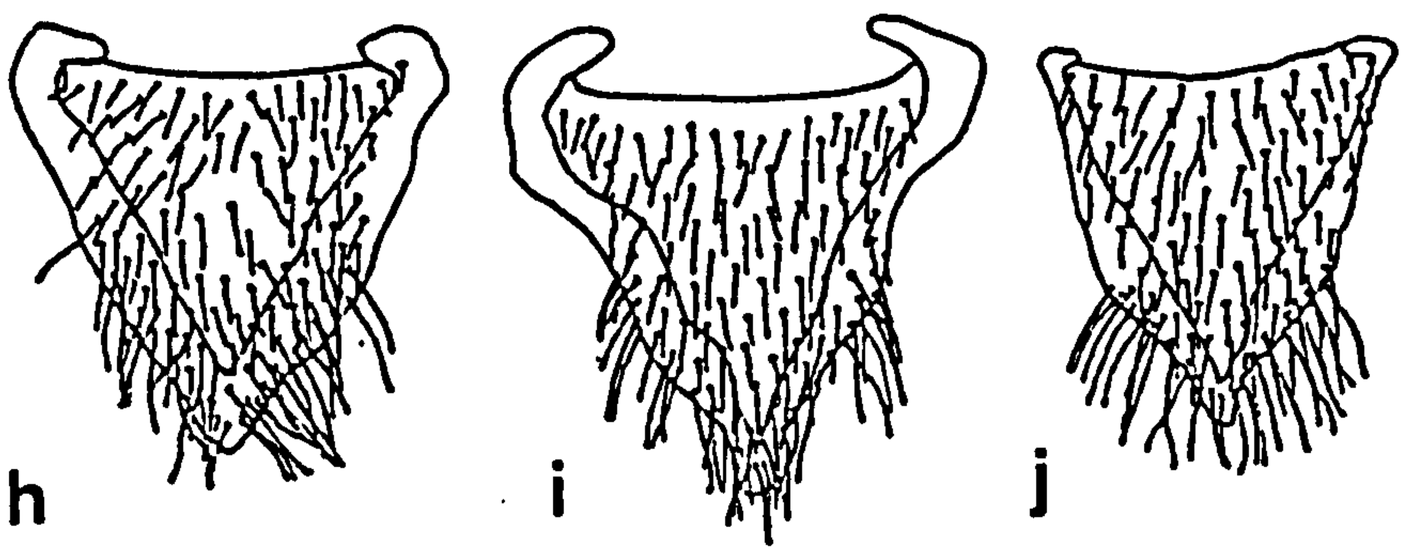
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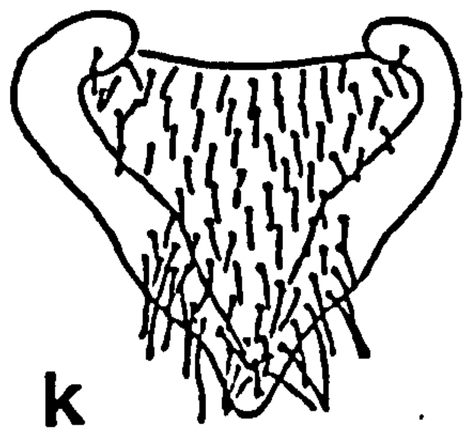
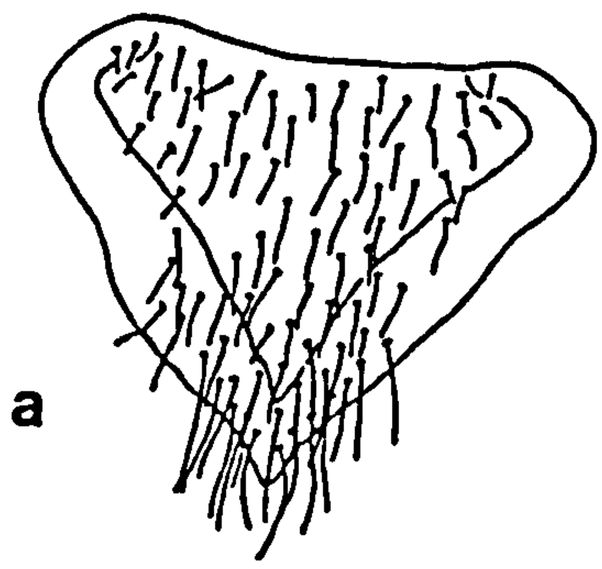

k

FIGURE 11 : Shape of sternum and bristle arrangements of *Trinoton* sp. from specimens collected in the present study.

(note, figures h, i, m, n, o and q exhibit the straight outer "rim" characteristic making it *T. anserinum* according to Eichler; figures a, d, g, j, k exhibit the curled outer "rim" characteristic. The remaining specimens show variations in the sternum shape)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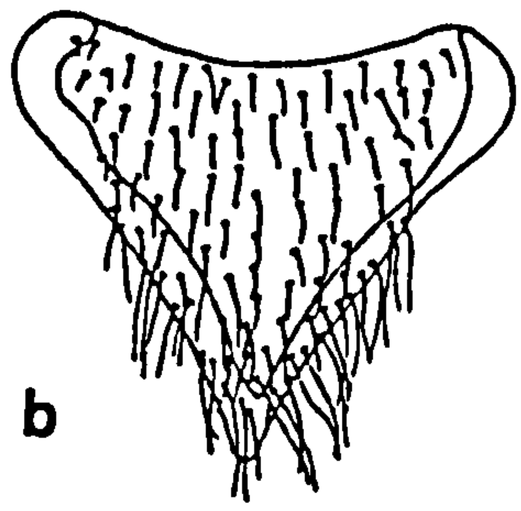
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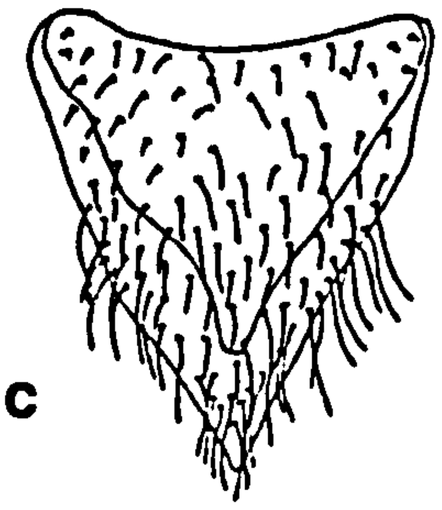
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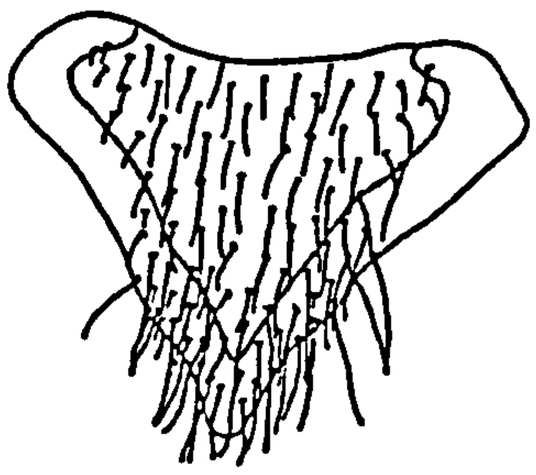
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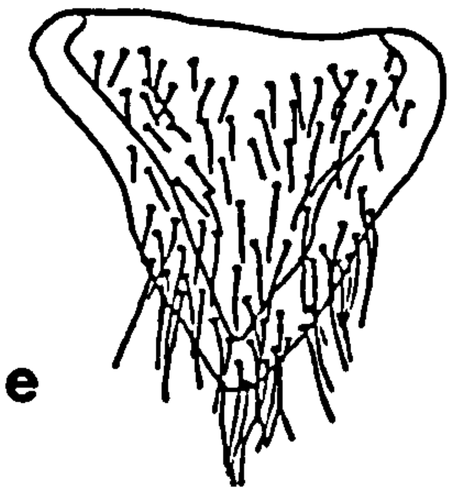
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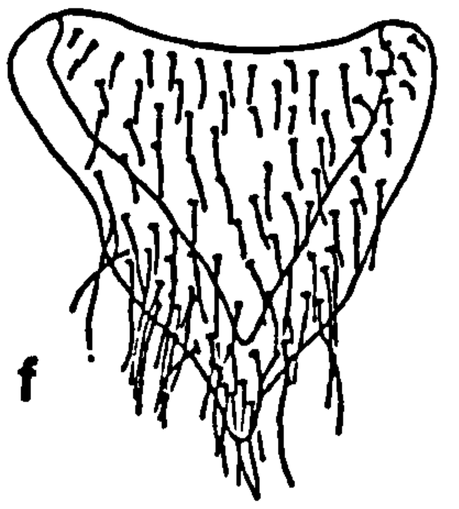
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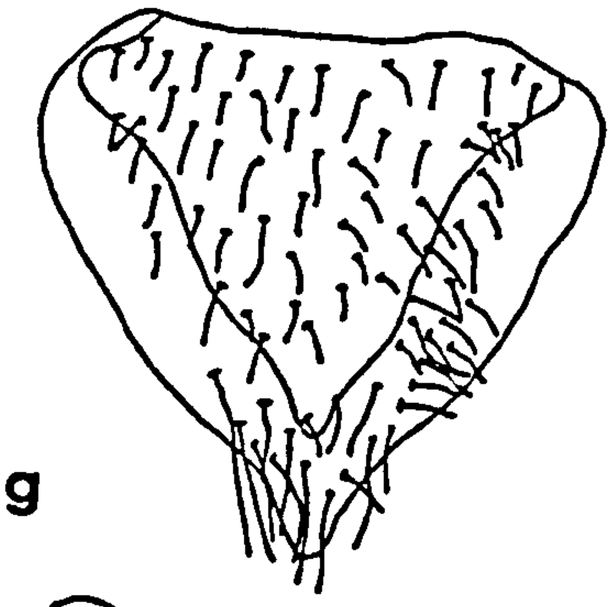
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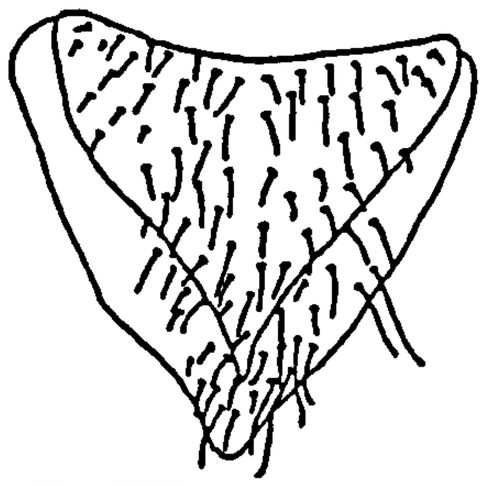
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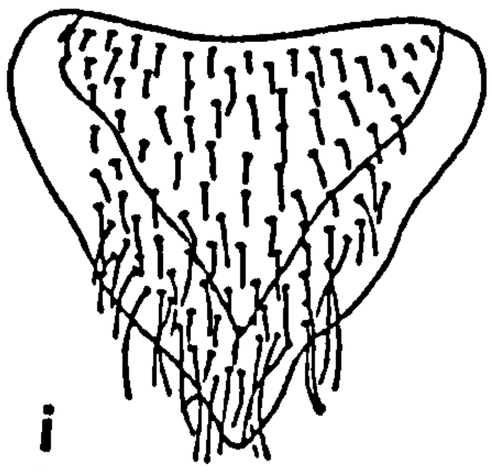
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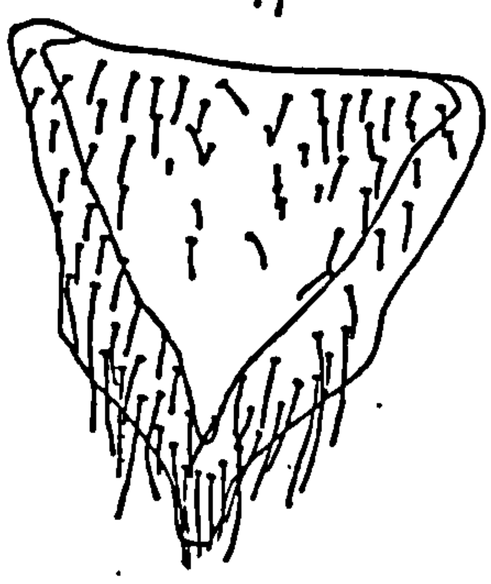
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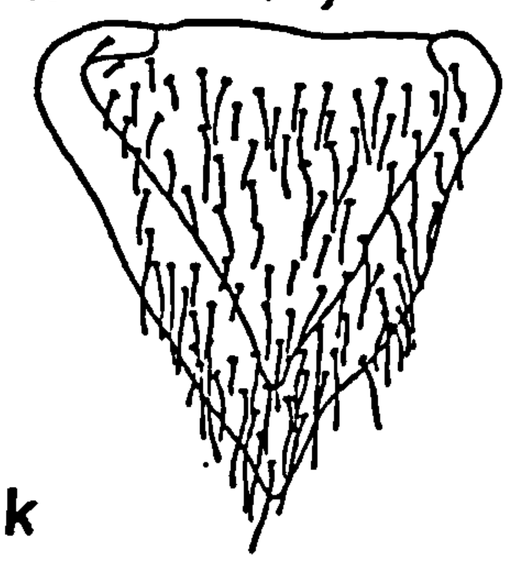
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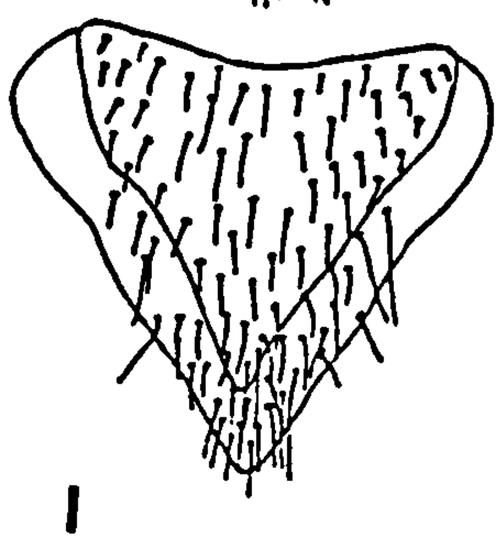
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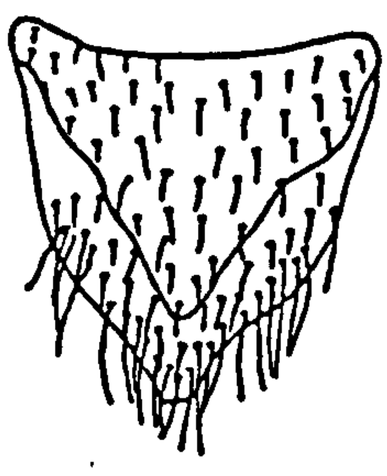
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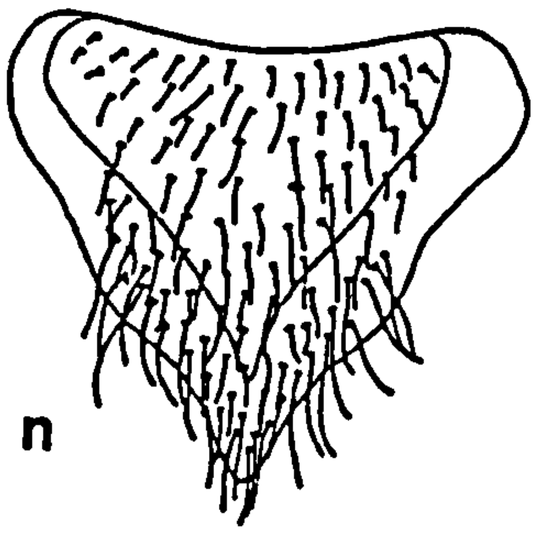
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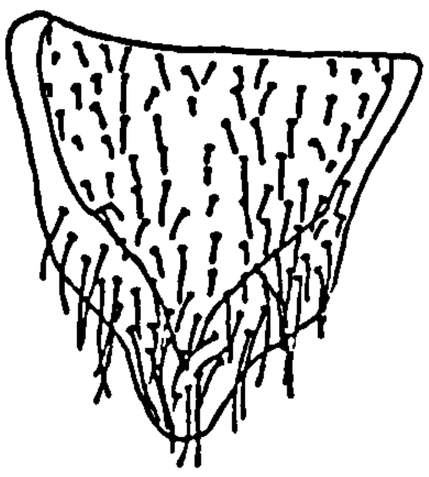
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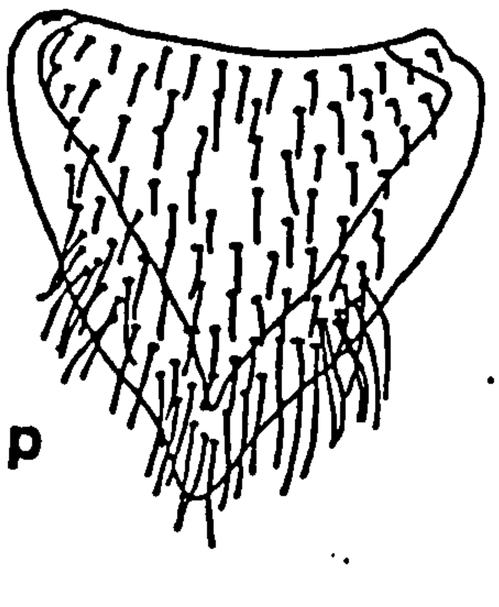
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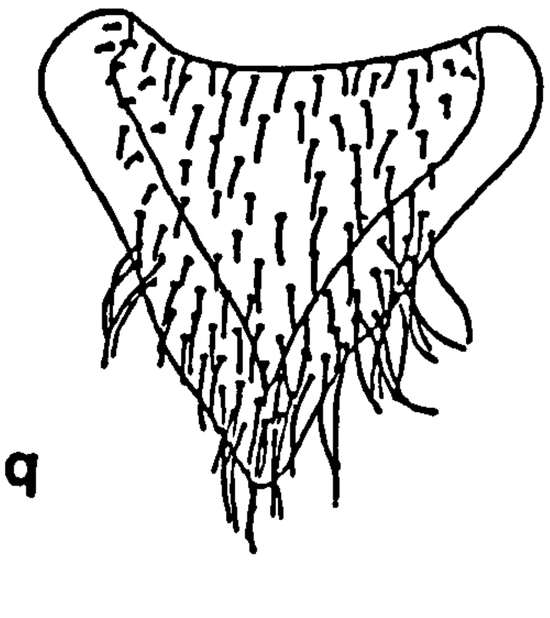
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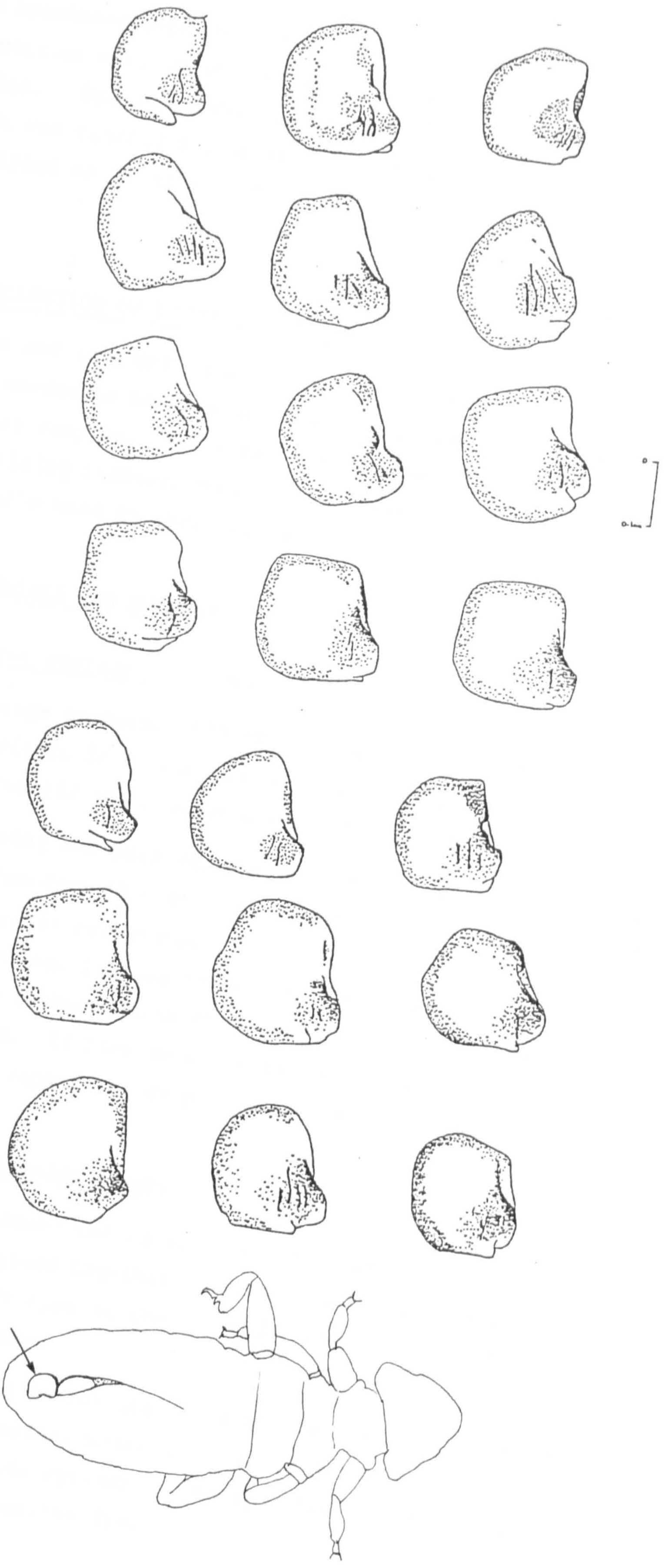
FIGURE 12 : Shape of posterior plate of male genitalia of *Trinoton* sp.

(Note, considerable variation exists in the shape of this chitinised area of the male genitalia. There is no distinct dichotomy suggesting the existence of two species. Whole diagram of louse indicates position of plate in male genitalia)



More specimens were available for comparison in this study and overall, no features were identified which consistently distinguished the two species. Specimens show individual variation within the same species and it was concluded that the species collected in this study should be classified as *T. anserinum*.

1.2 COLLECTION OF ECTOPARASITE SAMPLES

A simple and safe method was required for removing lice from swans. Samples needed to be obtained quantitatively in order to be comparable with other samples. Three techniques were considered: two were deparasitising chambers based on the method of Fowler and Cohen (1983) successfully used on small birds, and the third was a hand-search method.

1.2.1 MATERIALS AND METHODS

1.2.1.1 Water storage container

A water storage container (75 cm x 60 cm x 45 cm) lined with filter paper had placed within it a mesh cradle supported by hooks. A hole (10 cm^2) was cut in the lid and covered around the edge by foam.

During delousing the swan was placed on the cradle with its head through the lid. Chloroform (5.0 cm^3) on cotton wool was introduced into the container where it evaporated. The sides of the lid were clipped on to the base to prevent leakage of vapour. The maximum period for delousing was 20 minutes because swans showed signs of distress if maintained for a longer period. If lice were present on the swan they were killed by the chloroform vapour and dropped onto the filter paper.

1.2.1.2 Carbon dioxide chamber

A bag (1.7 m^3 volume) was constructed from "ground-sheet" material. It was sewn and glued together to form an air-tight seal. A hole (25 cm diameter) was left open at the top and a smaller inlet for carbon dioxide was made in the side.

The swan was placed inside the bag with its head projecting through the hole and carbon dioxide, which anaesthetises the insects, was introduced from a carbon dioxide cylinder. The swan had enough room to open its wings and shake parasites free.

1.2.1.3 Hand-search method

Feathers were searched for lice over the back, wings, head and neck. Lice were removed by hand and placed in pill boxes. To standardise this method it was necessary to determine an 'optimal searching time'. The optimum period was attained by delousing thirty swans each for 20 minutes. The number of lice collected in every 5 minute period was recorded.

1.2.2 RESULTS

1.2.2.1 Water storage container

This technique was unsuitable for delousing swans. Five swans were processed in the container but no lice were recovered and it was concluded that the chloroform vapour did not penetrate through the swan's dense plumage. It was later established that lice were present on two of the swans by a hand-search.

1.2.2.2 Carbon dioxide chamber

This method using an air-tight bag was not suitable for delousing swans. The major drawback was the difficulty in collecting ectoparasites from a large surface area of the bag after delousing. The technique proved inefficient if several swans were deloused in succession.

1.2.2.3 Hand-search method

A hand-search through the feathers was the best method of obtaining lice. The advantage of this method was the ease with which lice were removed once located on the bird. In addition, lice could be removed alive for use in further experiments.

1.2.2.4 Determination of optimal searching time

The 'optimal searching time' was 5 minutes. Thirty swans were each deloused for 20 minutes. Of all the lice collected, 89% were removed in the first 5 minute interval (Table 17). Only 11% were collected in the 5 to 10 minute interval, and no lice were found after 10 minutes of searching.

The hand-search method was adopted as the standard delousing procedure for this study.

TABLE 17: Determination of optimal searching time for removal of *T. anserinum* from swans

	TIME PERIODS (MINUTES)			
	0 - 5	5 - 10	10 - 15	15 - 20
NUMBER OF	42	5	0	0
% OF TOTAL	89	11	0	0

(* Number of swans deloused = 30)

1.3 ADAPTATION OF *T. anserinum* TO PARASITISM

Mallophaga are a group of obligate ectoparasites whose entire life-cycle from egg to adult is spent on the host (Clay, 1957; Ewing, 1929).

The louse population on one host is usually separable into a number of morphological types adapted to conditions specific to different ecological niches on the body of birds (Baum, 1968; Nelson and Murray, 1971; Nelson, 1972). This phenomenon is most obvious amongst the Ischnoceran species whose adaptive features are mainly associated with maintaining contact with the feathers and avoiding the preening bill of the host. On the head of a swan, for example, is found a short, round-bodied slow moving louse (*Anatoecus* sp.). It is adapted for movement on the shorter, narrower feathers of the head and being out of reach of the bill it does not need to move very fast. On the longer, broader feathers of the back and wings is found a flattened, elongate louse (*Ornithobius* sp.) which can easily slip sideways across the feathers. In Amblycera, diverse morphological types are less common and in general these lice rely on their greater speed for protection from preening. This certainly seems to be true of *T. anserinum* which shows a number of morphological features associated with rapid movement amongst the feathers.

1.3.1 MATERIALS AND METHODS

Specimens of *T. anserinum* were prepared for scanning electron microscopy. They were cleaned in acetone using ultrasonication (ultrasonic cleaner: Mettler Electronics). Specimens were mounted using araldite on

FIGURE 13 : Adaptation of *T. anserinum* to parasitism.

A. Tarsal claw at the end of the third leg of *T. anserinum*.

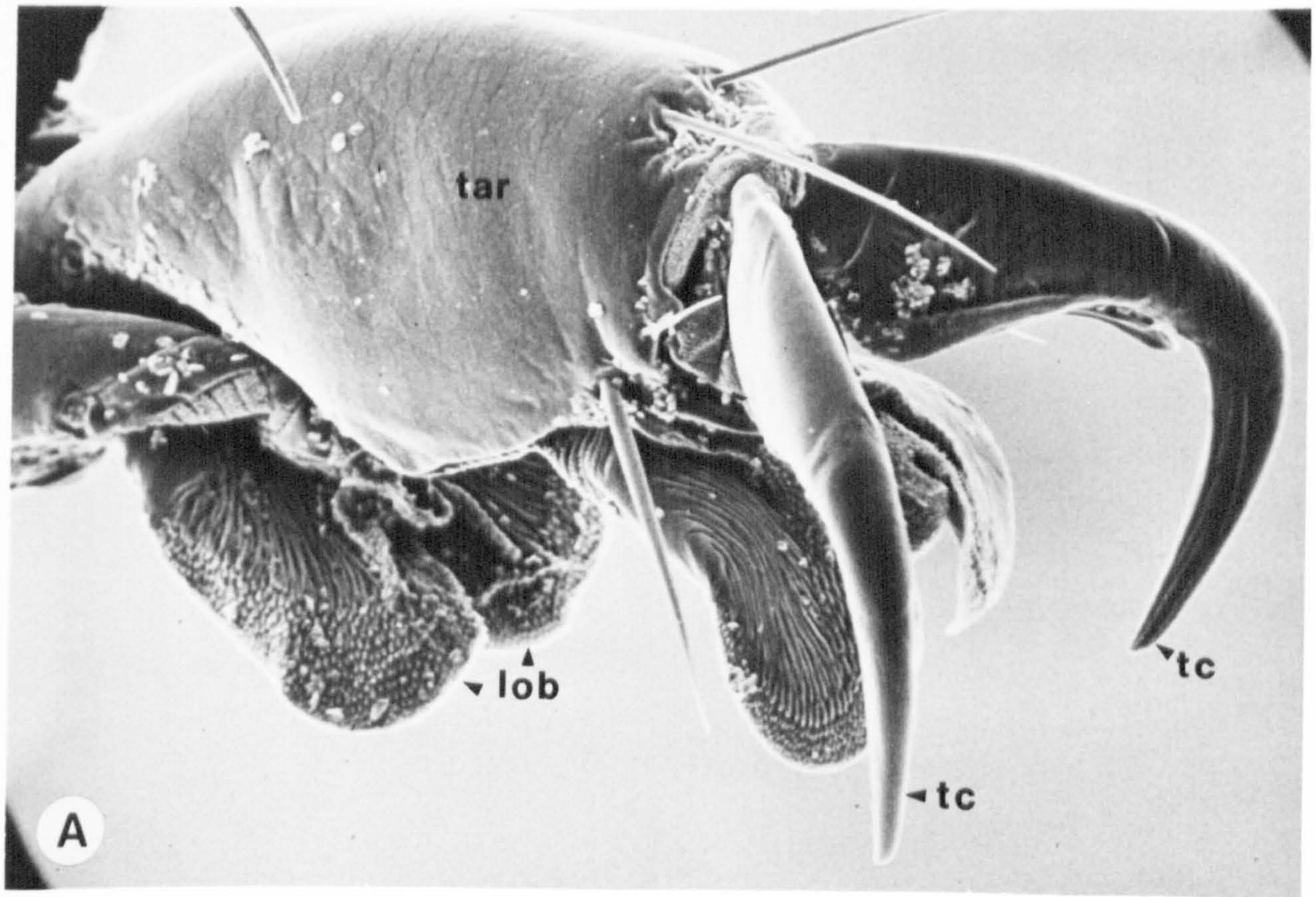
(Note, two pairs of lobed pads behind claws.
The large claws point backwards and close onto the
pairs of pads forming a gripping action)

0·1mm

B. Hook-like spicules of a lobed pad situated on the tarsus.

tar tarsus
tc tarsal claw
lob lobed pads
sp spicules

0·01mm


tar
lob
tc
tc
A

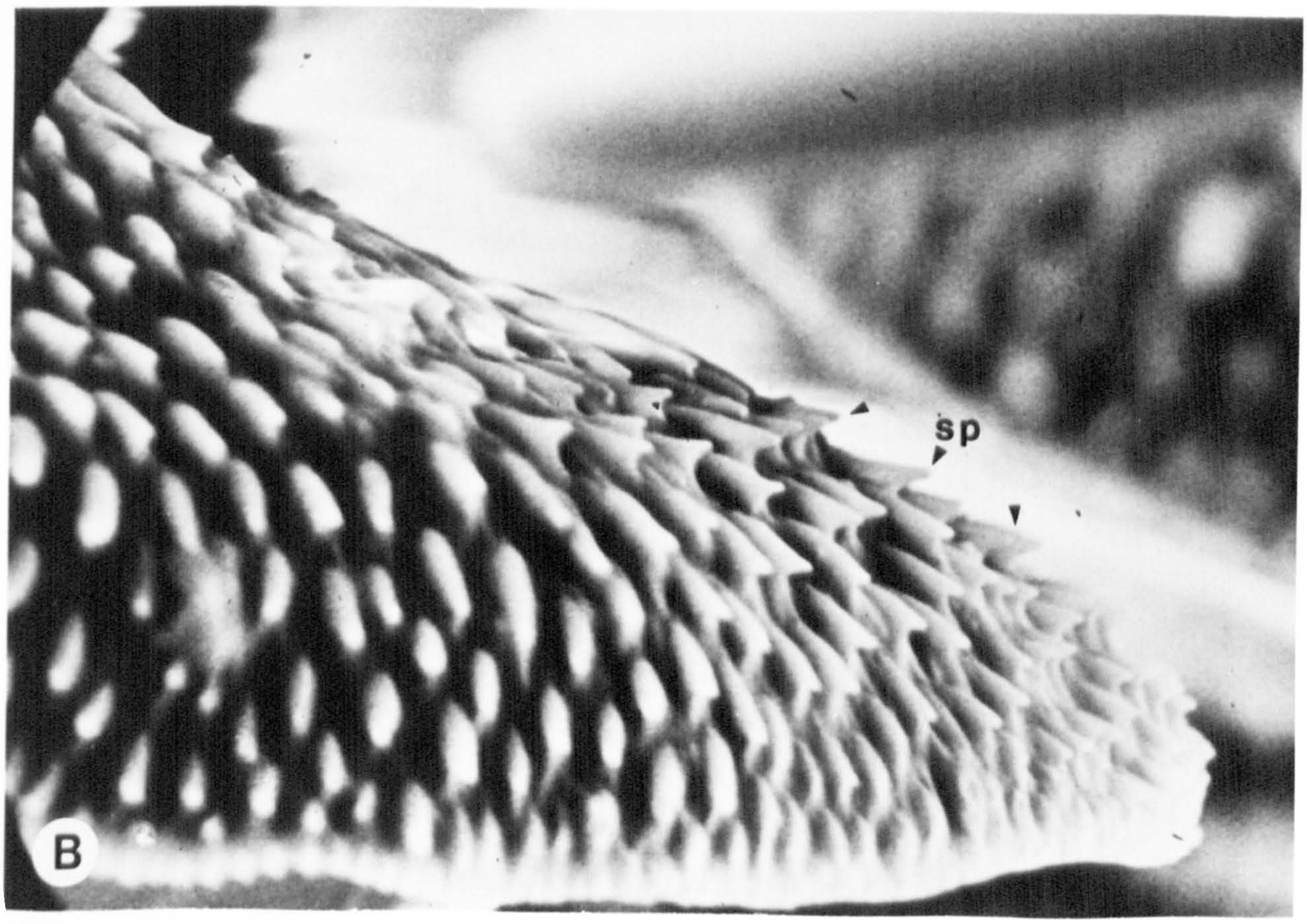

sp
B

FIGURE 14 : Adaptation of *T. anserinum* to parasitism.

A. Patch of setae on the ventral surface of the third femur. The setae are thought to be associated with clinging onto the host's feathers.

0·5mm

B. Left - patches of dense setae on ventral surface of fourth and fifth abdominal segments associated with anchorage to host.

Right - detail of highlighted area given in left-hand figure.

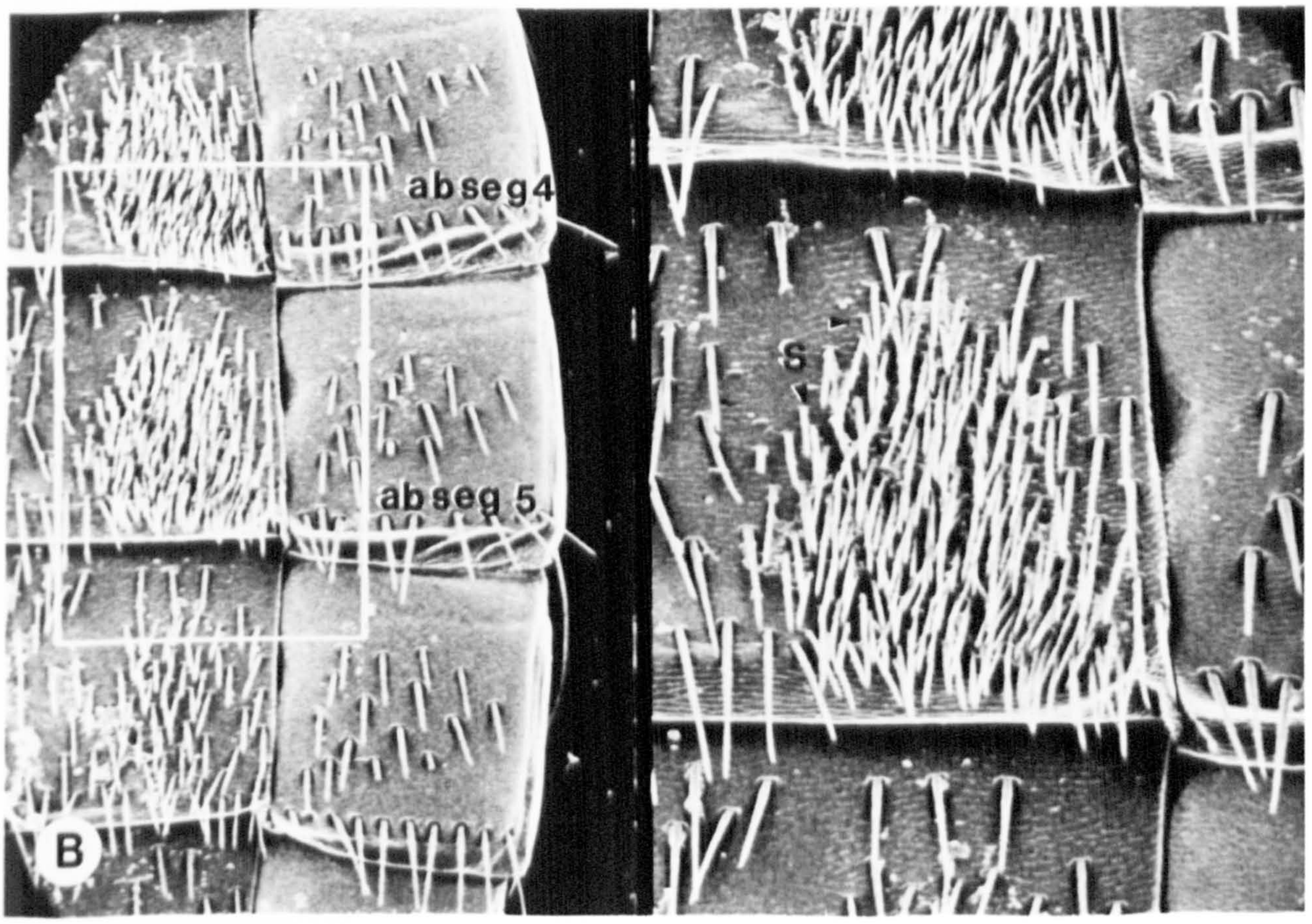
f femur

abseg 4 abdominal segment 4

s setae

0·5mm


A


abseg4
abseg 5
S
B

aluminium stubs and coated with a thin layer of gold (10 μm) using a Polaron sputter coating unit. Lice were viewed with a scanning electron microscope ISI-SS40 using an accelerating voltage of 10 Kv and the magnifications ranging from 50 X to 3000 X. Morphological features associated with an adaptation to parasitism were photographed.

1.3.2 RESULTS

The body of *T. anserimum* is dorso-ventrally flattened, the dorsal surface of which is relatively featureless apart from arrangements of setae projecting from it. On the ventral surface structures are present which may be associated with attachment to the swan.

The legs are robust ending in tarsal claws which are adapted for grasping onto feathers (Fig. 13A). There are two claws behind which are two pairs of lobed 'pads' (Fig. 13B). The pads are smooth on the undersides but have 'hook-like' spicules on the opposing sides. The roughened pads probably provide a gripping surface against which the claws can enclose feather barbs.

If setae are used in the anchorage of lice to the host, a patch of setae on the ventral surface of the third femur may be associated with attachment (Fig.14A). Similarly there are two patches of dense setae on the fourth and fifth segments of the abdomen which perform a similar function (Fig. 14B). In addition, *T. anserimum* has concealed antennae which are common to all Amblycerans.

DISCUSSION

Ectoparasite sampling

Total populations of lice are virtually impossible to remove from birds (Marshall, 1981). The only way to obtain all lice is to carry out a feather-by-feather examination of dead hosts.

Two of the techniques in the present study used deparasitising chambers but were not suitable for delousing swans. These methods were based on the technique of Fowler and Cohen (1983) where passerines were quantitatively deloused. Chloroform and carbon dioxide were unable to penetrate the swan's plumage. Waterfowl have a layer of down feathers

covering the skin surface together with secondary layers of contour feathers and semiplumes. Compared to the feather covering of passerines, swans have a much denser plumage which possibly restricts penetration of vapour and subsequent effects on the insect.

The hand-search method was suitable because it was safe, simple and reliable. In the field, the five-minute searching period was found to provide an 'optimal' time for examining some 40-50 swans in succession in any one catching location. The method provided a quantitative standard by which parasite loads on swans could be compared. It is likely that a longer searching time would have revealed a higher incidence of *T. anserinum* , but fewer swans would have been searched.

2. SPATIAL AND TEMPORAL RELATIONSHIP BETWEEN *T. anserinum* AND THE SWAN

To implicate an insect as a natural intermediate host of a filarial parasite, the insect must at some time come into contact with the definitive host.

If all life stages of the suspected intermediate host can be identified on the definitive host there must be a close spatial and temporal relationship between them. In addition, the incidence and degree of infestation of the insect would provide greater insight into its potential role as an intermediate host.

This section seeks to determine how closely the life-history of *T. anserinum* is associated with the swan by:

2.1) identifying all life stages of *T. anserinum* on the swans, and;

2.2) examining the incidence of *T. anserinum* in swan populations and the degree of infestation on individual birds.

2.1 IDENTIFICATION OF LIFE STAGES OF *T. anserinum* ON SWANS

Feather lice (Mallophaga) undergo an incomplete metamorphosis during their life-cycle. Eggs are laid and cemented onto feathers where they require the warmth of the host's body for development. The first nymphal instar emerges from the egg and after approximately 3 days it moults into an identical but slightly larger second nymphal instar

(Stockdale and Raun, 1965) working with *Menacanthus stramineus (Amblycera)*. A third instar nymph follows, which in turn moults to become and adult. The longevity of adults *M. stramineus* is approximately 20 days.

Because insect growth occurs in a stepwise manner, measurements of certain parameters can be taken to separate the population into different age-classes (for example, Fowler, Miller and Cohen, 1983).

2.1.1 MATERIALS AND METHODS

Five parameters of *T. anserinum* (N = 326) were measured using a binocular microscope (X40): a) total body length; b) head width; c) head length; d) third femur width; e) third femur length.

Measurements were plotted to determine the parameters which showed the greatest size variation between age-clases. Mean values for head width and total body length of each size class were calculated and Dyar's Law (Teissier, 1936) was applied to confirm that each size class corresponded in increasing size to first, second and third instar nymphs, adult male and female population classes.

Eggs were illustrated using a binocular microscope and further explored using a specialised cryogenic facility attached to the scanning electron microscope unit at MAFF (Slough).

2.1.2 RESULTS

Biometric measurements of lice were plotted and show three nymphal instars and the adult life stages. (Fig.15A, Table 18). The features which showed the greatest size variation and therefore the best separation of points, were head width and total body length. Three nymphal instars and adult male and female stages of *T. anserinum* (N = 326) were found on swans (Fig.17). Eggs of *T. anserinum* were also removed from swan's plumage. Developing stages of the embryo were visible within the eggs (Figs.15B and 18).

Dyar's Law is evidently held with respect to the three nymphal instars (Fig.16). The alignment of the three points representing the mean head width values for each instar shows that all nymphal instars of *T. anserinum* were collected.

FIGURE 15A : Locations of biometric measurements taken on *T. anserinum*.

Five measurements were taken from each specimen to separate them into different age-classes. Total number of specimens measured = 326.

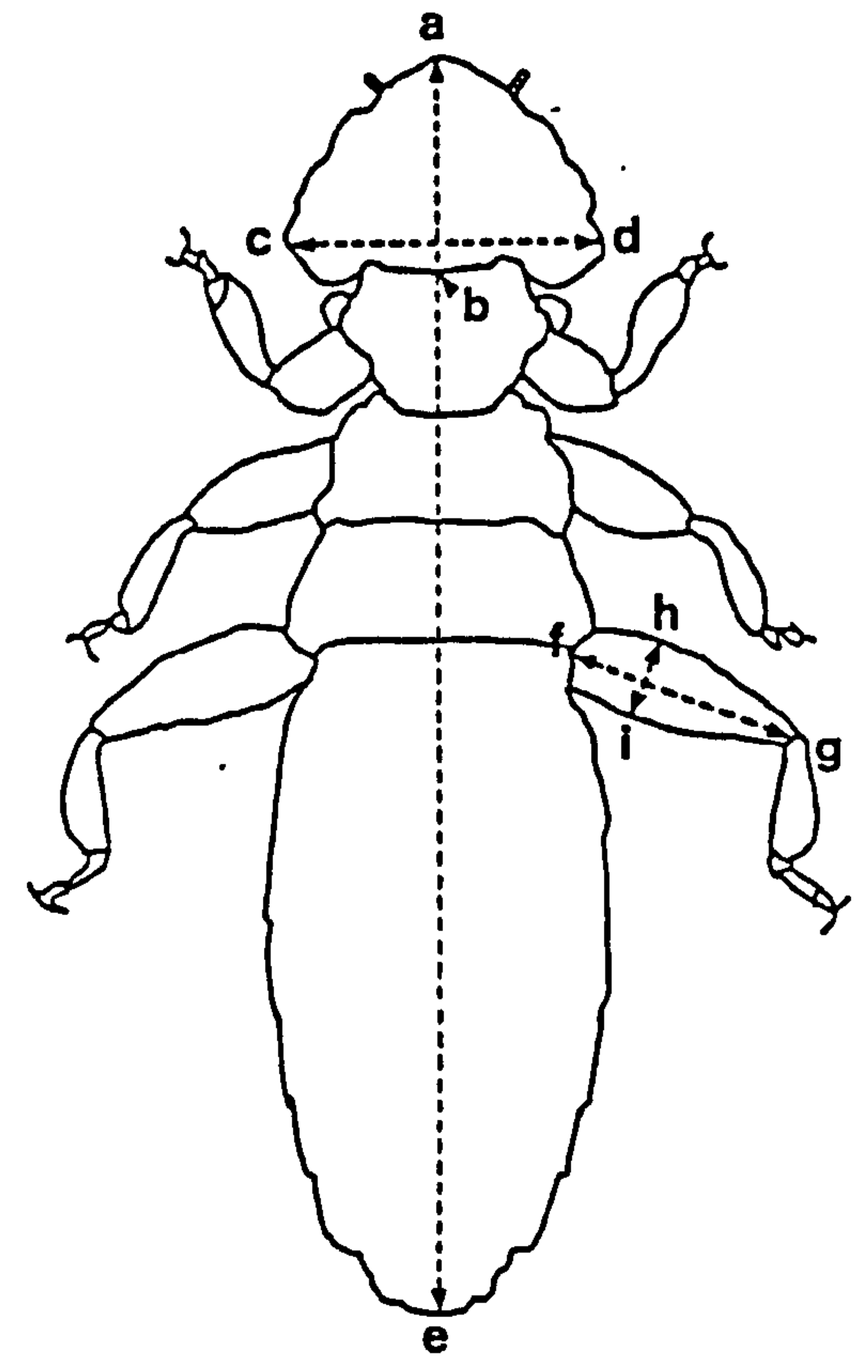
a-b head length (all measurements
a-e total body length were taken in mm)
f-g femur length
h-i femur width
c-d head width

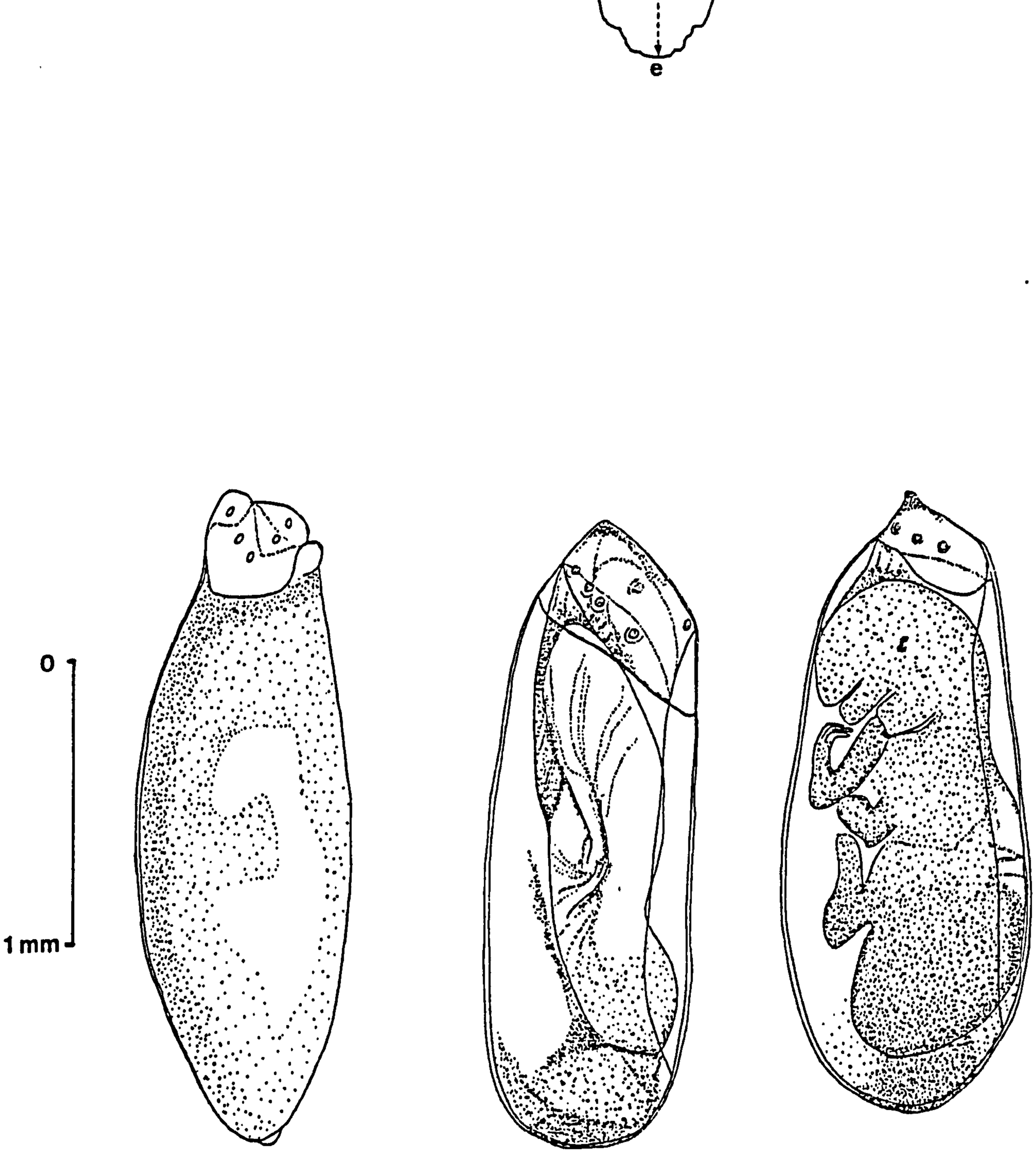
FIGURE 15B : Developmental stages of *T. anserinum* in the egg.

A Early developmental stage

B Embryo forming within the egg

C Embryo with early formation of eyes and limbs.





A B C

Figure 16 : Mean head width values for each size class of *T. anserinum*.

Alignment of these values confirms Dyar's Law (Teissier, 1936) which states that each size class corresponds in increasing size to first, second, third instar nymphs and adult male and female population classes.

● mean head width values

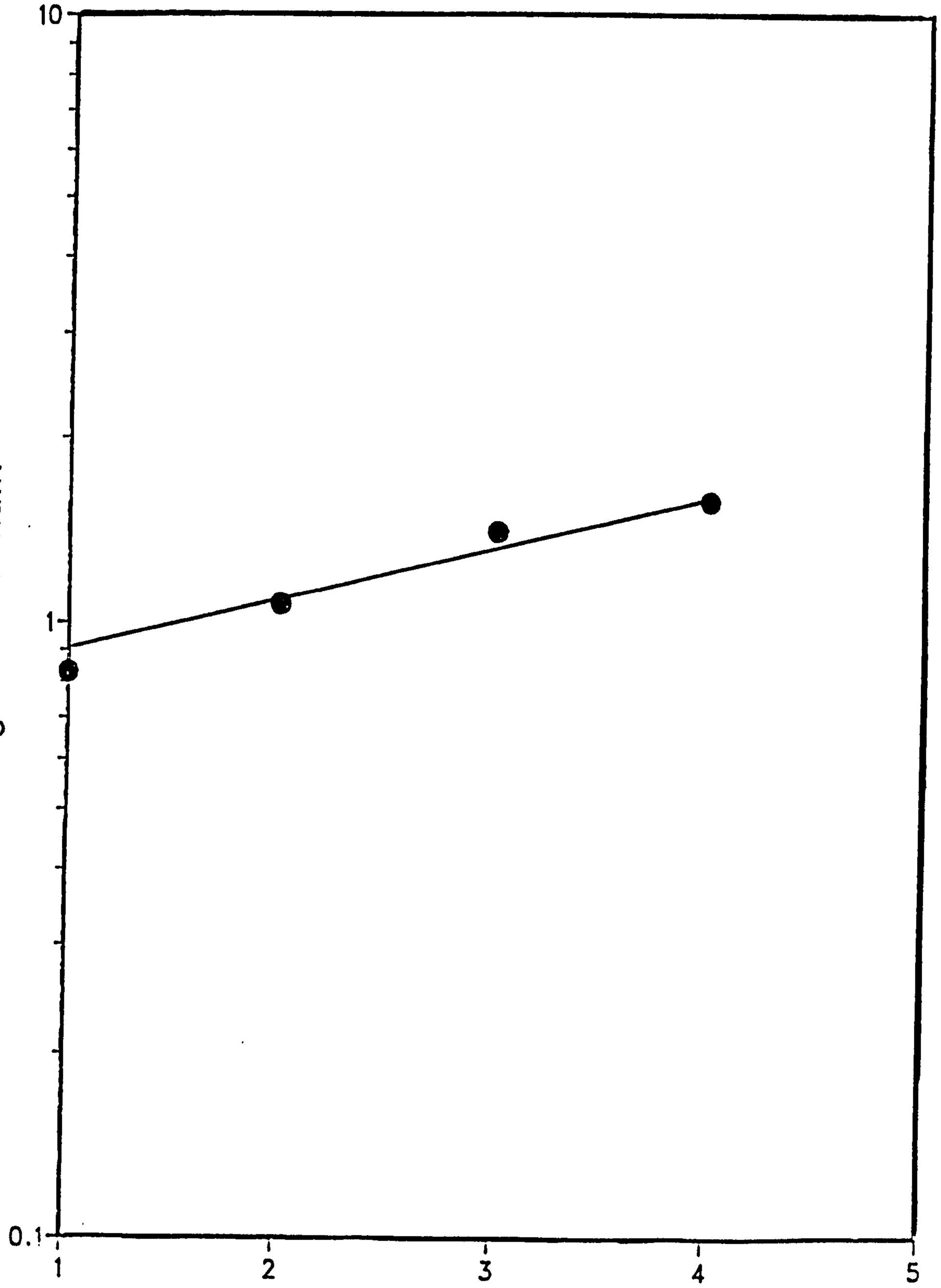

10
1
0.1
Log. of head width
1
2
3
4
5
Size class

FIGURE 17 : Head width and total body length of *T. anserinum* to show separation of specimens into different age-classes.

(Total number of specimens = 326)

Out of the five biometric measurements, head width and total body length produced the best separation of points.

- mean value for each age-class with 95% confidence limits.

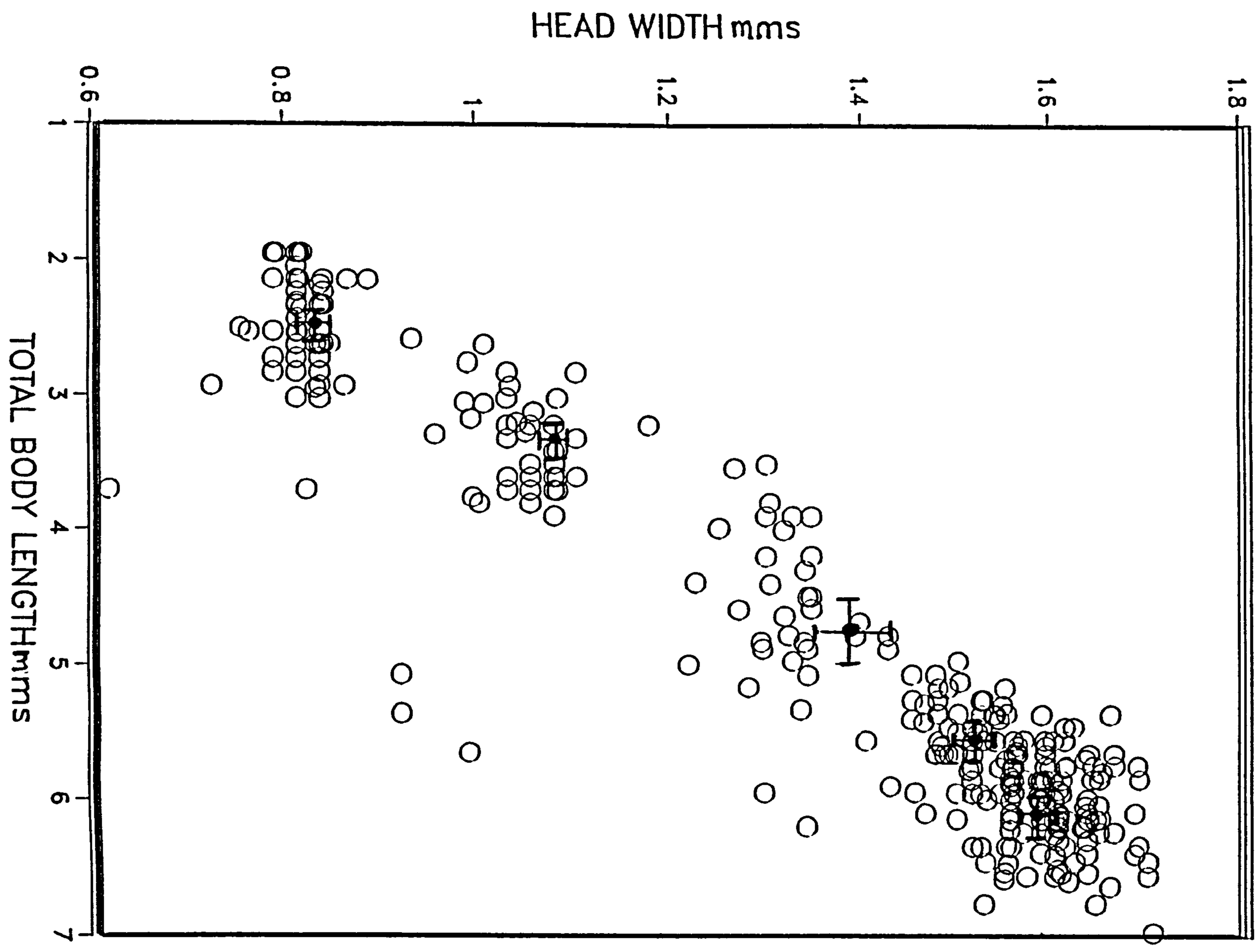

HEAD WIDTH mms
0.6
0.8
1
1.2
1.4
1.6
1.8
TOTAL BODY LENGTHmms
1
2
3
4
5
6
7

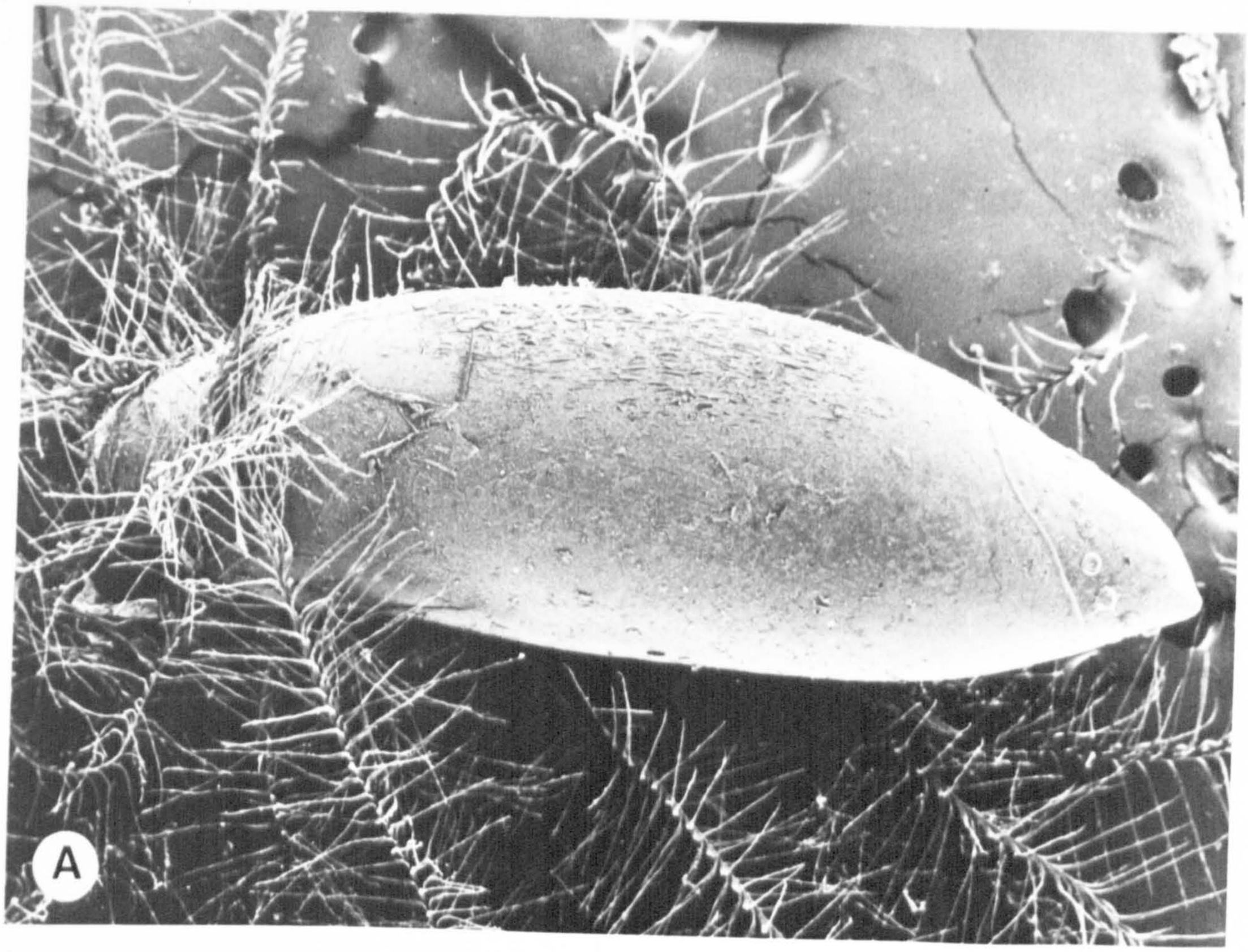
FIGURE 18 : Eggs of *T. anserinum* removed from swan's plumage.

A. Whole egg showing oval shape and egg cap on right hand side.

400µm

B. Detail of egg cap with twelve micropyles.

200µm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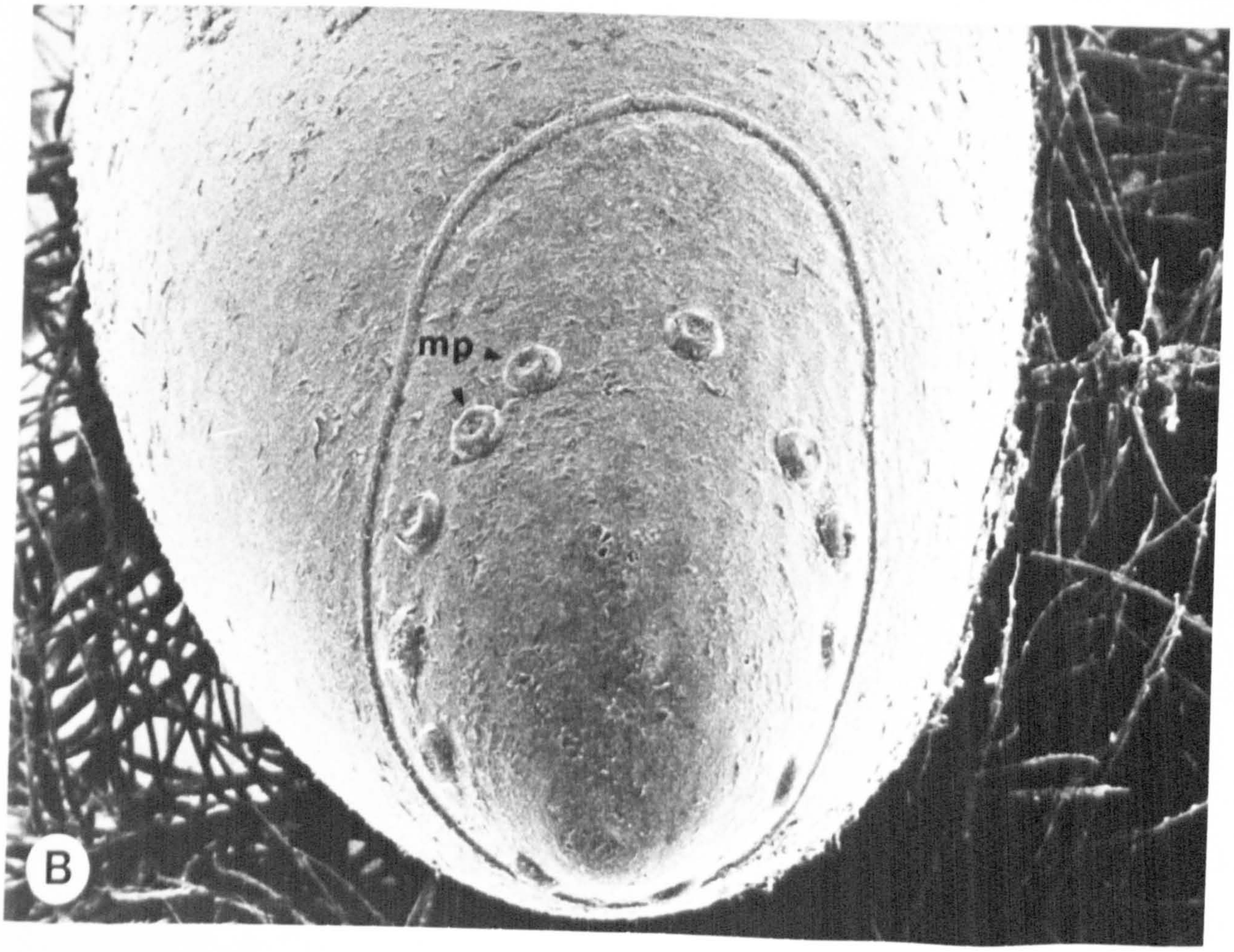

mp
B

TABLE 18: Biometrics of *T. anserinum*

BIOMETRIC MEASUREMENTS (mm)	N	Mean	SD	SE	Range
Nymph 1					
Head length	67	0.54	0.08	0.010	0.46 - 1.07
Head width	67	0.83	0.02	0.003	0.73 - 0.90
Total body length	67	2.49	0.31	0.040	1.95 - 3.02
Femur length	67	0.47	0.03	0.004	0.44 - 0.63
Femur width	67	0.22	0.02	0.003	0.17 - 0.34
Nymph 2					
Head length	47	0.65	0.13	0.020	0.49 - 1.34
Head width	47	1.07	0.10	0.010	0.63 - 1.34
Total body length	47	3.39	0.44	0.060	2.59 - 5.17
Femur length	47	0.63	0.09	0.010	0.44 - 0.85
Femur width	47	0.30	0.04	0.010	0.22 - 0.41
Nymph 3					
Head length	44	0.79	0.11	0.020	0.56 - 1.00
Head width	44	1.40	0.15	0.020	1.10 - 1.71
Total body length	44	4.79	0.79	0.120	3.41 - 6.51
Femur length	44	0.91	0.14	0.020	0.61 - 1.22
Femur width	44	0.42	0.07	0.010	0.27 - 0.56
Adult males					
Head length	77	0.91	0.10	0.010	0.76 - 1.56
Head width	77	1.54	0.10	0.010	0.93 - 1.68
Total body length	77	5.67	0.32	0.040	4.97 - 6.46
Femur length	77	1.05	0.06	0.010	0.80 - 1.17
Femur width	77	0.47	0.49	0.040	0.34 - 0.54
Adult females					
Head length	89	0.93	0.12	0.010	0.76 - 1.61
Head width	89	1.59	0.11	0.010	0.93 - 1.73
Total body length	89	6.05	0.39	0.040	5.07 - 5.98
Femur length	89	1.08	0.06	0.010	0.88 - 1.30
Femur width	89	0.48	0.04	0.005	0.39 - 0.71

2.2 INCIDENCE AND DEGREE OF INFESTATION OF *T. anserinum* ON SWANS

It has been established that the potential intermediate host *T. anserinum* is a permanent ectoparasite of the definitive host (p. 65). This relationship ensures that the insect has at least the opportunity of coming into contact with the filarial parasite circulating in the peripheral blood supply. To illustrate this association in a wider sense, it should be possible to show that the insect is in sufficient numbers on swans to guarantee continuation of the filarial infection within the swan population.

2.2.1 MATERIALS AND METHODS

Lice were collected in three ways:

1) the standard hand-search method (p. 59) applied to swans in Great Britain;
2) a hand-search with no standard time was used by other workers who obtained opportunistic samples of lice;
3) a complete body search of dead swans to determine total parasite counts.

2.2.2 RESULTS

2.2.2.1 Incidence of *T. anserinum* on swans

1. A total of 387 swans were searched for lice using the standard hand-search method (p. 59). Of these, 354 were Mute Swans, 9 were Whoopers, and 24 were of other species in Wildfowl Trust collections (Table 19). Overall, *T. anserinum* was removed from 32 swans (8.3%); 31 were Mute Swans and one was a Black Swan (Table 20). The identity of the specimen taken from a Black Swan is uncertain and still has to be confirmed.

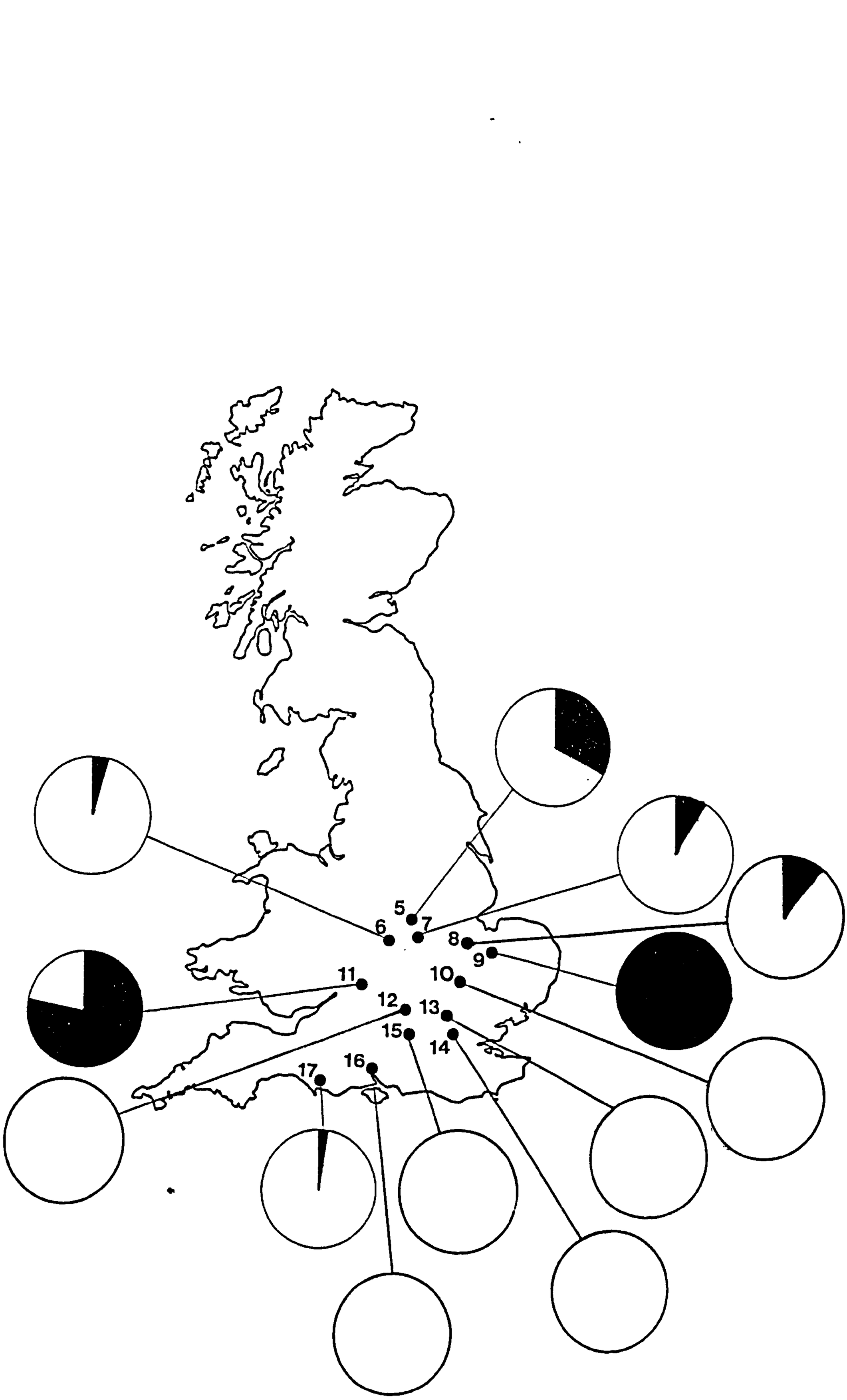
Twelve (37.5%) adult and 20 (62.5%) juvenile swans carried *T. anserinum* and equal numbers of males (N = 9. 30.0%) and females (N = 9, 30.0%) were infested; 12 swans were of unknown sex.

The geographical distribution of *T. anserinum* collected by the hand-search method (Fig. 19) is somewhat distorted by the large numbers of swans with lice in Cheltenham. All swans searched at this site were sick or injured and were kept in large aggregations in a Wildlife Hospital.

2. Opportunistic samples of *T. anserinum* were taken from Icelandic Whooper Swans and from Mute Swans in Denmark, Windsor, a veterinary centre

FIGURE 19 : Geographical location and incidence of *T. anserinum* on swans.

(lice collected by hand-search method)


5
6
7
8
9
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15
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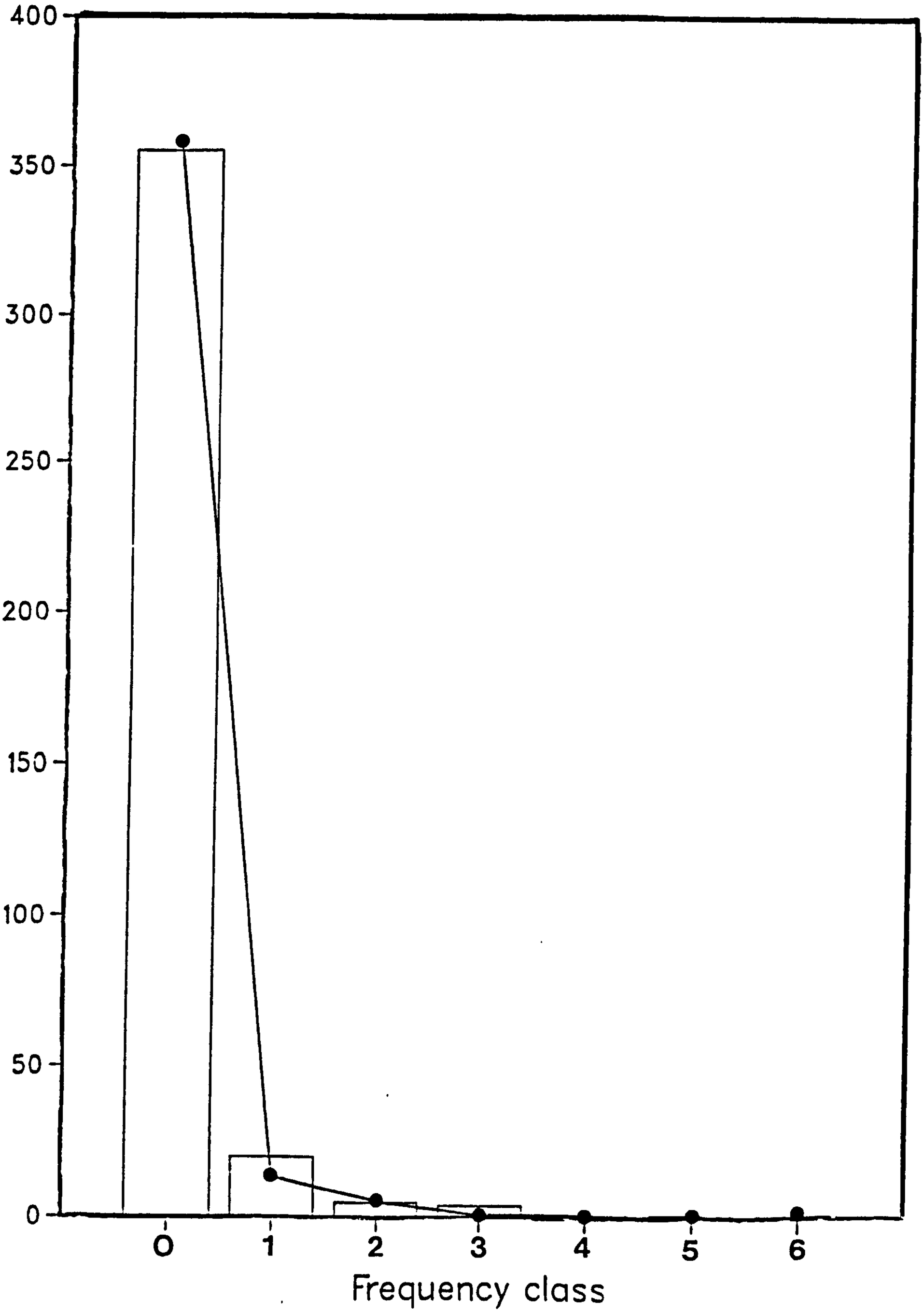
17

FIGURE 20 : Frequency distribution of *T. anserinum*. on swans.

● expected frequencies of a negative binomial distribution estimated from the sample statistics.


400
350
300
250
200
150
100
50
0
Observed frequency of T. anserinum
0
1
2
3
4
5
6
Frequency class

in Derby and a Nottingham R.S.P.C.A. station. A total of 43 specimens were collected opportunistically using the hand-search method without a standard searching time (Table 21).

3. Four dead Mute Swans obtained from the Veterinary Investigation Centre, Sutton Bonington, with particularly large numbers of lice were totally deparasitised to determine total louse counts (Table 22). From each swan, 109, 32, 53, and 26 *T. anserinum* were collected.

2.2.2.2 Incidence of both *T. anserinum* and *S. eurycerca* on swans

Out of the 32 hand-searched swans found to have *T.anserinum* , twelve (37.5%) of these also harbored *S. eurycerca*.

The overall incidence of the louse and nematode occurring together was 3.1%.

2.2.2.3 Infestation levels of *T. anserinum* on swans

The mean number of lice found on 32 hand-searched swans was 1.97 ranging from 1-12 lice per bird (Table 20). The number of lice on heavily infested dead swans, ranging from 26-109 lice per bird, are excluded from the mean value given above because they would distort the level of infestation for healthy swans.

Body populations of lice and mites on birds have been shown by researchers to conform well with a negative binomial distribution; that is, the majority of parasites are found on relatively few hosts (Cohen, 1983, unpubl.; Fowler and Miller, 1984; Fowler and Williams, 1985; Fowler and Price, 1987).

The frequency distribution of *T. anserinum* on swans is clearly an aggregated (contagious) type (Fig. 20). Expected frequencies were calculated (*) for a negative binomial distribution and shown to be in close agreement with the observed frequencies ($X^2_4 = 3.21$, $p>0.05$).

(* : an exponent, $\underline{k}$, is estimated from $\underline{k} = \overline{x}^2 / (\overline{s}^2 - \overline{x})$ and is used to calculate the expected frequencies for a negative binomial distribution based on the sample mean and variance.)

Table 19 : Indicence of *T. anserinum* on swans searched by hand

LOCATION	NO. OF SWANS WITH *T. anserinum*	NO. OF SWANS SEARCHED	% OF SWANS WITH *T. anserinum*
5. Nottingham	1	3	33.3
6. Alvecote,Staffs	7	149	4.7
7. Leicester	4	51	7.8
8. Peakirk,Cambs	2	18	11.1
9. St Neots,Cambs	1	1	100.0
10. Welney,Cambs	0	31	0.0
11. Cheltenham	16	20	80.0
12. Oxford	0	40	0.0
13. Hemel Hempstead	0	1	0.0
14. Thames	0	14	0.0
15. Reading	0	28	0.0
16. Lymington	0	1	0.0
17. Abbotsbury, Dorset	1	38	2.6
TOTAL	32	387	

Table 20 : Swans with *T. anserinum* (hand search method)

SITE	AGE OF SWAN	SEX OF SWAN	NO. OF TRINOTON	STATUS OF SWANS
Leics.	Juv.	-	1	A
Leics.	Ad.	-	1	A
Leics.	Juv.	-	2	A
Leics.	Juv.	F	1	A
Notts.	Ad.	-	3	-
St.Neots, Cambs.	Ad.	F	1	S
Alvecote, Staffs.	Juv.	F	1	MF
"	Ad.	M	1	MF
"	Ad.	M	1	MF
"	Ad.	M	1	MF
"	Ad.	F	1	MF
"	Juv.	M	1	MF
"	Juv.	M	1	MF
Abbotsbury, Dorset	Ad.	F	1	MF
Peakirk, Cambs.	Ad.	M	3	S
"	Ad.	F	1	P
Cheltenham, Glos.	Juv.	-	1	A
"	Ad.	F	1	A
"	Ad.	M	2	A
"	Juv.	F	3	A
"	Juv.	-	2	A
"	Juv.	M	2	A
"	Juv.	-	1	A
"	Juv.	-	1	A
"	Juv.	-	1	A
"	Juv.	-	3	A
"	Juv.	1	2	A
"	Juv.	-	5	A
"	Juv.	-	1	A
"	Juv.	-	12	A
"	Juv.	F	1	A
"	Juv.	-	4	A

A = Aggregates
MF = Moulting Flock
S = Single
P = Pair

Table 21 : Opportunistic samples of *T. anserinum*

SITE	SPECIES	AGE OF SWANS	SEX OF SWANS	NO. OF TRINOTON	NO. IN AGE-CLASSES Ad. ♀	Ad. ♂	N3	N2	N1
Iceland	Whooper	Ad.	M	1	1				
Iceland	Whooper	Juv.	M	1		1			
Iceland	Whooper	Ad.	F	1		1			
Iceland	Whooper	Juv.	M	1	1				
Windsor	Mute	Ad.	-	1	1				
Windsor	Mute	Juv.	F	7	1	2	2	1	1
Windsor	Mute	Juv.	F	7	3	4			
Windsor	Mute	Juv.	F	5	3	2			
Denmark	Mute	Ad.	-	1	1				
Denmark	Mute	Ad.	-	1	1				
Denmark	Mute	Ad.	-	1		1			
Denmark	Mute	Ad.	-	1		1			
Vet.Centre, Derby	Mute	Juv.	-	2	2				
RSPCA, Nottm.	Mute	Ad.	-	13	4	6	0	2	1

Table 22 : Total body searches of dead Mute Swans for *T. anserinum*

SITE	AGE OF SWANS	SEX OF SWANS	NO. OF TRINOTON	NO. IN AGE-CLASSES Ad. ♀	Ad. ♂	N3	N2	N1
Leics.	Ad.	-	109	9	14	10	29	47
Notts.	Ad.	-	32	--14---		9	8	1
Notts.	Ad.	-	53	7	10	18	6	12
Notts.	Ad.	F	26	--11---		3	11	1

DISCUSSION

Spatial and temporal relationship between *T. anserinum* and swans

Nelson (1964) describes the ideal vector-host-parasite relationship as one where there is a long established obligatory association between the vector and the definitive host. *T. anserinum* and the swan exhibit such an association because the louse is a permanent ectoparasite and has been shown, in this section, to be in contact with its host, the swan, during all stages of the life-cycle. Lice in general have a much more intimate spatial and temporal relationship with their hosts than more temporary ectoparasites. Winged insects such as mosquitoes, blackflies, midges and sandflies are all significant vectors of filarial parasites but only temporarily contact the host when they require a blood meal.

If *T. anserinum* is an intermediate host, its incidence and infestation in swan populations would directly influence the continuation of *S. eurycerca* infections. Since there was no information available on the incidence and infestation rates of *T. anserinum* these factors were investigated in this study. The results of these investigations are now considered in relation to aspects of the swans' status,condition and behaviour.

An overall incidence of 8.3% is probably an underestimate of the "real" incidence of *T. anserinum* on swans. There are always errors in parasite collection and the inefficiencies of the hand-search technique which could contribute to these errors have already been discussed.

The incidence and infestation levels of *T.anserinum* on swans are dependent on many factors including the hosts' age, health, behaviour and abundance. Species other than the host may also influence louse abundance. Interspecific and intraspecific competition with other ecto-parasites and associations with pathogens and predators can affect abundance. However, it is thought that in general most of these factors are not of great importance apart from intraspecific competition which has rarely been observed but could play a critical role in population regulation (Marshall, 1981).

The host appears to exert the greatest influence on its ectoparasitic population. In theory, the older the host is, the longer it has had to make contact with infested hosts and acquire parasites (Kennedy, 1975). But Hopkins (1949) observes that there is a tendency for young animals to

be more heavily infested than adults due to a relative immunity on the part of the adults. For swans it seems that the opportunities for acquisition of lice are greatest early on in the bird's life. Young cygnets will probably become infested with lice if their parents are infested. There is close contact between the cob, pen and cygnets for 4-5 months before the young depart (for Bewick's Swans the association is only for 40-45 days and for Whooper Swans it is 70 days). It is common to observe Mute Swan cygnets riding on their parents' backs (Birkhead and Perrins, 1986). Indeed, more than 60% of the swans with *T. anserinum* in this study were juveniles.

Once young swans have left their natal site, they join immature flocks where there is physical contact between many swans and therefore opportunity for parasite transfer. Birkhead and Perrins (1986) report that some juveniles begin to engage in courtship behaviour whilst still in their dark brown plumage and this continues until they find a mate and leave the flock at 3-4 years old.

Once an established pair have dispersed to find a breeding territory the opportunities for louse transfer are much reduced. Indeed, the only contact a pair of swans has with others may be during territorial disputes. Serious fights are rare but involve considerable contact between opponents; necks become entwined, wings are beaten and the winner usually mounts the opponent in a display of supremacy. The paired swans searched in this study from the Thames, Oxford and Reading did not have *T.anserinum*.

Any kind of aggregation of the host which will increase the probability of bodily contact is critical to louse populations (Marshall, 1981). *T. anserinum* was found on the swans in immature flocks at Alvecote and Leicestershire and amongst the moulting flock at Abbotsbury an incidence of 2.6% was recorded. Swans were sometimes placed in artificial aggregations in RSPCA and swans rescue centres. At the British Wildlife Hospital, Cheltenham 80% of swans had *T. anserinum*. At St.Neots RSPCA centre and the Windsor Swan Rescue centre too, the birds were sick or injured and louse transfer was probably enhanced within the confines of the enclosures.

Swans also form aggregations when they collect in wintering flocks at Caerlaverock (Dumfries) and Welney (Cambs.). Due to the organisational circumstances at these catching sites none of the migrant swans were searched for lice. However, it is likely that the opportunitie s for louse transfer in such an aggregated population are similarly enhanced. Lice

which spend their whole lives on their hosts are generally highly species-specific and are usually found only on one or a few closely related hosts (Hopkins, 1949; Clay, 1949; Marshall, 1981). *T.anserinum* is less host-specific and has been recorded from five swan species (Seguy, 1944; McKelvey and MacNeill, 1980; Seegar, 1977). Wintering flocks of Mute, Whooper and Bewick's Swans are ideal situations for interspecific parasite transfer. Although this has not been directly observed, *T. anserinum* has been found on Mute and Whooper Swans in this study. The degree of interspecific competition between Mute, Whooper and Bewick's Swans during feeding has been compared (Black and Rees, 1984). When grain was provided (in morning and afternoon feeds) all three species contested for space and most of the aggression and bodily contact was recorded during feeding times. Swans were observed to press against each other whilst competing for food providing opportunity for louse transfer (Rees, pers.comm.). Nelson (1972) describes the occurrence of interspecific transfer of lice with numerous examples of mixed-flock species such as passerines and migrant birds.

Host behaviour can affect the incidence and infestation of lice in other ways. It is well known that preening reduces parasite load on birds (Kartman, 1949; Rothschild and Clay, 1952; Baum, 1968; Nelson and Murray, 1971). Swans spend much of their time preening. Black and Rees (1984) report that Mute Swans spend 25 per cent of their time engaged in "comfort" activities - that is, preening, sleeping, sitting or bathing. For healthy swans, preening is a normal daily activity but is notably reduced or absent in sick or injured birds. Unhealthy hosts therefore have larger populations of ectoparasites than healthy ones (Marshall, 1981). Hopkins (1949) observed sick animals are more heavily infested with both Mallophaga and Anoplura (sucking lice) than are those in good health. Eichler (1942) records many instances of sick mammals and birds with abnormally heavy louse infestations. Ash (1960) records 10,000 individuals of *Austromenopon* sp. taken from a gull (*Larus* sp.) in poor health. The higher incidence and infestation levels of *T. anserinum* found on sick swans may be attributable to a decrease in preening activities. High infestations were recorded from four Mute Swans which had all died from lead poisoning. The deterioration in neuro-muscular function particularly of the neck from lead may prevent the swan from preening and consequently allow the louse population to increase unchecked.

Times of moulting may play a role in the control of louse populations. Swans moult once a year in July and August, flight is prevented and a great many feathers are lost at this time (Birkhead and Perrins, 1986). Three populations of swans were searched for lice during the moult. Incidence levels of *T. anserinum* of 2.6% were recorded at Abbotsbury, 4.7% at Alvecote and 0.0% for swans on the Thames. Spring moults in some bird populations cause drastic falls in louse populations (Ash, 1960; Baum, 1968; Boyd, 1951; Cohen, 1983 (unpubl.); Dogiel, 1964; Foster, 1969; Woodman and Dicke, 1954). It is uncertain to what extent the summer moult in swans affects the population of *T. anserinum* but it could be suggested that the moult causes a seasonal fluctuation in the louse population.

The first attribute required on an intermediate host of *S. eurycerca* was that the insect must exhibit a spatial and temporal relationship with the swan. *T. anserinum* has been shown unequivocally to have such an association.

3. INGESTION AND TRANSMISSION OF THE FILARIAL PARASITE

Because microfilariae of *S. eurycerca* inhabit and circulate in the vascular system, one attribute an intermediate host must have is the ability to ingest blood.

To determine whether *T. anserinum* is capable of fulfilling the requirement of penetrating the skin and taking a blood meal, an examination was made of the following morphological and behavioural aspects associated with feeding:

3.1 structure of the mouthparts;
3.2 morphology of the alimentary canal;
3.3 crop contents and feeding methods;
3.4 location and timing of feeding of *T. anserinum* on Mute Swans.

3.1 STRUCTURE OF THE MOUTHPARTS

It is now widely accepted that Amblyceran lice feed on blood (Appendix 3). Members of the Ricinidae feed entirely on blood (Askew, 1971) as does *Piagetiella* which inhabits the pouches of pelicans. What has not been studied is the mechanism of blood-feeding in Amblycerans. To elucidate this it is essential to understand the structure of their mouthparts.

3.1.1 MATERIALS AND METHODS

1. Preparation of mouthparts for light microscopy

Lice were dissected in alcohol (70%) and dehydrated in a succession of alcohol solutions (80%, 95%, 100%) for 5 minutes in each solution. Specimens were cleared in terpineol for 1-2 hours before mounting in Canada balsam. They were viewed on a binocular microscope (x10 - 65) and drawings of dissected mouthparts were made using a camera lucida.

2. Preparation of mouthparts for scanning electron microscopy

Lice were prepared for the scanning electron microscope as described on p.60.

3.1.2 RESULTS

Structure of the mouthparts of Amblycera

The mouthparts of Mallophaga are of the biting type (Snodgrass, 1899) and consist of the labrum, mandibles, hypopharynx, maxillae and labium. In Amblycera, the labrum is very close to the front of the head, adjacent to which is a pair of large dentate mandibles which lie parallel to the ventral surface of the head. The hypopharynx is situated between the maxillae and mandibles and under the light microscope it appears as a transparent membranous structure covered with tufted projections. The elements of the maxillae are the stipes, palpi, lacinae and galea. In Amblycera maxillary palpi are four-segmented and provide a useful diagnostic feature in separating Amblyceran from Ischnoceran lice. The labium consists of a sub-mentum and mentum; the palpi are reduced to small lobes and the ligula is represented by a pair of membranous processes (Imms, 1948; Wigglesworth, 1940).

Structure of mouthparts of *T. anserinum*

Labrum

The labrum is situated anteriorly on the ventral surface of the head (Fig.21A & B). It is lined with a row of setae: some are peg-like (7µm - 20 µm in length) and are interspersed with needle-like setae (35 µm - 40 µm).

Mandibles

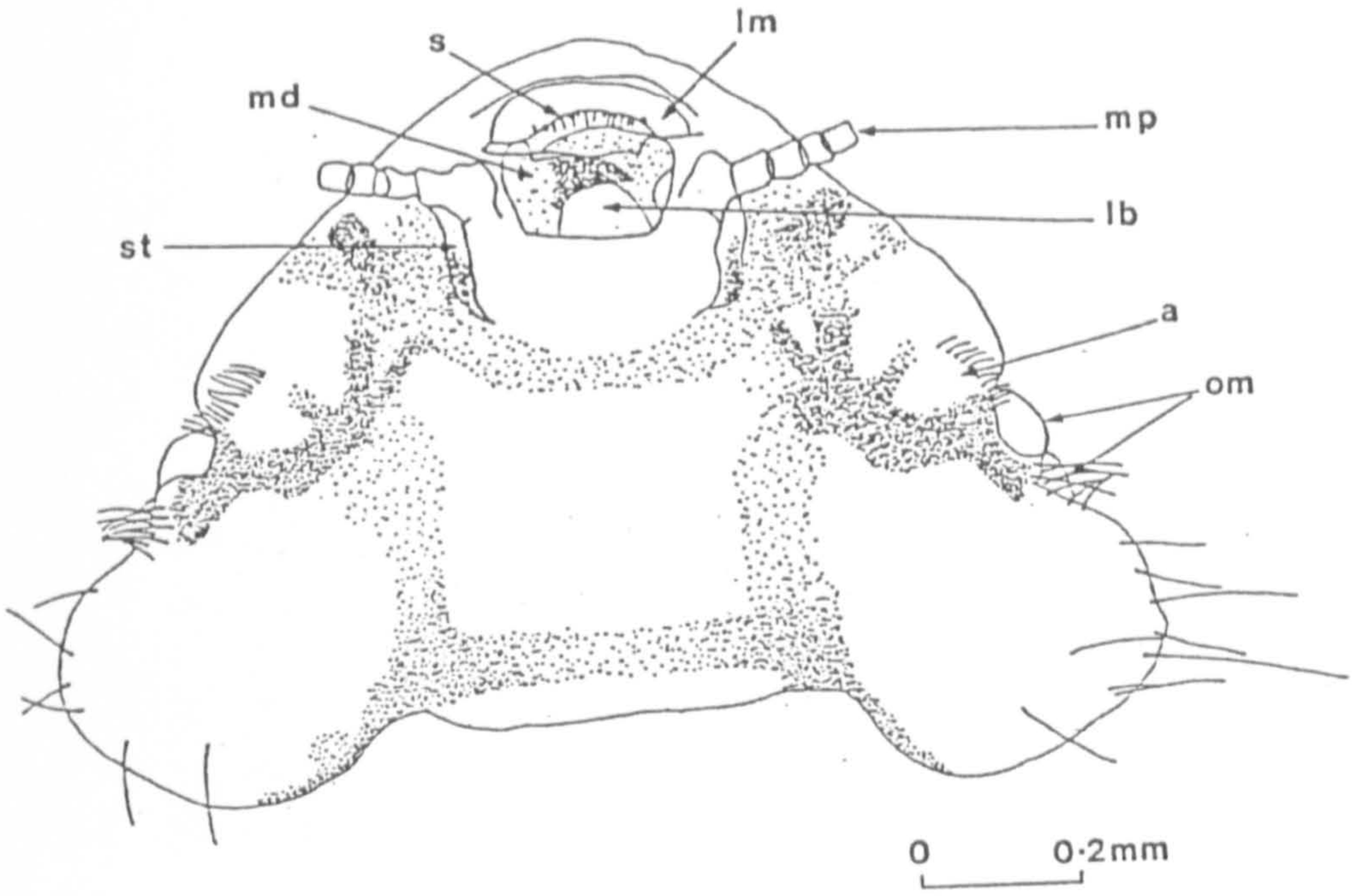
The mandibles of *T. anserinum* are large (0.4 mm), asymmetric and each has two apices (Fig.22C, D). The right mandible is stouter than the

FIGURE 21 : Structure of the mouthparts of *T. anserinum* viewed through light microscope.

A. Ventral view of head showing mouthparts.

B. Section of labrum lined with setae.

s	setae	st	stipes
lm	labrum	a	antenna
md	mandible	om	ommatidia
mp	maxillary palp	lb	labium
ps	peg-like setae		
ns	needle-like setae		



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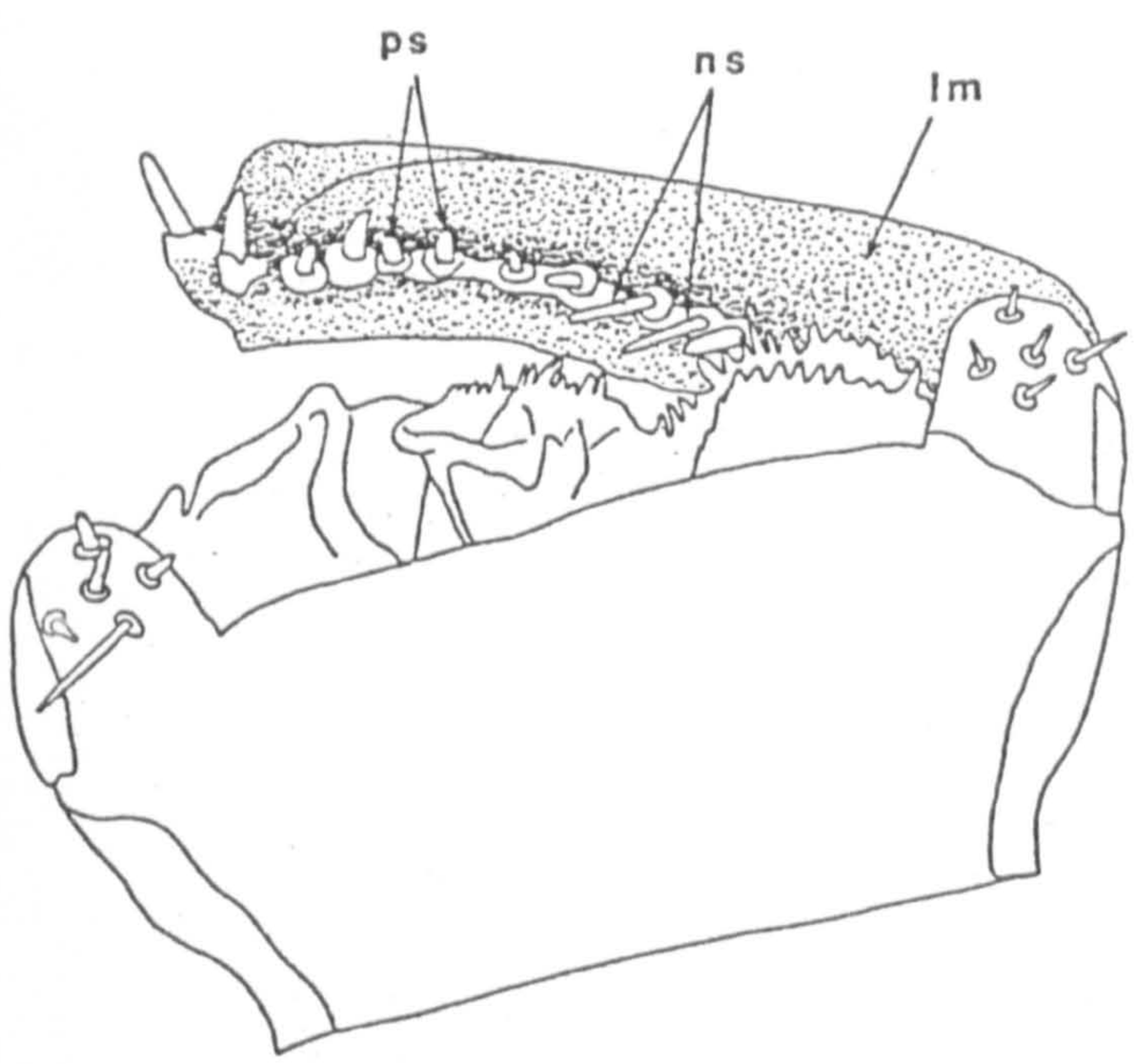
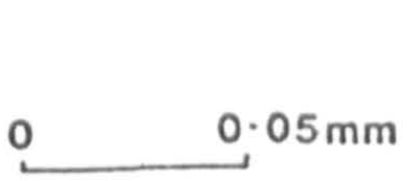




B

FIGURE 22 : Electron micrographs of the mouthparts of *T. anserinum*

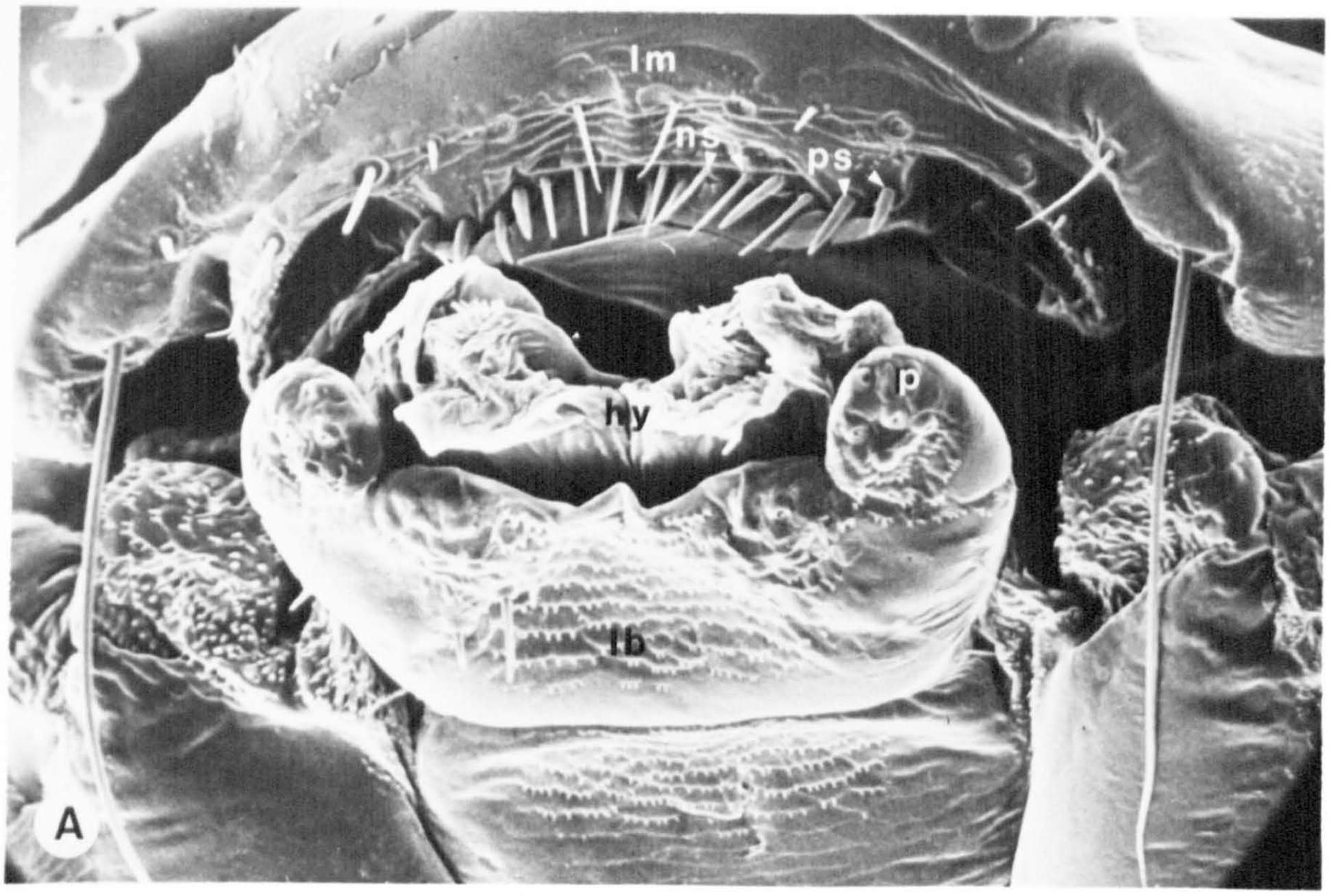
A. View of mouthcavity with labrum, hypopharynx and labium.

B. Mandibles in position within mouth cavity (labium and hypopharynx have been dissected to expose mandibles - note - left mandible has a deep cavity which receives the dorsal tip of the right mandible)

C. Right mandible with pointed apex.

D. Left mandible with deep cavity.

lm	labrum	ap	apex
ns	needle-like setae	dc	deep cavity
ps	peg-like setae		
hy	hypopharynx		
p	palp		
lb	labium		
rmd	right mandible		
lmd	left mandible		


lm
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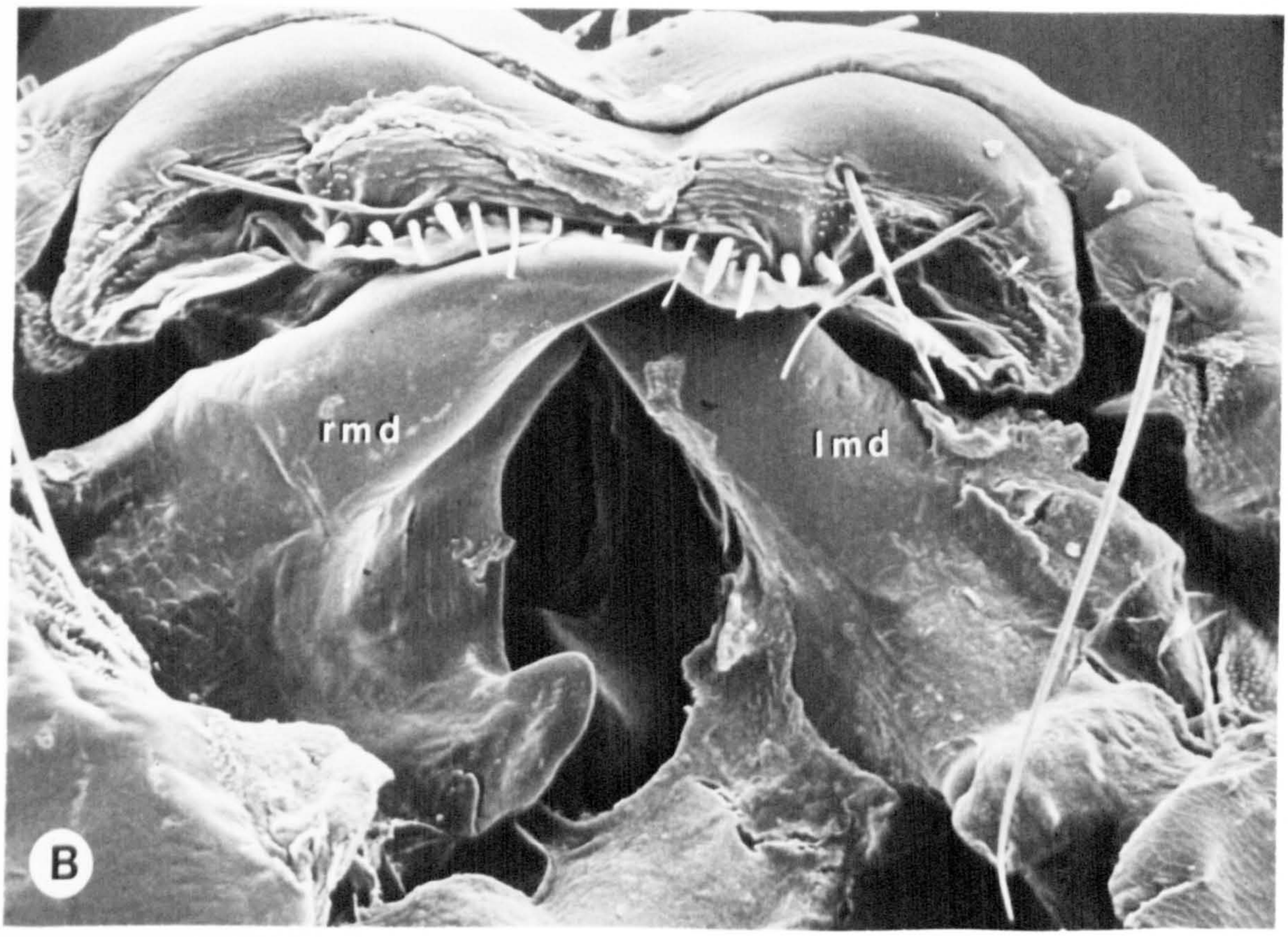
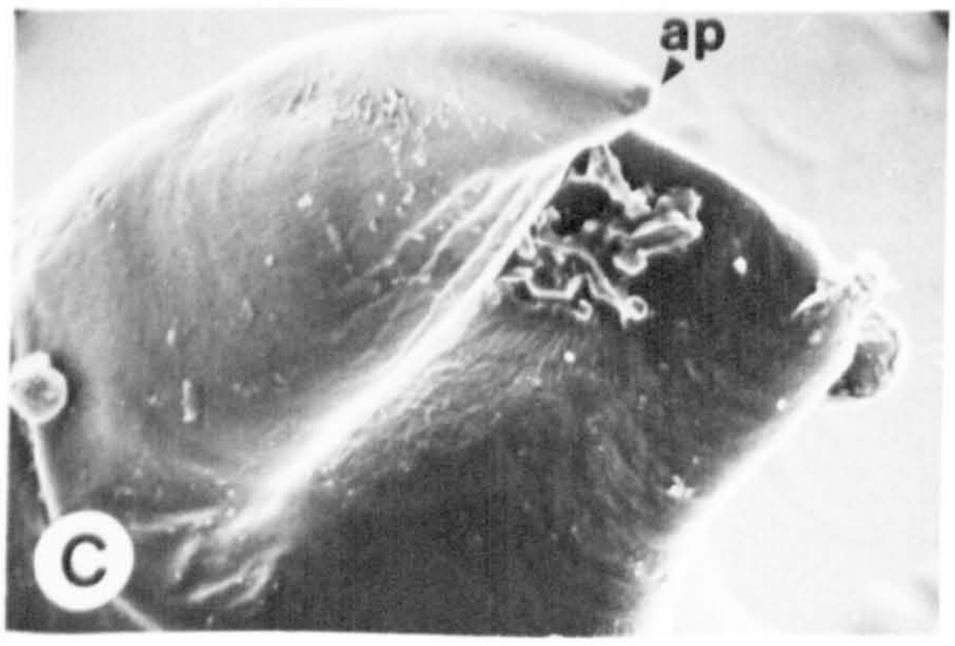

rmd
lmd
B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C


dc
D

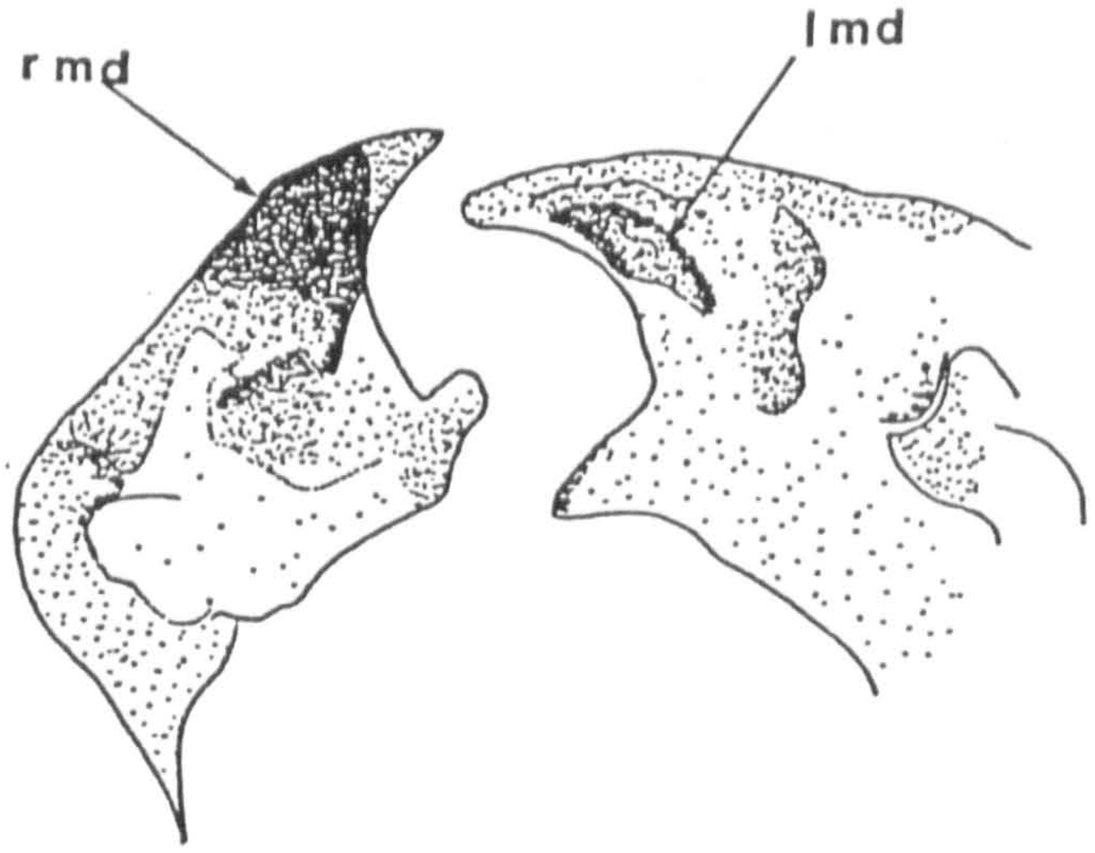
FIGURE 23 : Light microscope views of mouthparts of *T. anserinum*.

A. Right and left mandibles.
(note, more chitinisation of right mandible)

B. Position of mandibles in relation to the maxillae and labium.

C. One maxilla with components - galea, maxillary palp and stipes.
(note, cuticular teeth on edge of galea)

rmd right mandible
lmd left mandible
ga galea
lb labium
mp maxillary palp
st stipes
ct cuticular teeth

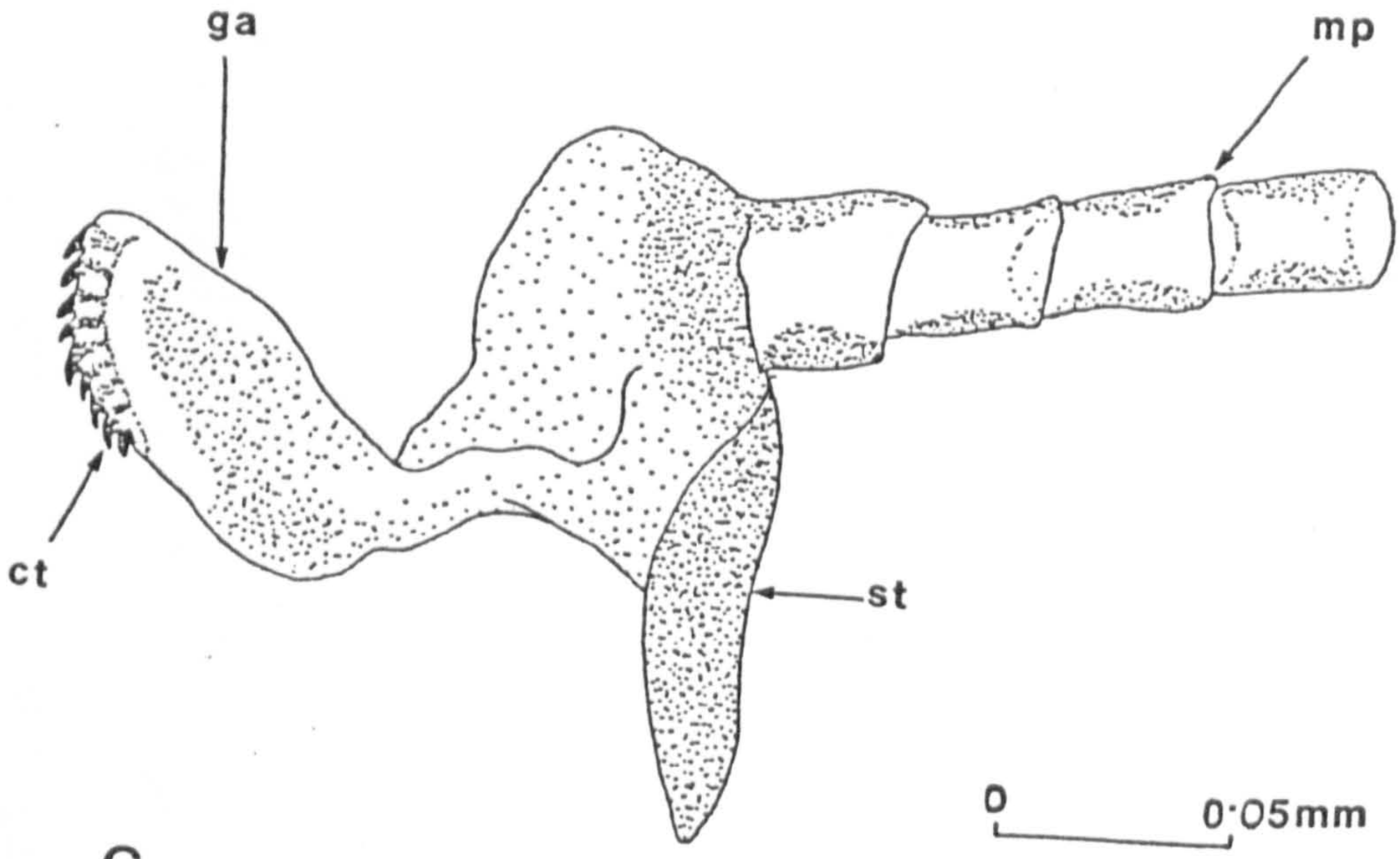


A

0 0·1mm

B

0 0·2mm



C

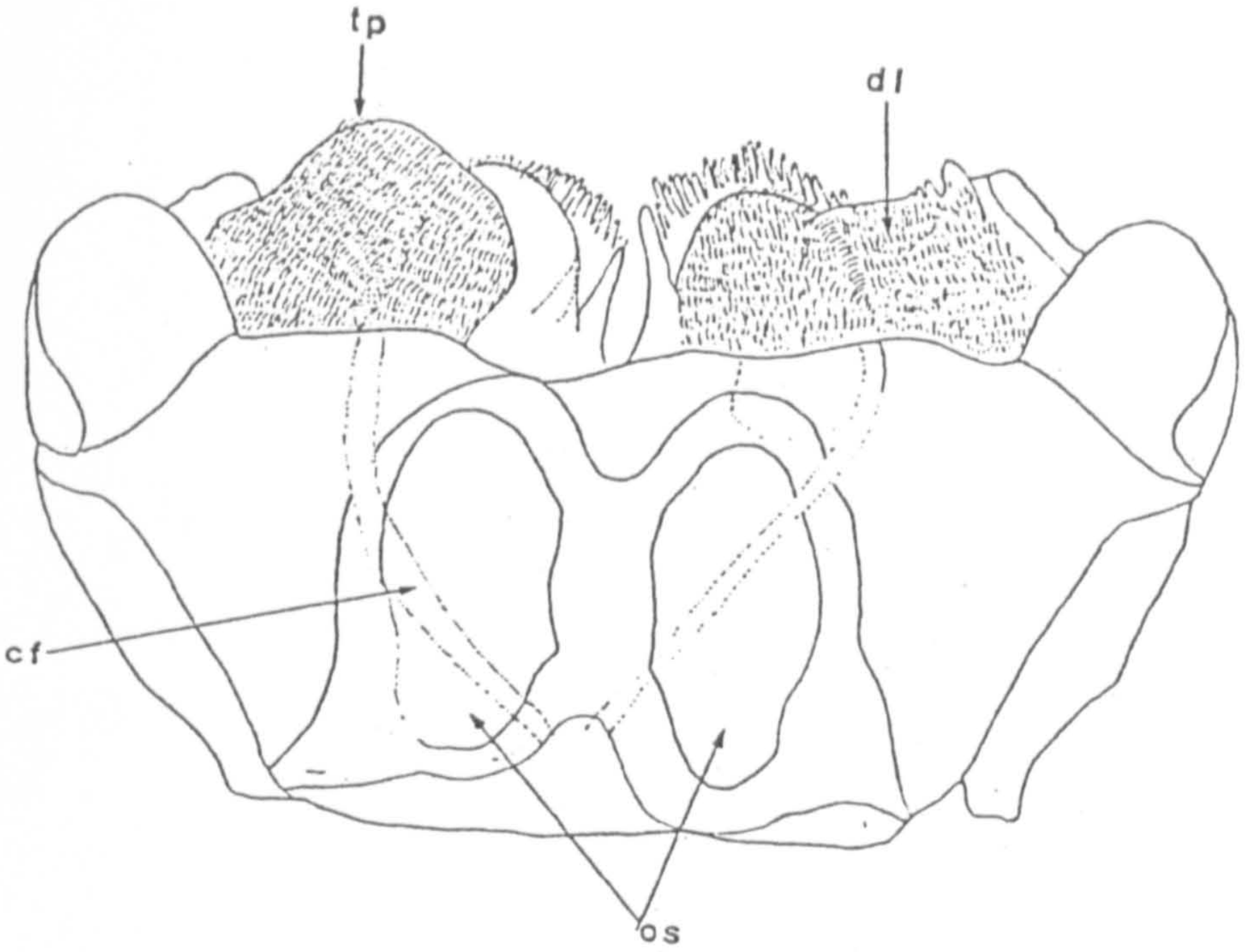
0 0·05mm

FIGURE 24 : Light microscope views of mouthparts of *T. anserinum*.

A. Position of hypopharynx relative to galea (maxillae) and labium.
(note, two lobes of hypopharynx with tufted projections)

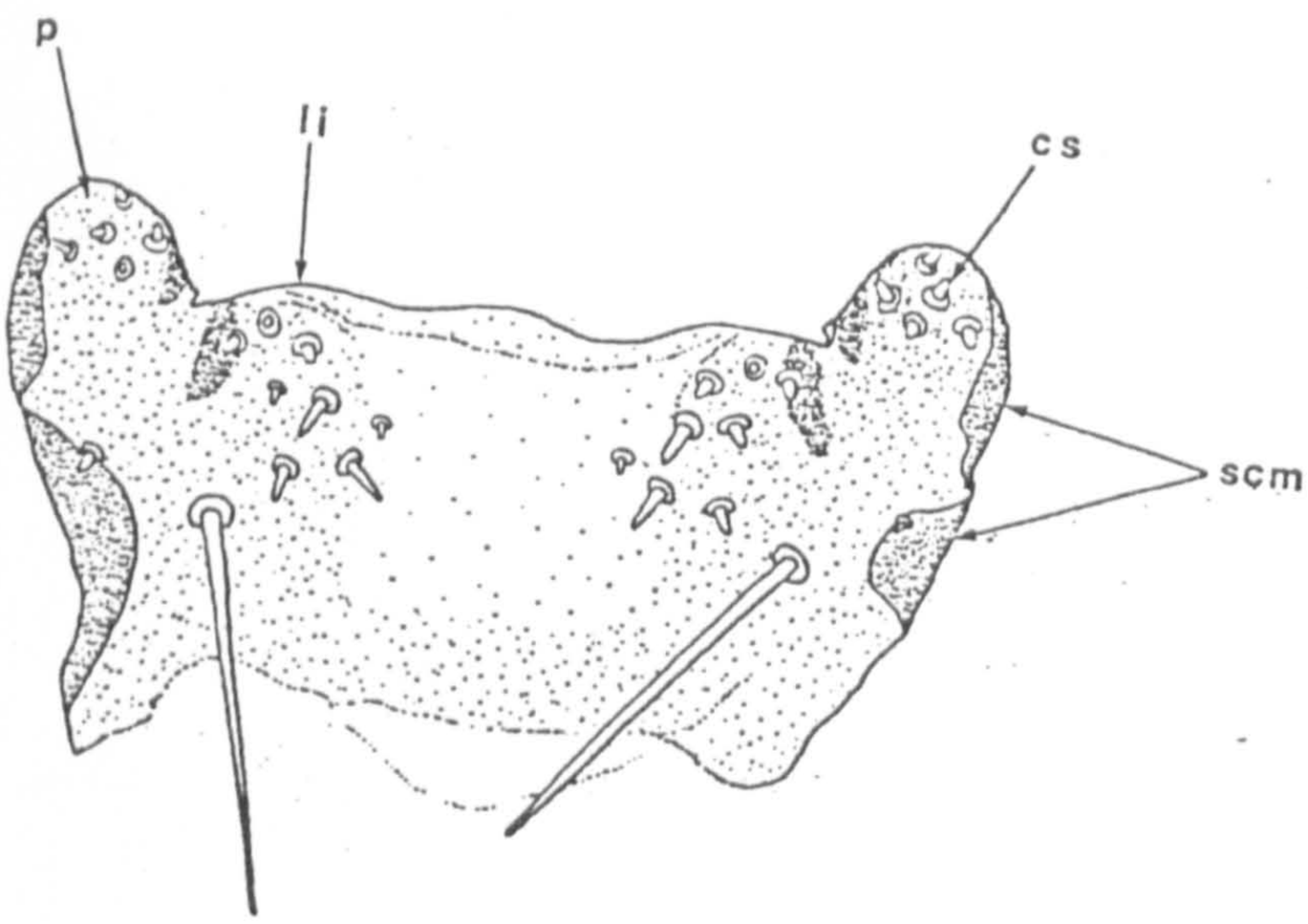
B. Labium with lateral sclerotised margins.
(note, conical spines on palps at either edge of labium)

tp	tufted projections
dl	distal lobe
cf	connecting filaments
os	ovoid sclerites
p	palp
li	ligula
scm	sclerotised margin
cs	conical spines



A

0 0·02 mm



B

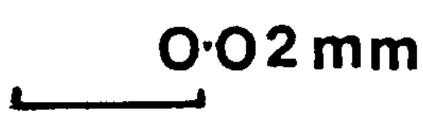
0 0·05 mm

FIGURE 25 : Electron micrographs of mouthparts of *T. anserinum*.

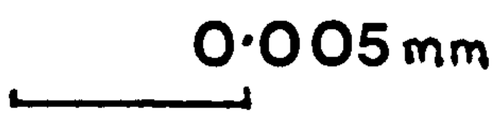
A. Four-segmented maxillary palp.

B. Tip of maxillary palp showing peg-like receptors.

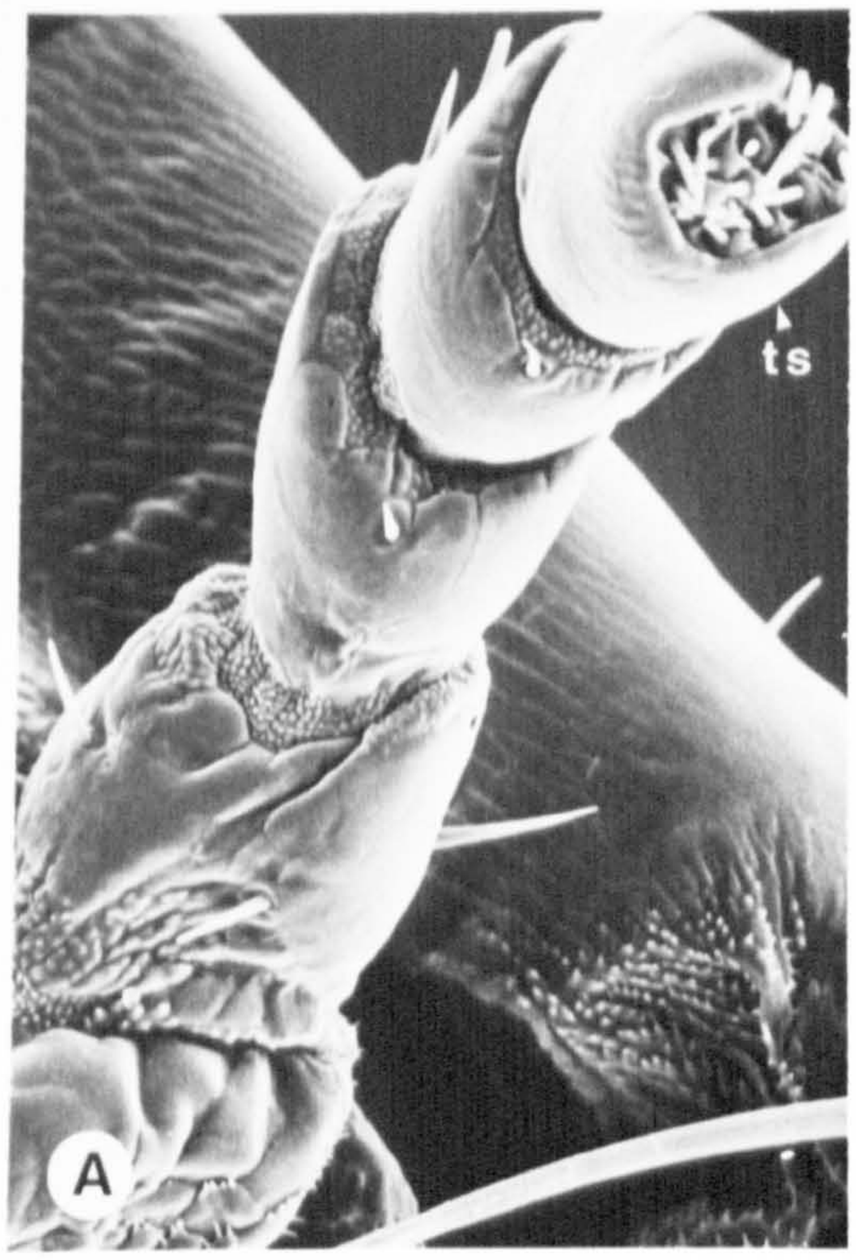
C. Distal lobes of hypopharynx.

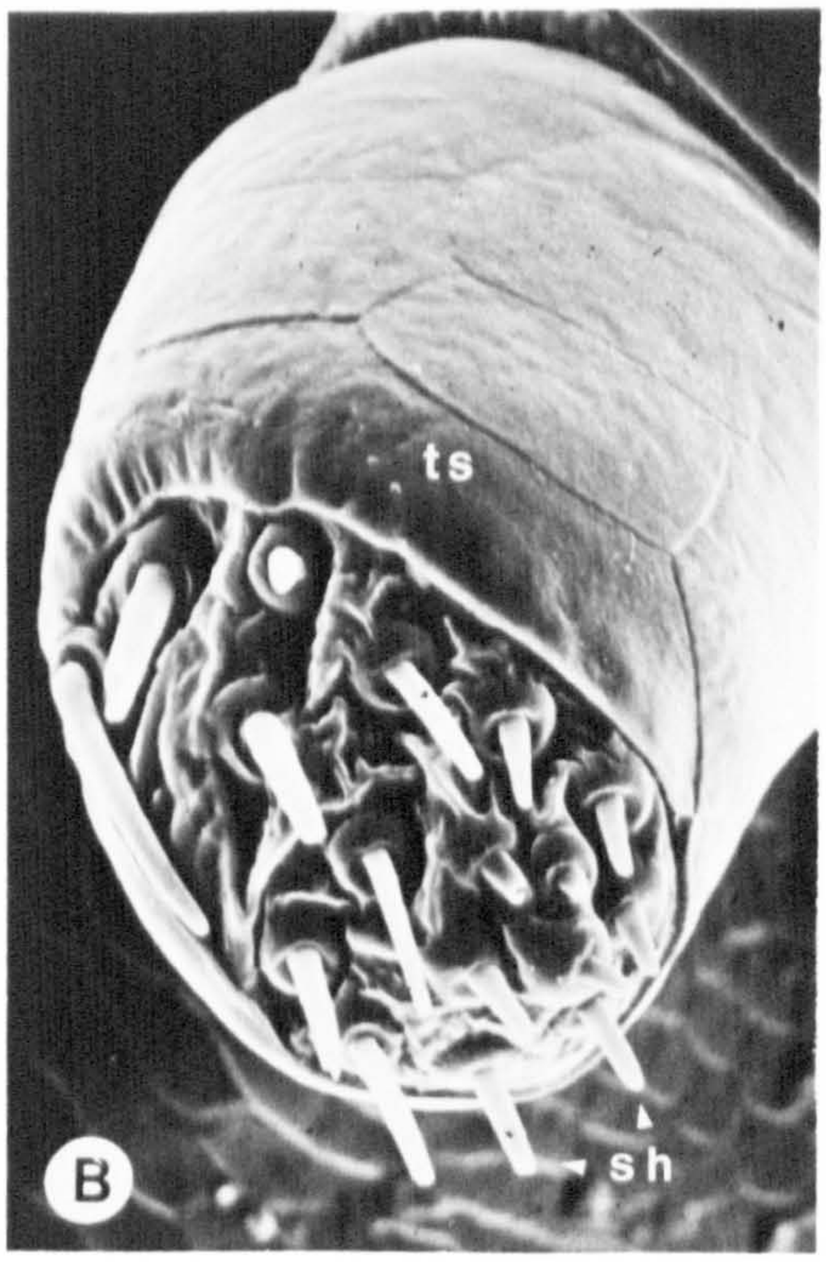


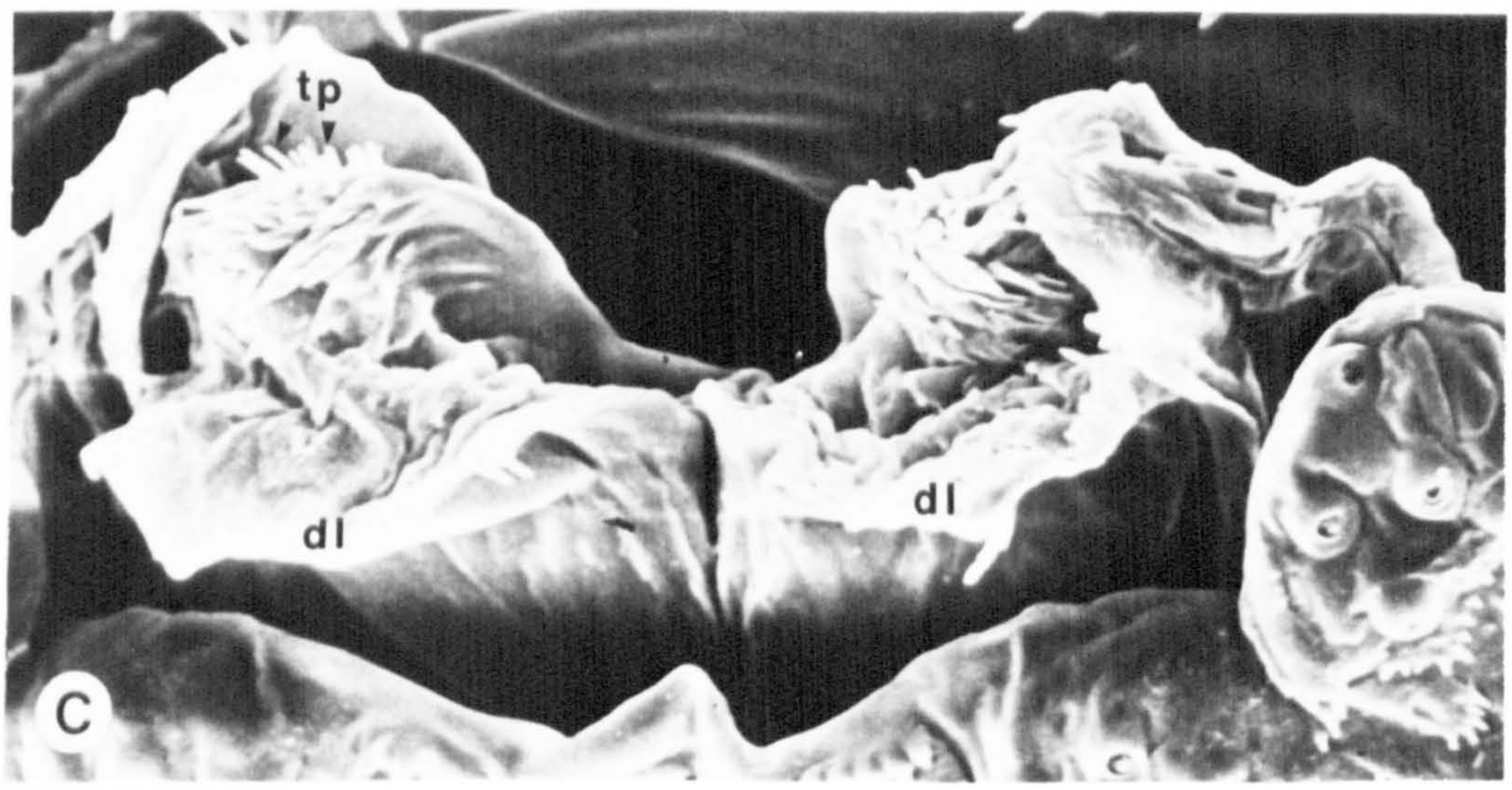
D. Tufted projections of hypopharynx.

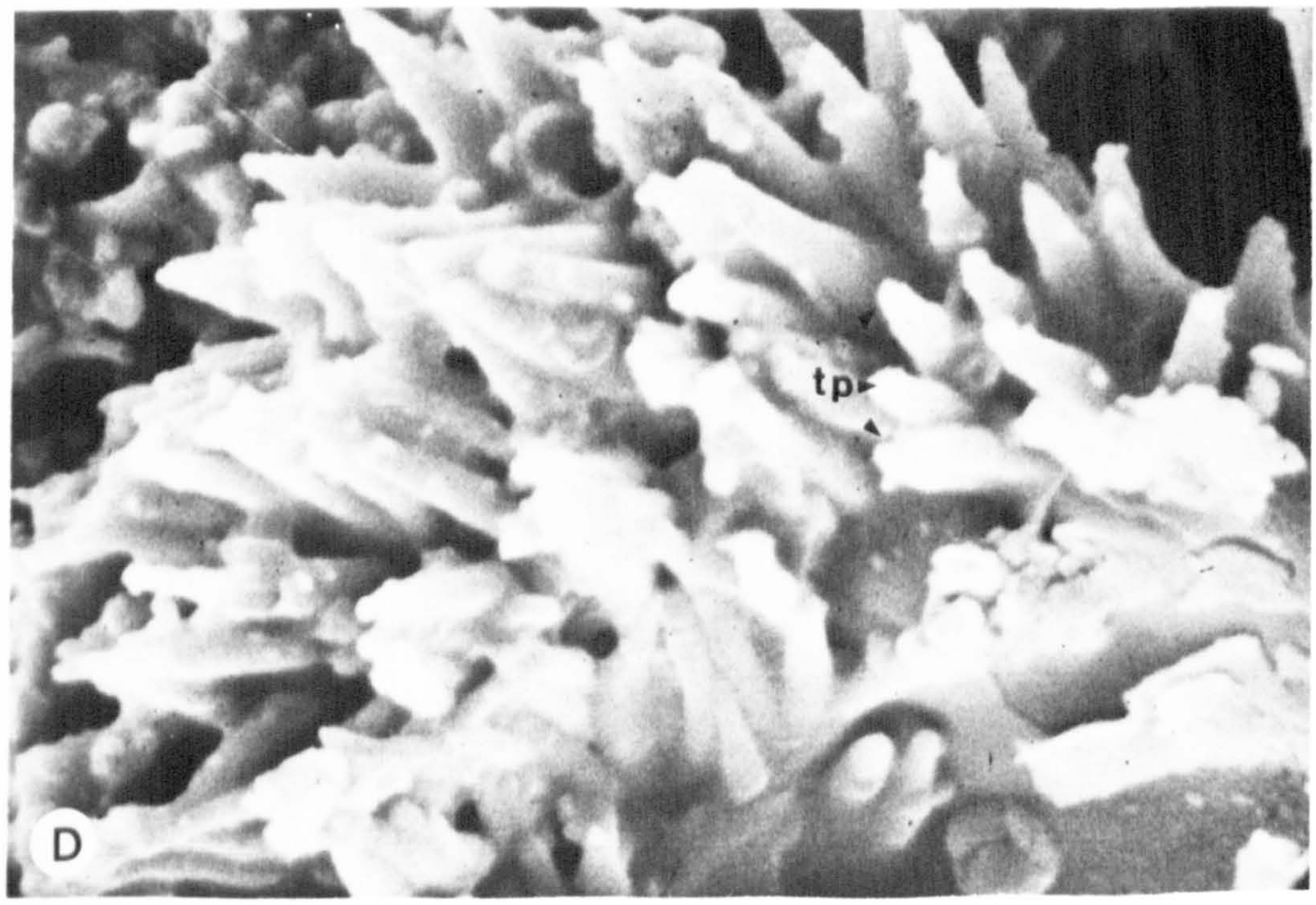


ts terminal segment of maxillary palp
sh sensory hairs
tp tufted projections
dl distal lobe of hypopharynx


ts
A


ts
sh
B


tp
dl
dl
C


tp
D

left and the apices are more heavily chitinised (Fig. 23A). One apex is blunt and the other is sharply pointed. The left mandible has a deep cavity (Fig. 22D) which receives the dorsal tip of the right mandible and when they interlock, the tips of the right close outside the tip of the left mandible (Fig. 22B).

Hypopharynx

The hypopharynx consists of a distal tongue-like lobe attached at the posterior end by muscles of the lingual sclerite. The lingual or ovoid sclerites of *T. anserinum* are clearly visible under the light microscope (Fig. 24A). Connecting filaments from the lingual sclerites cross the distal end of the hypopharynx and join the sitophore sclerite. The sitophore sclerite is situated on the anterior hypopharyngeal surface and is not readily seen from a ventral view. The anterior end of the hypopharynx diverges into two serrate lobes each covered in tufted projections (Fig.25C & D).

Maxillae

Maxillae of *T. anserinum* are visible in cleared specimens under the light microscope. The galea and stipes are more difficult to discern using a scanning electron microscope because they are thinly chitinised (Fig.23B & C).The galea has an inter-cutting surface with a row of cuticular teeth and each maxillary palp is composed of four segments: the terminal segment has 17 hairs at the tip which may have a sensory function (Fig. 25A &B).Under the light microscope the stipes are faintly chitinised and are closely associated with the sides of the labium. The lacinae persist only in a few species of Mallophaga and are thought to be absent in *T. anserinum*.

Labium

The labium is bordered laterally by a sclerotised margin. The prementum carries one palp on either side, each of which has a field of stout conical spines at its tip. The ligula is much reduced and also has a number of setae on it (Figs.22A & 24B).

3.2 MORPHOLOGY OF THE ALIMENTARY CANAL

The morphology of the alimentary canal of lice has been widely described (Snodgrass, 1944; Buxton, 1947; Imms, 1948; Waterhouse, 1953). In Amblycera, the oesophagus expands into a crop before leading directly

into the mid-gut. With the exception of Trichodectidae (Ischnocera) the crop always possesses teeth (proventriculus) (Waterston, 1926). In Amblycera the teeth are situated in a circular or semi-circular pattern near to the posterior end of the crop. In both Ischnocera and Amblycera, the oesophageal invagination is poorly developed.

The mid-gut of Mallophaga is simple and straight (*Columbicola columbae*) or has a single loop at its posterior end (*M.stramineus*). There are sometimes two anterior caecae (*M.stramineus*). There are four malpighian tubules, one pair is often longer than the other and the basal half of each tubule is differentiated by its greater pigmentation. The hind-gut narrows after the entrance of the tubules and expands again in a conspicuous rectum with six rectal pads.

3.2.1 MATERIALS AND METHODS

Freshly killed lice were dissected in physiological saline and observed under a binocular microscope (x40). The alimentary canal was dissected and drawn using a camera lucida and photographs were taken of dissected material stained with Giemsa (Appendix 1).

3.2.2 RESULTS

The alimentary canal is typical of a blood-feeding Amblyceran (Fig.26A&C). The proventriculus is clearly visible as two semi-circular combs of teeth in the stained preparation (Fig.27A&B) A crop is absent in most lice which feed solely on blood including *Pediculus humanus* (Anoplura) (Fig.26D). *T. anserinum* retains the crop as a storage organ for both blood and feathers. The crops of Ischnoceran lice, which feed solely on feathers, are more defined structures in the alimentary canal (Fig. 26B). The mid-gut of *T. anserinum* leads from the crop into two caecae. From two pairs of malpighian tubules leading from the mid-gut, the hind-gut constricts to the rectum.

3.3 CROP CONTENTS AND FEEDING METHODS

The crop contents of many Mallophaga have been described by researchers (Appendix 3). Waterston (1926) recorded a variety of materials found in the crop of *Gonoides bicuspidatus* : feather fibre, down, skin, scurf and blood.

Crops of poultry lice were dissected to determine the presence of

FIGURE 26 : Diagrammatic representation of the alimentary canal of Mallophaga.

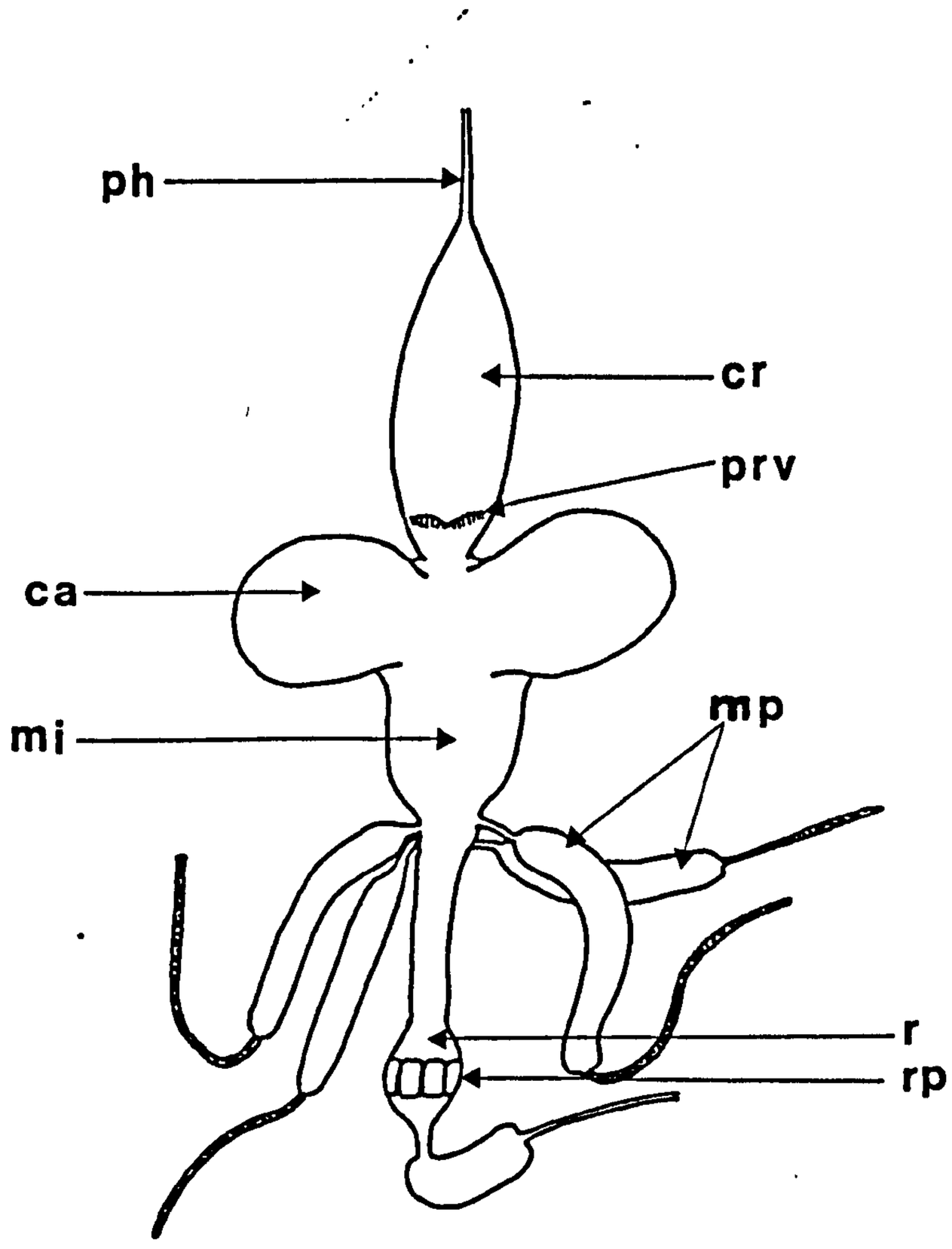
A. Alimentary canal of *T. anserinum*.

B. Alimentary canal of *Columbicola columbae* (Ischnoceran feather-feeding louse - note - separation of crop from rest of canal) (after Waterhouse, 1953)

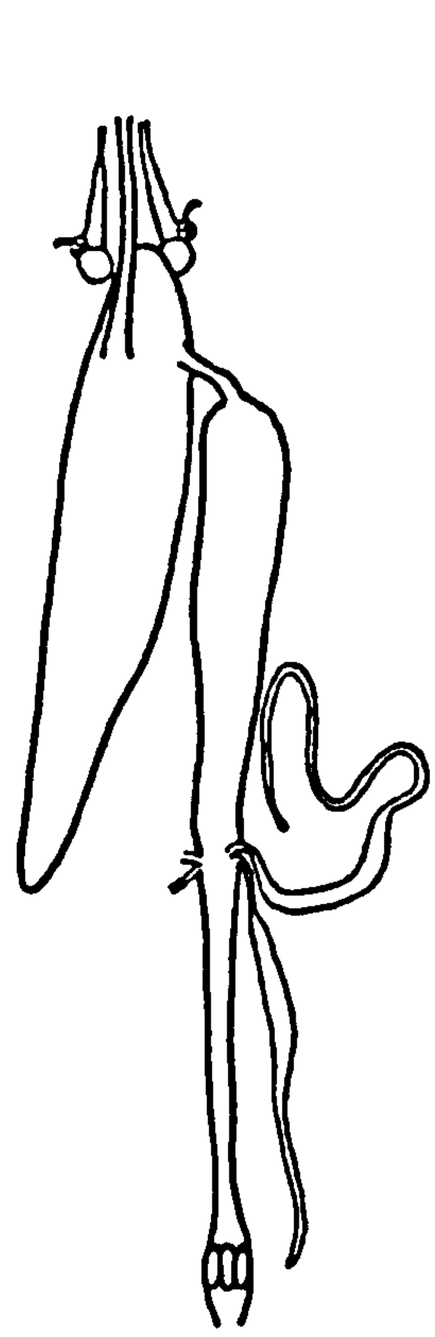
C. Alimentary canal of *Menacanthus stramineus* (Amblyceran louse feeding on blood and feathers - note - similarity of alimentary components of *T. anserinum)* (after Waterhouse, 1953)

D. Alimentary canal of *Pediculus humanus* (Anopluran louse feeding solely on blood - note - absence of defined crop and proventriculus used to separate and store solid food particles in Amblyceran and Ischnoceran lice) (after Snodgrass, 1944)

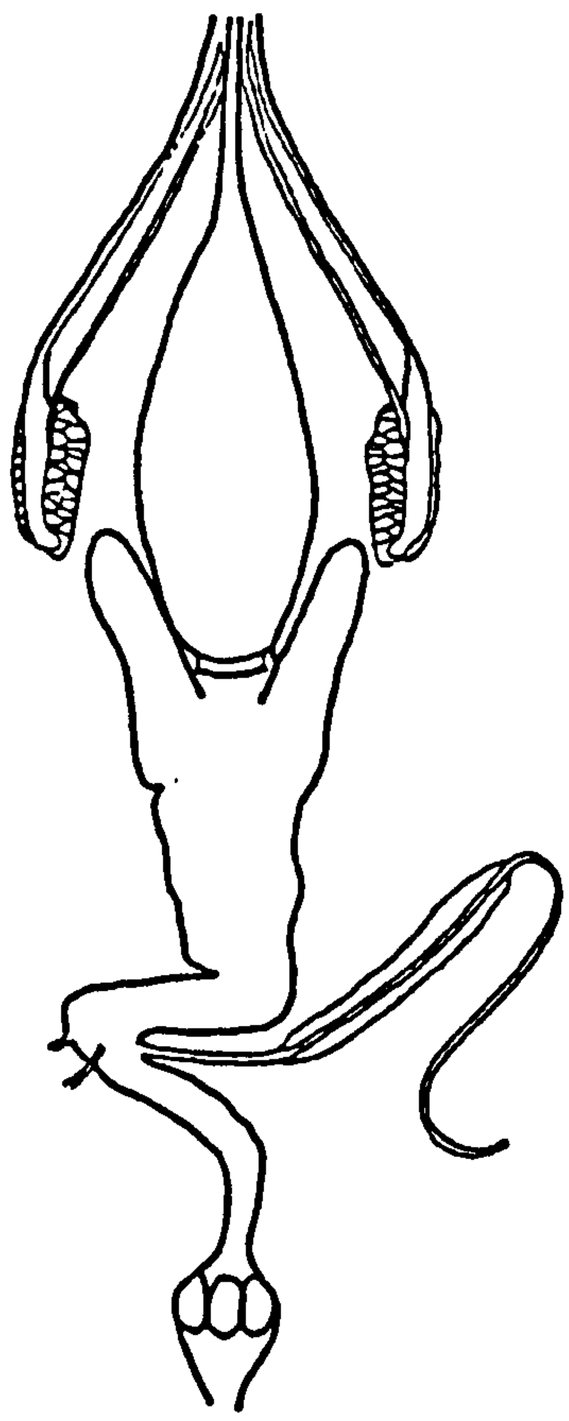
ph	pharynx
cr	crop
prv	proventriculus
ca	caecum
mi	mid-intestine
mp	malpighian tubules
r	rectum
rp	rectal pads



A



B



C

D

FIGURE 27 : Alimentary canal of *T. anserinum* viewed through light microscope.

A. Proventriculus of *T. anserinum* stained with Giemsa. (note, cuticular teeth inform of two "combs" which separate the crop from mid-gut)

0·1mm

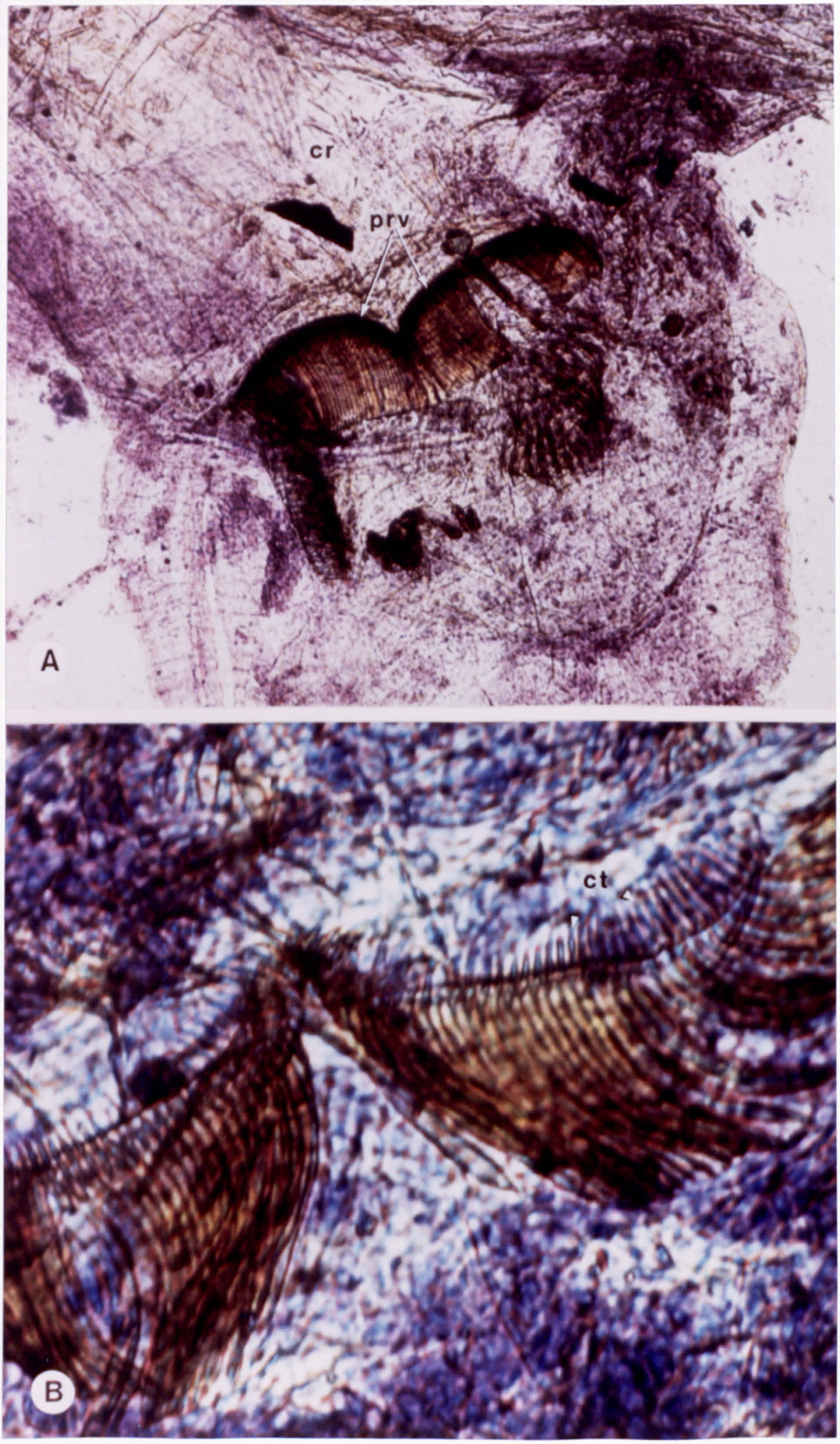
B. Detail of cuticular teeth of proventriculus.

0·05mm

prv proventriculus

cr crop

ct cuticular teeth


cr
prv
A
ct
B

blood and feathers (Crutchfield and Hixson, 1943). It was found that most specimens of *Menacanthus stramineus* (chicken body louse) fed on blood. This was determined by staining for red blood cells with Wright's stain. The types of feathers consumed by Ischnoceran species were identified. *Menopon gallinae* (the shaft louse) fed exclusively on barbs and barbules, whilst the diet of the wing louse, *Lipeurus caponis* , consisted almost entirely of hooklets. It is clear from these investigations that food type reflects the location of feeding of each louse species on its host.

The feeding habits of lice on auks were recorded by observing feathers and blood in the alimentary canal (Eveleigh and Threlfall, 1976). Similar studies have shown that species of pigeon lice feed only on down feathers (Barber, 1921; Nelson and Murray, 1971). Seegar (1977) dissected 89 lice (*T. anserinum*) from Whistling Swans and found 66% had consumed a blood meal.

In addition to identifying food materials consumed by avian lice, researchers have also examined the mechanisms of feeding and digestion, in a detailed account of the anatomy and histology of the alimentary canal of three bird lice, Waterhouse (1953) includes a description of the movement and microscopic examination of food undergoing digestion. He suggests that more important than the mechanical breakdown of food particles, vigorous peristaltic action causes a continuous circulation of digestive juices from mid-gut to crop, aiding digestion.

Although blood has been detected in many mallophagan species, the actual mechanism of blood ingestion has not been reported.

3.3.1 MATERIALS AND METHODS

3.3.1.1 Blood-feeding

Identification of blood in the crop of *T. anserinum*

Several techniques for blood detection were considered in this study:

a) a chemical reaction of chromagen solution and iron in blood (Appendix 4).

b) a reagent strip based on the peroxidase-like activity of haemoglobin (Hemastix, Ames Division, Miles Laboratories Ltd., Stoke Poges, Slough).

Both the chromogen and hemastix methods were unreliable in detecting blood. Chromagen detects concentrations of iron down to 5 ppm; but if any iron-based contaminants other than blood were present in the insect crop, erroneous positive readings were produced. Chromagen gave positive readings for iron when applied to feathers from a swan, to filter paper containing small iron components and to feathers contained in the crops of feather-feeding Ischnoceran lice (*Ornithobius sp* .). The hemastix method is used commercially to detect fresh, free haemoglobin in urine. Preserved insect specimens did not give positive results with this test even when blood was visible in the crop.

c) The X-ray facility on a scanning electron microscope for microanalysis of iron was also considered as a blood detection method.

Crops were dissected from *T. anserinum* specimens and mounted on aluminium stubs for analysis by the X-ray facility on a scanning electron microscope. This technique detects single elements but did not trace iron consistently even in samples of fresh swan blood.

d) The Giemsa stain method for detecting red blood cells microscopically.

This method was adopted for detecting blood in *T. anserinum* . Fresh and preserved specimens were dissected. The head was removed and the alimentary canal teased out. Diluted Giemsa stain (ratio of stain to buffered water pH 7.2, 1:5) stained the gut contents to show red blood cells. The crop contents were recorded for 259 specimens.

Examination of blood-feeding mechanism

A video camera was attached to a binocular microscope to film the mechanism of *T. anserinum* feeding on blood. Double-sided cellotape attached to the dorsal surface of a louse, was secured on a slide. The feeding mechanism was then filmed.

Fresh swan blood was heated in a beaker of water (20°C). A calibrated micropipette (5µdm³) was rinsed with an anticoagulant (sodium citrate 3.8%) and filled with blood. Blood was released from a pipette placed adjacent to the mouthparts and the volume of blood ingested by each louse was measured.

The process of ingestion of a blood meal was observed in detail on slow motion recordings.

Fresh infected blood and centrifuged plasma containing live microfilariae was fed to lice and observed using a binocular microscope.

3.3.1.2 Feather feeding

Lice were dissected as described on p.88 and the presence or absence of feathers was recorded for each specimen.

A more detailed analysis was performed on feather types from the crops of nine lice, the feather particles were measured and as far as possible, the feather types identified.

Different feather types isolated from lice may indicate the location of lice whilst feeding on the swan. To assist in the identification of feather types, feathers from different body regions of a swan were measured and drawn for comparison with feather fragments from crops. Down feathers have a general distribution all over the bird and the lengths of segments of down from the back, side and wing regions were measured in an attempt to distinguish between them.

3.3.2 RESULTS

3.3.2.1 Blood-feeding

Identification of blood in crops

Of 259 specimens, 56 (21.6%) had fed exclusively on blood and 85 (32.8%) consumed both blood and feathers. The food preferences of each age-class were examined (Table 23 , Fig. 28). The proportions of adult, third and second instar lice feeding on blood were not significantly different, but the proportion of first instar lice feeding on blood was significantly lower than for other age-classes (Appendix 5).

Blood-feeding mechanism of *T. anserinum*

Eight *T. anserinum* were filmed feeding on blood in vitro. Slow motion recordings were repeatedly played to observe the feeding mechanism and to derive an accurate description of the process.

Blood in contact with the mouthparts immediately induced ingestion. The mandibles moved rapidly from an "open" to a "closed" position initially to draw in small volumes of blood. The drops of liquid were observed passing through the pharynx at a mean rate of 7 per second. As a constant flow of blood was ingested the mandibles remained in an "open" position only sometimes closing slightly to possibly regulate the flow. As the blood was being ingested there was an observable "lapping" motion occurring adjacent to the mandibles. It was not clear from the video recordings

Table 23 : Crop contents of different age-classes of *T. anserinum*

AGE-CLASS		CROP CONTENTS			
		FEATHERS	BLOOD	BOTH	EMPTY
ADULT FEMALES (N = 62)	N	16	13	25	8
	%	25.8	21.0	40.3	12.9
ADULT MALES (N = 59)	N	14	15	21	9
	%	23.7	25.4	35.6	15.3
ADULTS (TOTAL) (N = 121)	N	30	28	46	17
	%	24.8	23.2	38.0	14.0
INSTAR 3 (N = 35)	N	9	10	14	2
	%	25.7	28.6	40.0	5.7
INSTAR 2 (N = 41)	N	11	13	13	4
	%	26.8	31.7	31.7	9.8
INSTAR 1 (N = 62)	N	42	5	12	3
	%	67.7	8.1	19.4	4.8
TOTAL (N = 259)	N	92	56	85	26
	%	35.5	21.6	32.8	10.0

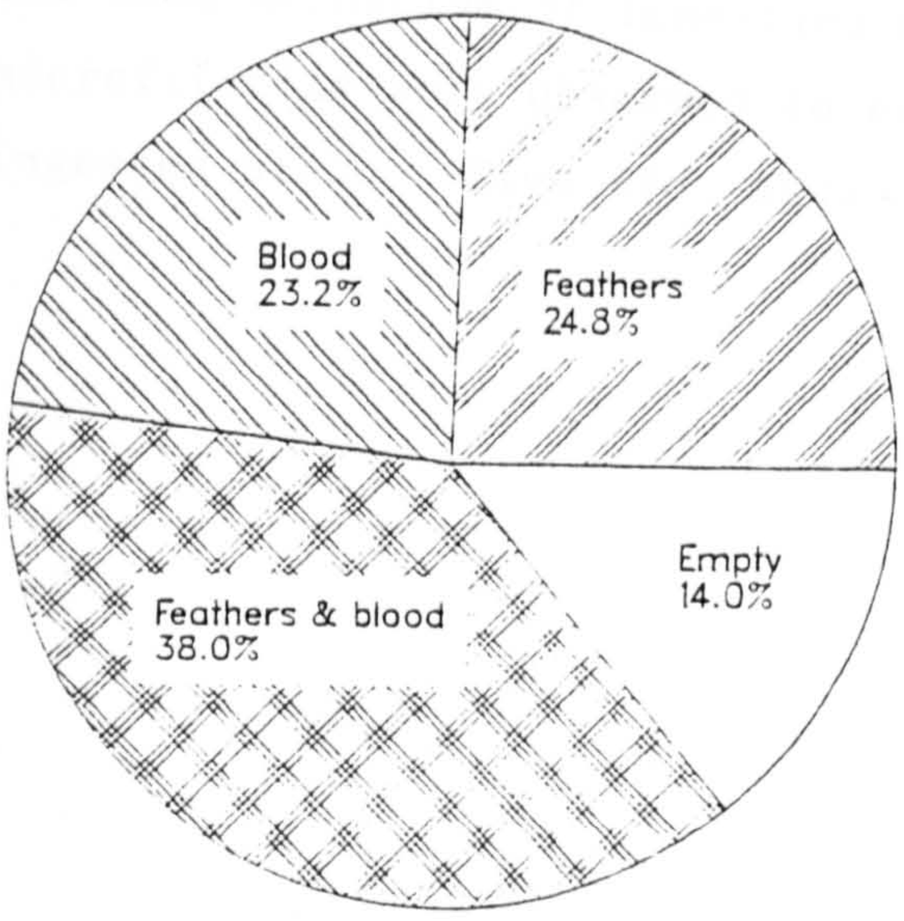
FIGURE 28 : Crop contents of each age-class of *T. anserinum* (N = 259)

A. Proportion of adult lice with crops containing blood, feathers, feathers and blood or no food.

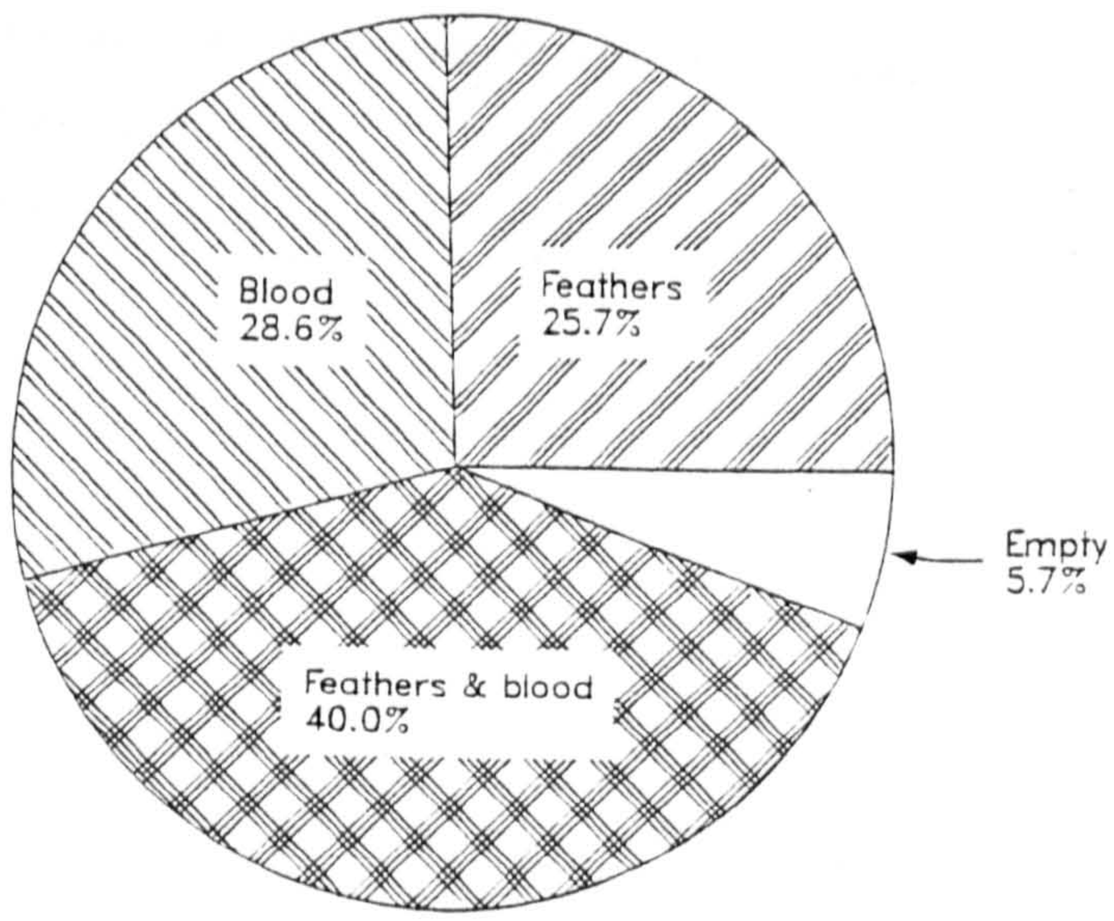
B. Crop contents of third instar lice.

C. Crop contents of second instar lice.

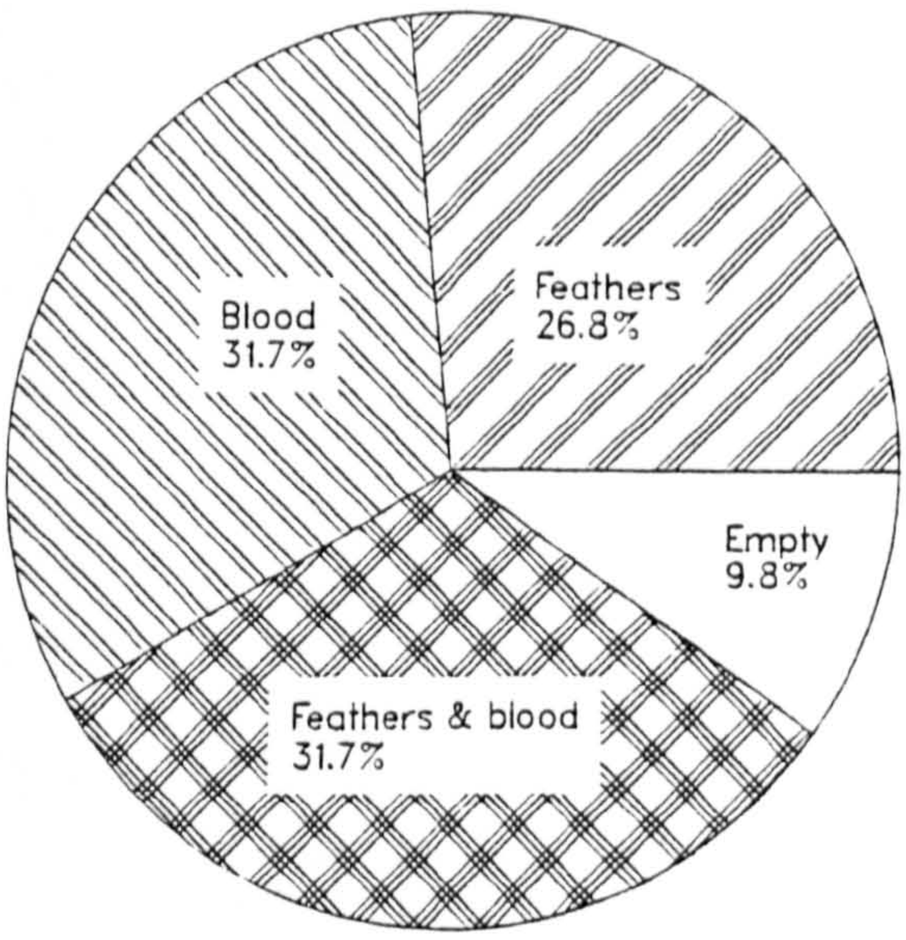
D. Crop contents of first instar lice.


Blood
23.2%
Feathers
24.8%
Empty
14.0%
Feathers & blood
38.0%

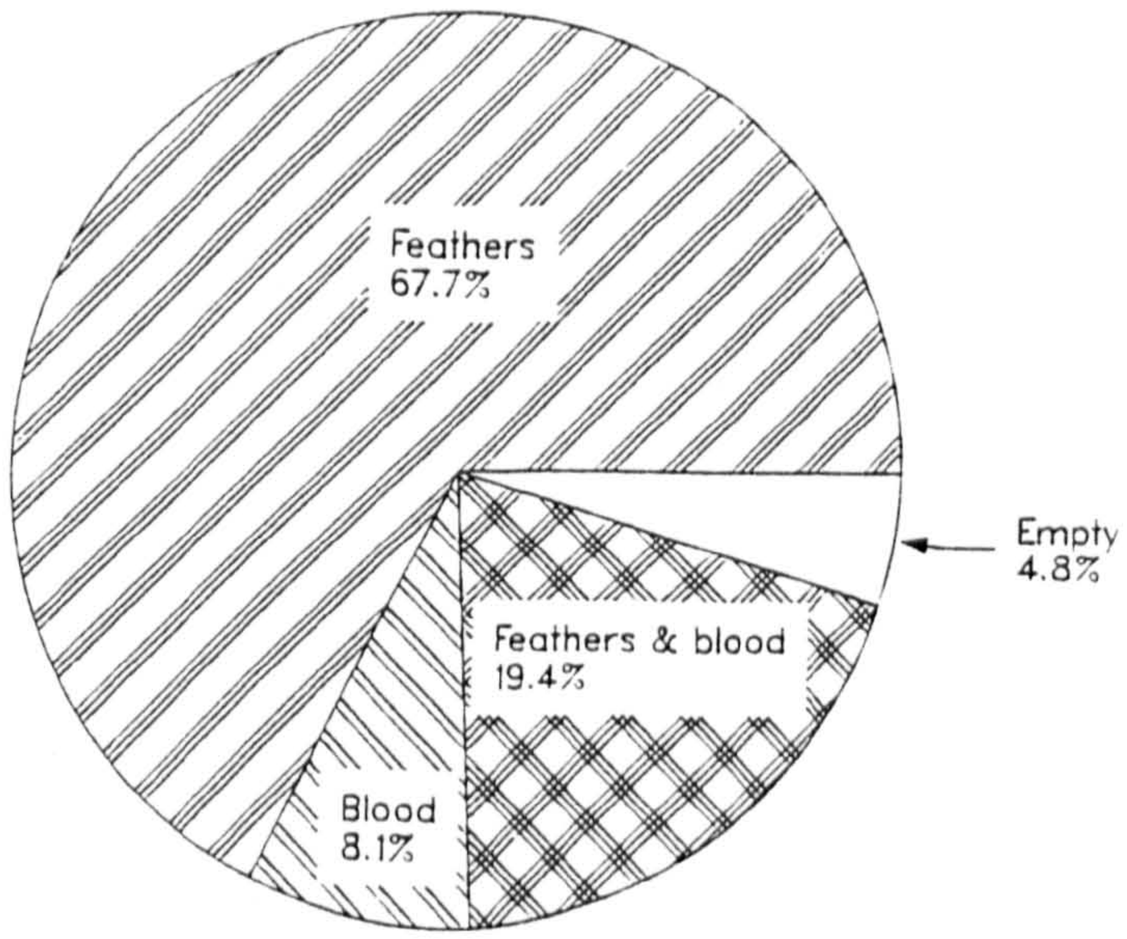
A


Blood
28.6%
Feathers
25.7%
Empty
5.7%
Feathers & blood
40.0%

B


Blood
31.7%
Feathers
26.8%
Empty
9.8%
Feathers & blood
31.7%

C


Feathers
67.7%
Empty
4.8%
Feathers & blood
19.4%
Blood
8.1%

D

which structure was involved but from the morphology of the hypopharynx (Fig.25C&D) this structure is thought to be implicated. Blood was observed to fill the crop and then fill the alimentary canal (Fig. 29). The mean ingestion time was 15 seconds after which the whole abdomen was distended.

When blood and plasma containing live microfilariae was fed to lice, the same mechanism of ingestion was observed as described above. Wriggling microfilariae were observed to enter the buccal cavity with the flow of ingested blood. Five lice were observed to ingest microfilariae.

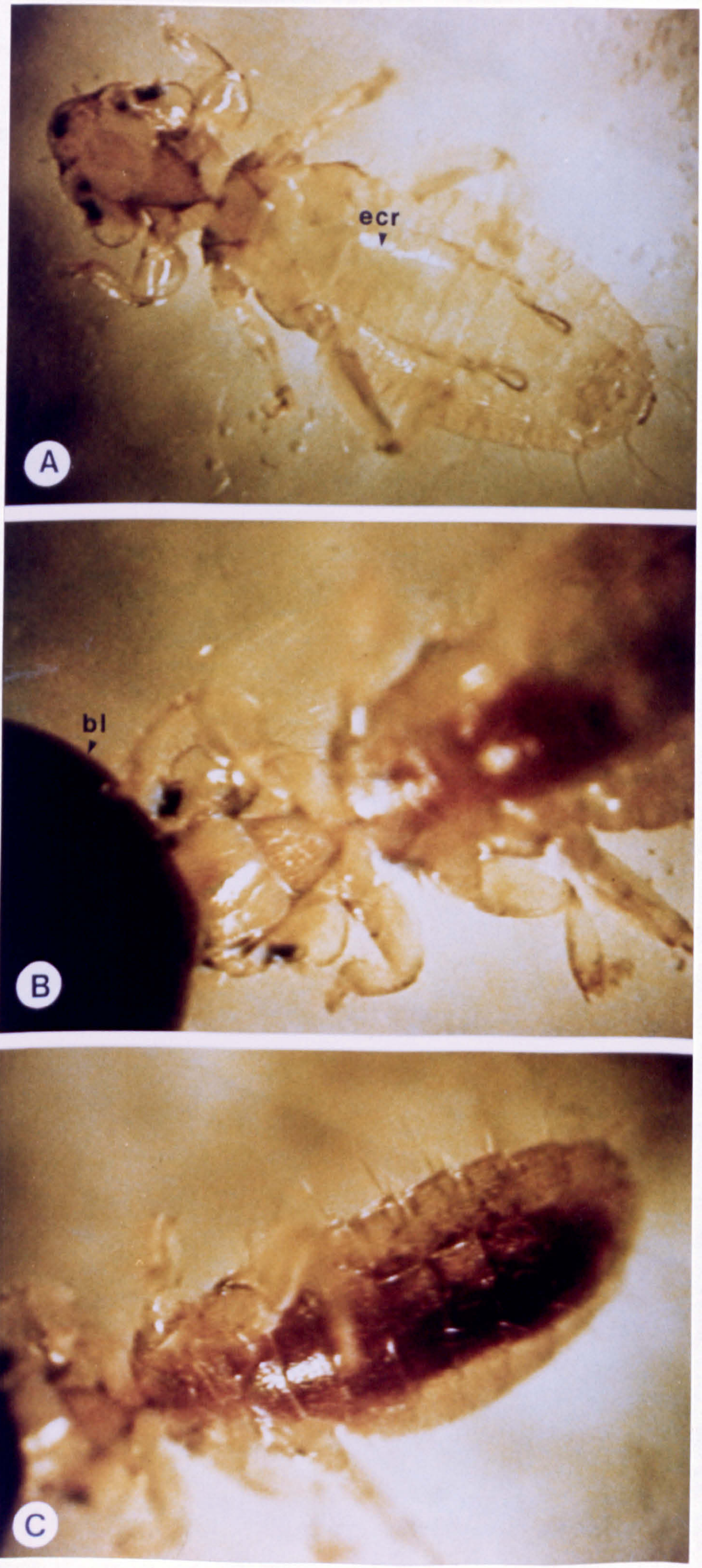
FIGURE 29 : Sequence of photographs of *T. anserinum* ingesting a blood-meal.

A. Third instar nymph with an empty crop prior to feeding.
(note, lack of chitinisation in exoskeleton)

B. Louse during ingestion of blood.
(blood droplet in bottom left-hand corner of photograph)

C. Fully satiated louse at end of blood meal.

Compare photographs A and C.


ecr
A
bl
B
C

Volumes of blood consumed by *T. anserinum*

Whilst observing the feeding mechanism and attempting to maintain *T. anserinum* in vitro (p.122) the volume of fresh swan blood ingested by lice was measured. The mean ingested volume was 1.42 μdm^3 (N = 30; SD = 1.19 μdm^3). Fresh blood would only be ingested when warmed. Some lice were offered cold blood serum and the mean volume ingested was 2.75 μdm^3 (N = 4).

3.3.2.2 Feather-feeding

Of 259 lice dissected, 92 (35.5%) fed on feathers and 85 (32.8%) consumed blood and feathers. The crop contents of different age-classes were investigated (Table 23 ; Fig. 28). The proportions of adult, third and second instar lice feeding on feathers were not significantly different (Appendix 5). However, significantly more first instar lice (67.7%) fed on feathers than did adults (25.0%), third (25.7%) or second (26.8%) instar lice. A similar situation was found to exist for the proportions of lice feeding on both feathers and blood. Adult, third and second instars did not differ significantly from each other, but when compared with first instar lice each consumed significantly larger proportions of feathers and blood.

Digested feathers from lice

Eight of the nine lice studied had consumed individual down barbule segments. The lice had probably cut each segment at its junction and followed the barbule along towards the attachment to the barb. Consequently most crops were packed with small pieces of feather of the same size and shape (Fig.30A).

The crop contents of a first instar nymph consisted of down barbule segments, and longer pieces of barbule (3-6 segments long) (Table 24). The second instar nymph had consumed a similar type of down feather ranging from 70 μm - 500 μm in length (Fig.30D). Some barbs with the remains of barbules had also been eaten (Fig. 30B). Both down barbule segments and barbules had been consumed by the third instar nymph. The range of feather particle sizes was larger than the instar one and two nymphs. Larger pieces of barbs (950 μm) were found in the third instar nymphs crop, but the mean length of feather was smaller than that for the instar one and two nymphs.

Table 24 : Measurements of feather particles from crops of *T. anserinum*

AGE-CLASS	MEASUREMENTS OF FEATHER PARTICLES (µm)				FEATHER TYPES
	N	$\bar{x}$	95% CL	Range	
N1	50	150	± 34.5	70 - 400	Down barbs, barbules & barbule segments
N2	25	150	± 54.9	70 - 500	Down barbules and segments
N3	50	120	± 21.0	50 - 950	Down barbs, barbules & segments
Ad ♀	63	69	± 2.5	50 - 100	Down barbules, hooklets
Ad ♂	121	128	± 12.3	49 - 170	Barbules, hooklets
Ad ♀	86	135	± 34.3	50 - 900	Barbules, segments
Ad ♂	20	84	± 15.8	50 - 150	Barb sections, hooklets
Ad ♀	100	85	± 1.6	70 - 90	Barbules
Ad ♂	94	95	± 19.6	50 - 1580	Hooklets, down segments

FIGURE 30 : Feather components in the crop of *T. anserinum*.

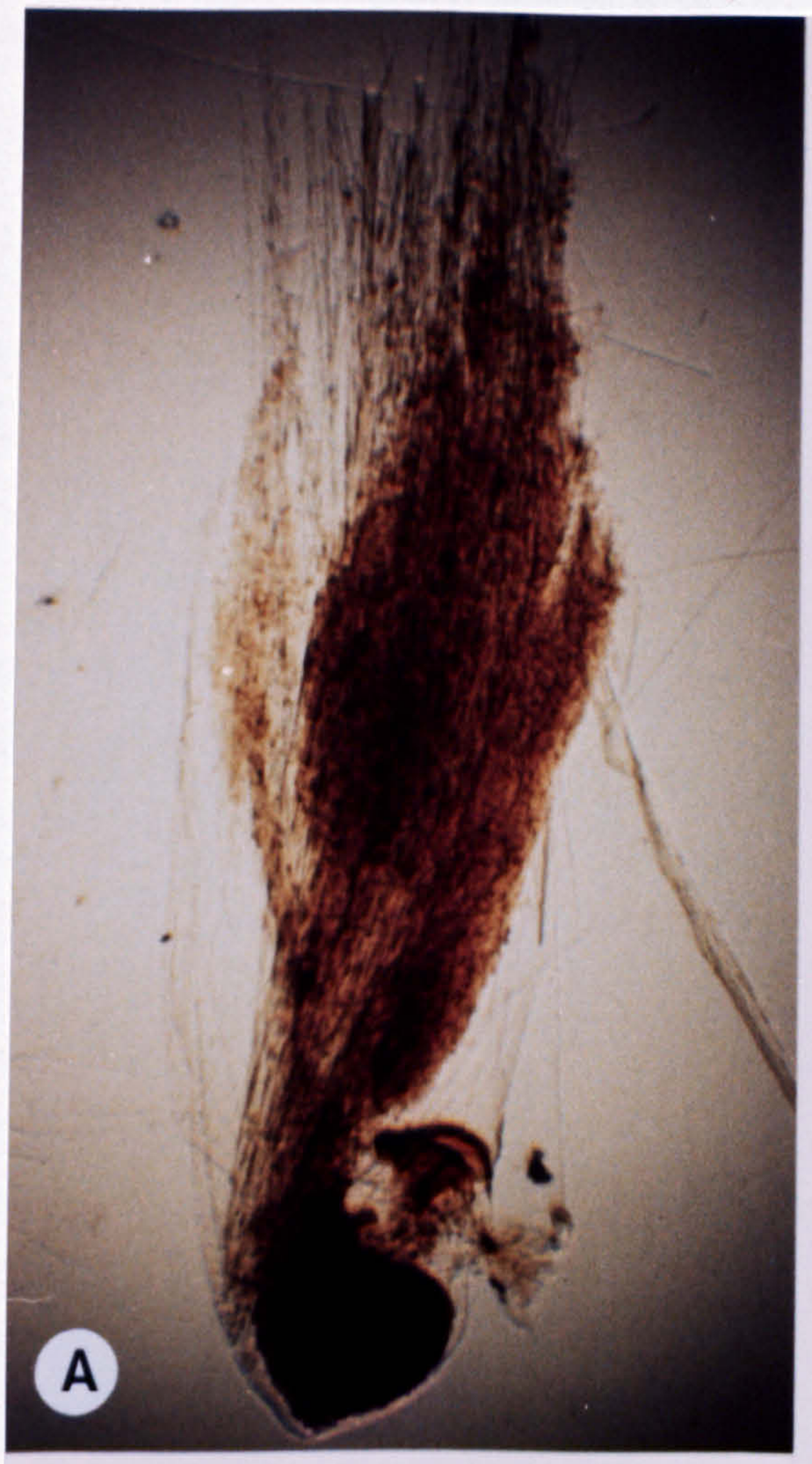
A. Crop packed with pieces of feather all of similar size and shape.
(note, proventriculus near base of crop provides a "sieve" to prevent large pieces of feather from entering the mid-gut)

B. Barb from a swan feather with barbules still attached.

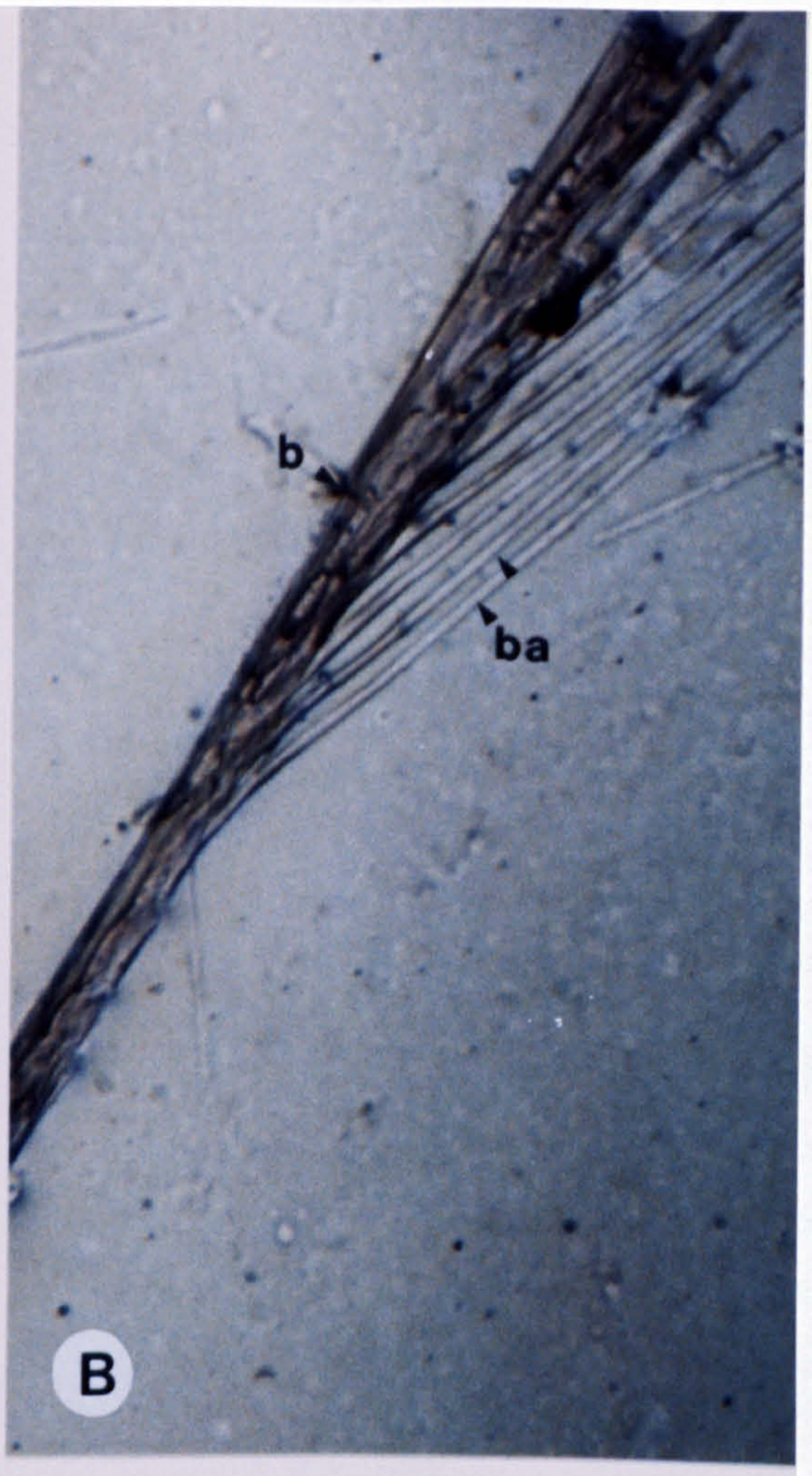
C. Hooklet from a contour feather.

D. Barbules from down feathers.
(note, barbules were found singly or in longer pieces consisting of 3-6 barbules)

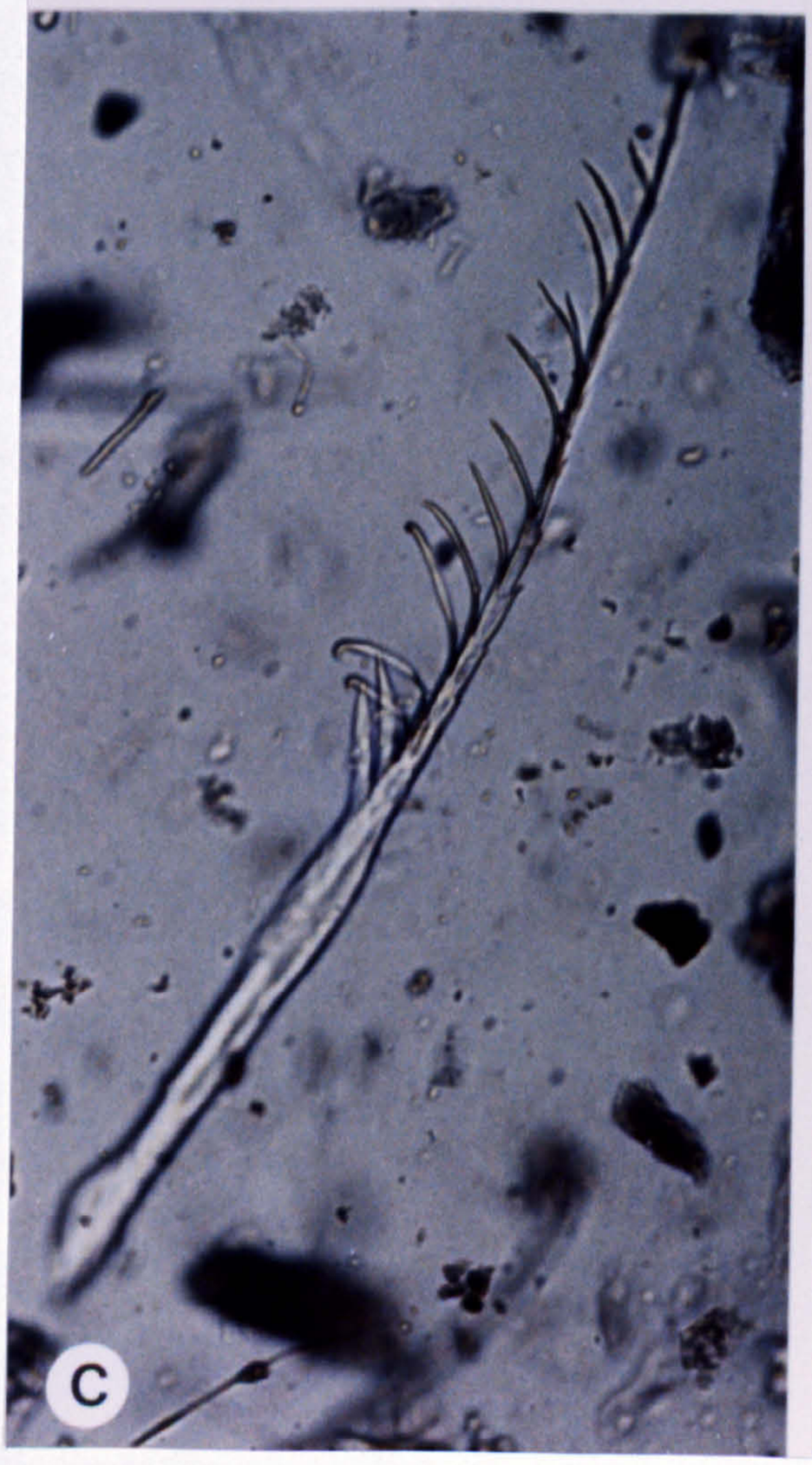
b barb
ba barbule



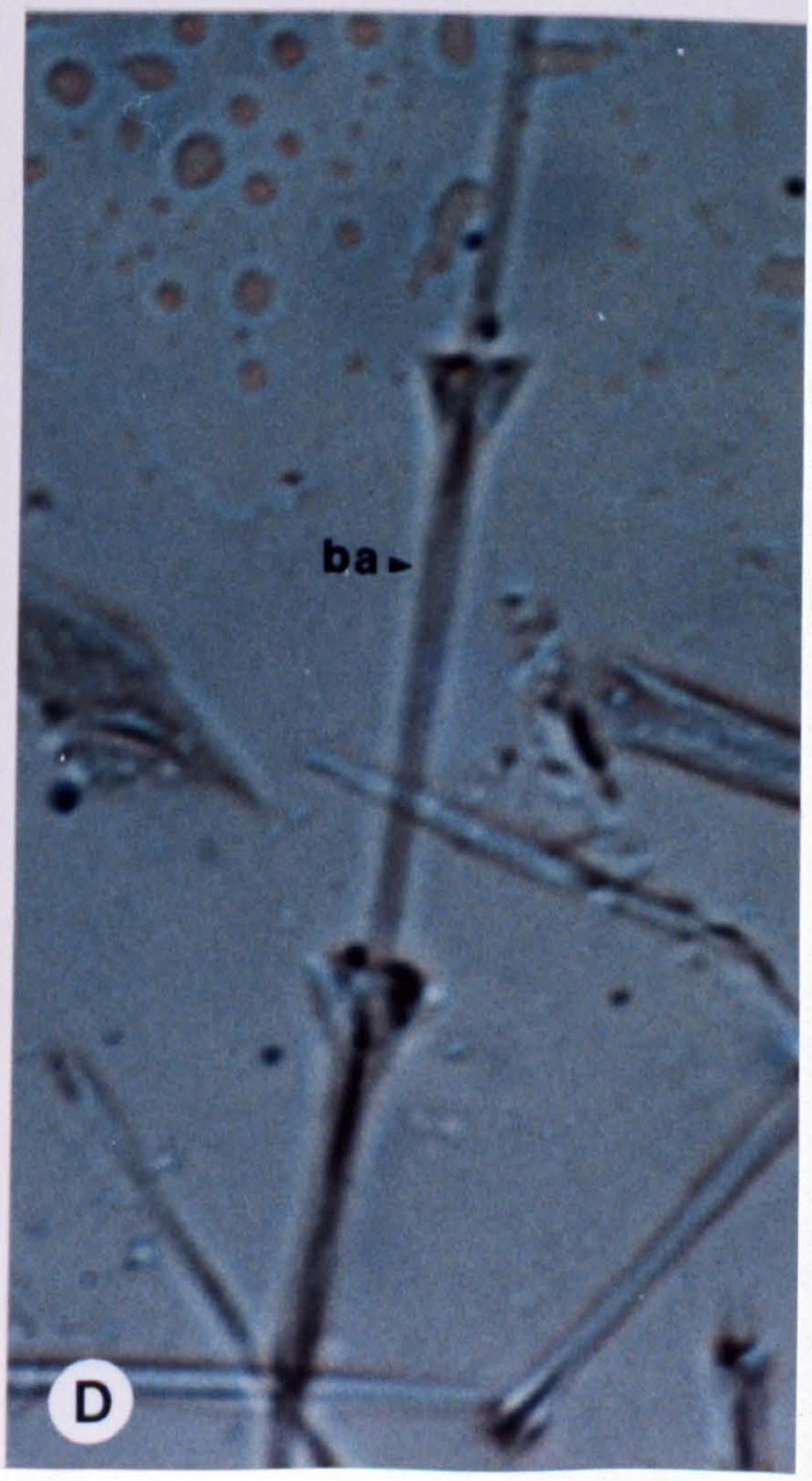
A



B



C

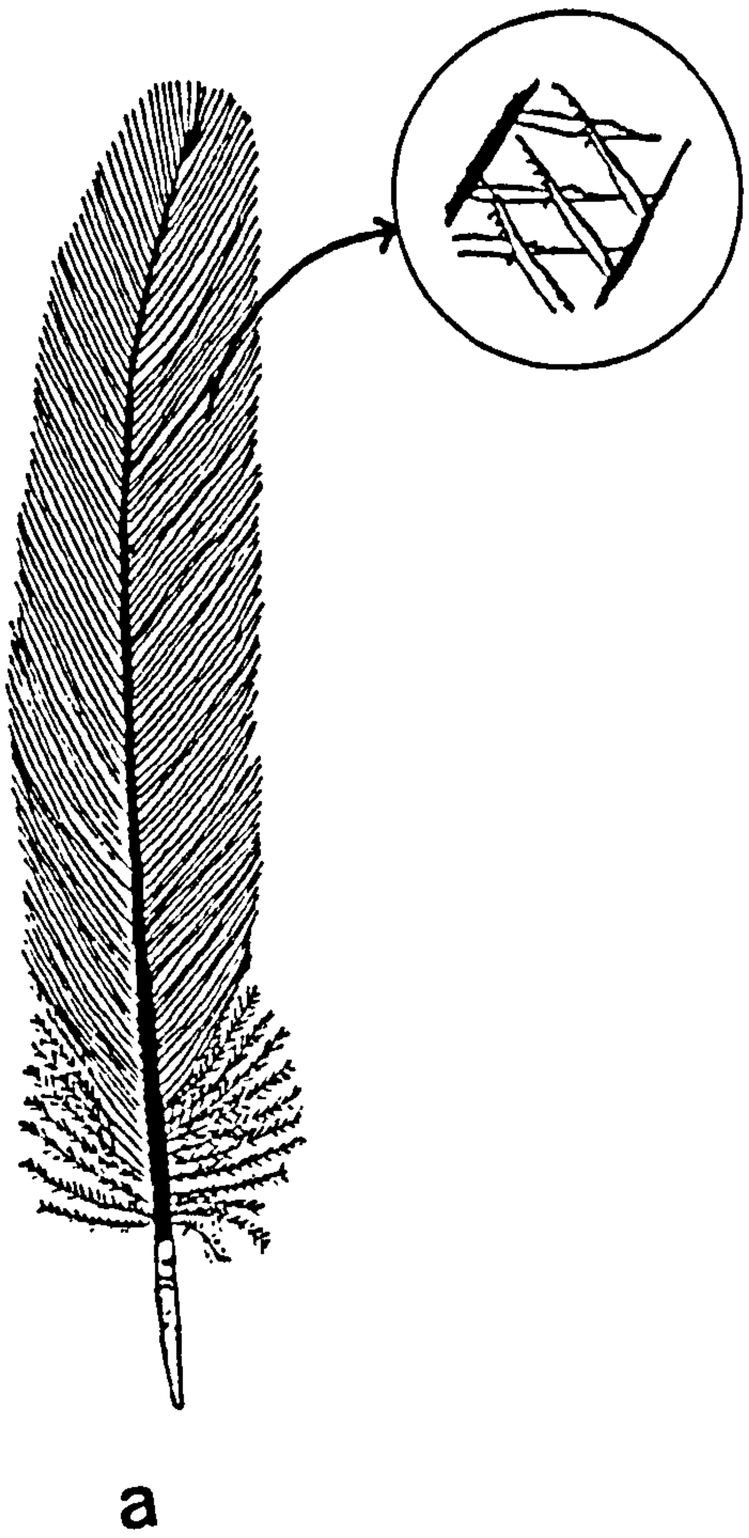


D

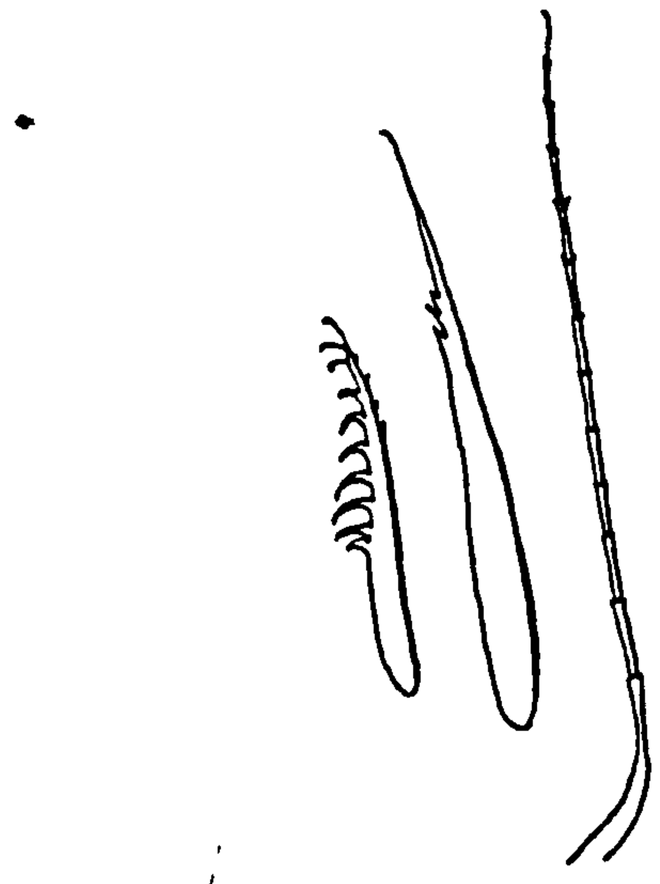
FIGURE 31 : Structure of bird feathers.
(after Marshall, 1981)

a. Contour feather with detail of interlocking hooklets.

b. From left to right
hooklet, semiplume and barbule
(with several segments of barbs) from down feathers.



a



b

There was no consistent feather particle size consumed by adult lice. The mean lengths ranged from 68 µm - 135 µm. There was also no similarity in the sizes consumed by adult females. One consistent characteristic of adult crop contents was the presence of barbules from contour feathers (hooklets) (Fig. 30c). The hooklets were present in all but two of the adults examined. The remaining contents of adult crops consisted of longer pieces of barbs with barbules and down barbule segments.

Identification of feather types from swans

In an attempt to identify feeding locations of lice, feathers from different body regions of a swan were measured and compared. The differences between contour and down feathers were clear. Hooklets from contour feathers have a distinctive shape (Fig.31a&b) and down barbules are divided into segments (Fig. 31b).

The lengths of down segments were compared from different body regions but were not significantly different. It was not possible to distinguish between down from different body regions of a swan.

3.4 LOCATION AND TIMING OF FEEDING OF *T. anserinum* ON MUTE SWANS

The types of feathers consumed by *T. anserinum* give some indication of its general location on swans. But these investigations do not show the precise feeding sites nor do they indicate the timing for feeding on the bird.

Feeding sites have been observed for a number of insect vectors of nematodes. The blackfly vectors of onchocerciasis, *S. damnosum* and *S. naevei* , bite those parts of the body where microfilarial densities are highest. (Crosskey, 1955; Duke and Beesley, 1958). Wharton (1962) observed that the vectors of *B. malayi* prefer to bite low and produce a prevalence of elephantiasis in the lower extremities. It is well known that the distribution of microfilariae in capillaries is uneven in different parts of the body (Nelson, 1964). Nelson frequently observed more microfilariae of *W. bancrofti* in blood from the ear than in the finger. Although Diptera possess the capability of feeding at almost any site on the host's body, various spatial preferences have been observed. Tabanids feeding on ungulates tend to prefer the limbs, abdomen, head and neck. But larger forms (*Tabanus borinus* and *T. sudeticus*) more frequently feed on the back (Balashov, 1984).

The feeding activities of intermediate hosts of many nematode species (*W. bancrofti* , *B. malayi* , *L. loa* , *D. immitis* , *D. repens* , *L. carinii* , *D. witei* , Hawking, 1967) correlate with diurnal periodic cycles of microfilariae in the peripheral blood of vertebrate hosts. There is some indication that the microfilariae of *S. eurycerca* in Mute Swans also exhibit a periodic cycle (p. 36). If *T. anserinum* is an intermediate host of *S. eurycerca* , it would be reasonable to hypothesize that the louse takes a blood meal when the nematode is circulating in superficial capillaries and that the blood-feeding activities of the louse may be related to the periodicity of the nematode. An attempt was made to study this relationship by marking lice and establishing their location and time of feeding throughout 12 hour periods.

There are many different methods for marking insects and their use is well documented in behavioural studies. Fluorescent paints and powders are commonly applied to winged insects (Harris, 1979 honeybees; Berry, 1961 stableflies; Rawlings, 1981 Anopheles; Chamberlain, 1977 hornflies). The success of this method however depends on the marked insects being visible to the observer. Fluorescent powders (Industrial

TABLE 25 : Radioisotopes used to mark insects

ISOTOPE	EMMISSION	LABEL TYPE	INSECT	AUTHOR	DATE
Co^{60}	γ	Ext.	Weevils	Sullivan	1953
NaI^{131}	β	Ext.	Engelmann Spruce Beetles	Davis & Nagel	1956
P^{32}	β	Int.	Blackflies	Bennett	1963
S^{35}	β	Int.	Fleas	Sviridov	1963
P^{32}	β	Ext.	Blackfly larvae	Baldwin	1975
C^{14}	β	Int.	Squirrel fleas	Sonenshine & Lauer	1976
Cs^{137}	γ	Int.	Mole crickets	Hudson & Cromroy	1985
Zn^{65}	γ	Ext.	Plum curculio	Lafleur	1985
Tc^{99M}	γ	Ext.	Swan lice	Cohen	1988

Colours Ltd., London) were used initially in this study to mark but they proved to be unsuitable because lice burrow deep into the feathers out of sight of the observer.

An alternative marking method is to use radioisotope labelling. Even though this is a comparatively new technique, it has been widely used in insect behavioural studies (Table 25). Radioisotope labels have an advantage over other methods of marking because they can be detected without the insect being visible. There are 44 principal isotopes which have been used in entomological work. Of these P^{32} is the most popular because it is the optimum for safety, activity and ease of detection (Marshall, 1981). Radiolabels can be applied in two ways; either externally on the exoskeleton, or in food to become incorporated in tissues. Weevils (*Pissodes stobi*) were externally labelled with radioactive cobalt (Co^{60}) in the nitrate form which was mixed with a resin glue (Sullivan, 1953). Bloodsucking insects can be labelled internally by injecting the hosts with radioisotopes. This method has been used for marking blackflies with P^{32} (Bennett, 1963).

MATERIALS AND METHODS

3.4.1 Marking *T. anserinum*

A method of marking *T. anserinum* was required which had the following attributes:

1) it detects lice on swans even when the insects are out of sight of the observer;
2) it remains detectable over a period of 12-15 hours;
3) it does not affect the behaviour or mortality of the lice;
4) it is quick and easy to apply.

A radioisotope label was the only marking method which satisfied the first attribute. Further investigations were made to find a suitable isotope which fulfilled the three remaining requirements.

3.4.1.1 Technetium-99M as a radioisotope market

Technetium-99M (Tc 99M) was the first artificially produced radioisotope and is now widely used as a radiopharmaceutical in diagnostic medicine. It is inexpensive and readily available for use in hospitals where it is

produced on the premises from a molybdenum generator (Mo99-Tc 99M generator).

Molybdenum-99 decays with a half-life of 67 hours to technetium-99M. The radioisotope is removed from the Mo99-Tc99M generator by eluting with saline which passes through sterile filters and is collected in a liquid form. Technetium-99M itself decays rapidly ($t_{\frac{1}{2}}$ = 6.02 hrs) by emitting gamma rays to produce a daughter isotope Tc99 - a relatively stable element with a half-life of 210,000 years.

Gamma rays from Tc99M which are more penetrating than alpha or beta rays are detected with a scintillation tube.

3.4.1.2 Methods of marking with Tc99M

Two methods of marking *T. anserinum* with Tc99M were evaluated: an internal label which was incorporated in food and an external label applied to the exoskeleton.

Internal label

Tc99M (2.0 cm^3) was eluted in saline solution from a generator by hospital staff at Leicester Royal Infirmary. The radioisotope solution (0.1 cm^3) was mixed with 2.0 cm^3 fresh swan blood (collected two hours before). Lice taken from swans were kept overnight in pill boxes containing feathers at room temperature. An attempt was made to feed six lice with Tc99M blood solution using the method described on page 92.

External label

A saline solution of Tc99M (0.1 cm^3) was mixed with a small volume of 'Araldite' glue on a microscope slide. Lice were placed dorsal side upwards on a piece of cellotape attached to a clean microscope slide and positioned under a binocular microscope. A trace volume of isotope-labelled araldite was placed on the prothorax using a fine needle. The glue was allowed to set on the insect before it was handled and released on to the swan. Initially the specific activity of each label was approximately 5μCi (185 kBq).

FIGURE 32 : Body regions of a swan divided to record movement of *T. anserinum* over time.

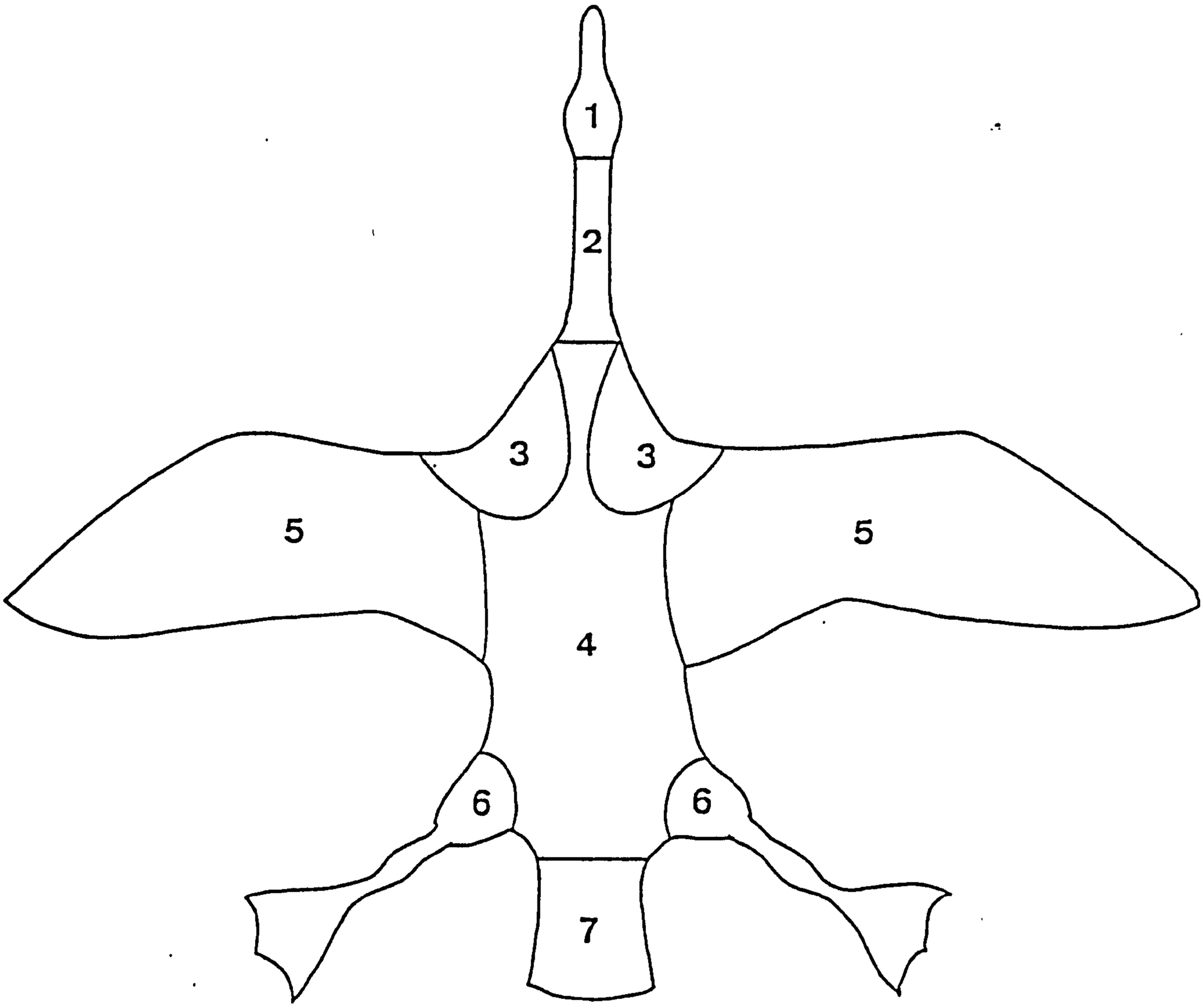


TABLE 26 : Times of experimental observations of *T. anserinum* on swans

Time of experiments	Number of lice observed
12.00 - 24.00	7
15.30 - 24.00	3
15.30 - 06.30	4
18.30 - 06.30	6

3.4.1.3 Effect of radioisotope label on *T.anserinum*

Five lice were labelled externally with Tc99M. They were maintained in pill boxes for 24 hours to determine what effect, if any, the isotope had on their behaviour and survival.

3.4.2 Behavioural studies of *T. anserinum* labelled with Tc99M

The activities of 20 lice (externally labelled) were observed on Mute Swans. Experimental lice were not fed on blood from the time they were removed from their original hosts until they were placed on the swans for observation, a period of approximately 12 hours.

Lice were placed on the head, back and wings of swans and their positions were noted every half-hour for approximately 12 hours using a portable scintillation counter. The actual observation times are shown in Table 26 . The swan's body was divided into regions for recording the position of lice (Fig. 32). After the end of each experiment, the lice were removed from swans and checked to determine that the label was still secure.

Two factors were investigated:

1) The location of lice on Mute Swans,
   (a) by direct observation and recording movements of lice from different starting locations;
   (b) by observing any possible relationship between location and surface temperature of the swan;
2) Feeding times of lice,
   (a) by determining the distances moved by lice during day and night;
   (b) by determining the location of lice during day and night.

3.4.3 Measurement of surface temperatures of swans

The skin surface temperatures were taken of five Mute Swans using an electronic analogue thermometer* operated with a copper/constantan thermocouple to give direct readings in °C.

The thermocouple was made by positioning a length of copper wire (30.0 cm) and an equal length of constantan wire along a piece of wood (0.5 cm in diameter and 15.0 cm in length). The ends of the copper and constantan wires were joined and soldered to form a flattened metal surface which would be placed in contact with the skin surface of the swan.

At the other end of the thermocouple, a plastic tube was placed over the wood to hold the wires in position. The ends of the wires were connected to the terminal of the electronic thermometer.

The temperature of different body regions of the swan were measured to the nearest °C.

(* Electronic analogue thermometer Type 1625 : Comark Electronics Ltd., Littlehampton, West Sussex.)

RESULTS

3.4.4 Suitability of Tc99M as a marker for *T. anserinum*

Four attributes were required of a marker for observing the activity of *T. anserinum*.

1) Tc99M satisfied the first attribute because it detected the lice even when they were not visible to the observer.

2) Tc99M has a half-life of 6.02 hours and is still detectable after 12-15 hours. It is therefore suitable for monitoring lice over this time interval.

3) An experiment was performed to determine the effects, if any, of the isotope on lice. The five lice remained alive and their behaviour appeared unaltered by the radiolabel. During this time the lice consumed feathers and blood (fed *in vitro* as described on p. 92) determined later by examination of their crop contents.

4) The quickness and ease of application of the isotope was considered.

Internal label

None of the six lice ingested radiolabelled blood. Prior to these experiments, the lice had not had a blood meal for over 24 hours and it is unlikely that the reason for not feeding was that they were satiated. It was concluded that the internal label was an unsuitable labelling technique.

External label

Araldite glue mixed with Tc99M was a successful labelling technique. It took only 10 minutes to label one insect ready for experimentation once the radioisotope had been collected.

3.4.5 The location of lice on Mute Swans

3.4.5.1 Location of lice on swans from different starting locations

The locations and movements of 20 lice were observed individually on swans. All lice exhibited some movement over the bird except one which remained in its starting position.

Lice were placed in three starting locations: back, head and wings. The extent of movement of lice placed in each position is illustrated (Fig. 33).

For lice placed on the back (Fig.33A), a range of movement occurred onto wings, up the neck onto the head and over most of the back and scapular regions. More details of movement can be obtained by looking at individual maps of the position of each louse on a swan (Fig.33B). In general, when lice were placed on the back, the direction of movement was towards the scapular region or onto the wing itself. Three lice were observed to move directly towards the scapular region without moving to any other part of the bird. Others took an indirect route but ultimately arrived at the wing/scapular area. Four lice moved from one shoulder to the other. One louse moved from the back up to the crown of the head, returned to the back and then to the wing.

The range of movement of lice placed on the wings was confined to the scapular regions, the middle of the back and the wings (Fig.33C). Individual maps of louse movement show three out of four lice remained on the wing and shoulder during the whole period of observation. Only one louse moved from the wing to the middle of the back and returned almost to the same point on the wing again (Fig.33D). Whilst on the wing, the lice rested upon the primary flight feathers.

The extent of movement of lice placed on the head (Fig.33E) shows the lice moved down the neck to the scapular region, top of the back and onto parts of the wings. The detailed movements of each louse show that three out of five lice placed on the crown of the head did not move from this position at all. One of these lice moved from the crown to the gular (throat) underneath. Two other lice however, left the head and moved down the neck to the scapular region; one louse remained here, the other moved onto the back (Fig.33F).

FIGURE 33 : Movement of *T. anserinum* on Mute Swans.

A. Range of movement of eleven lice placed in middle of back.

B. An individual map of movement of one louse over a 12 hour period (placed on the back)

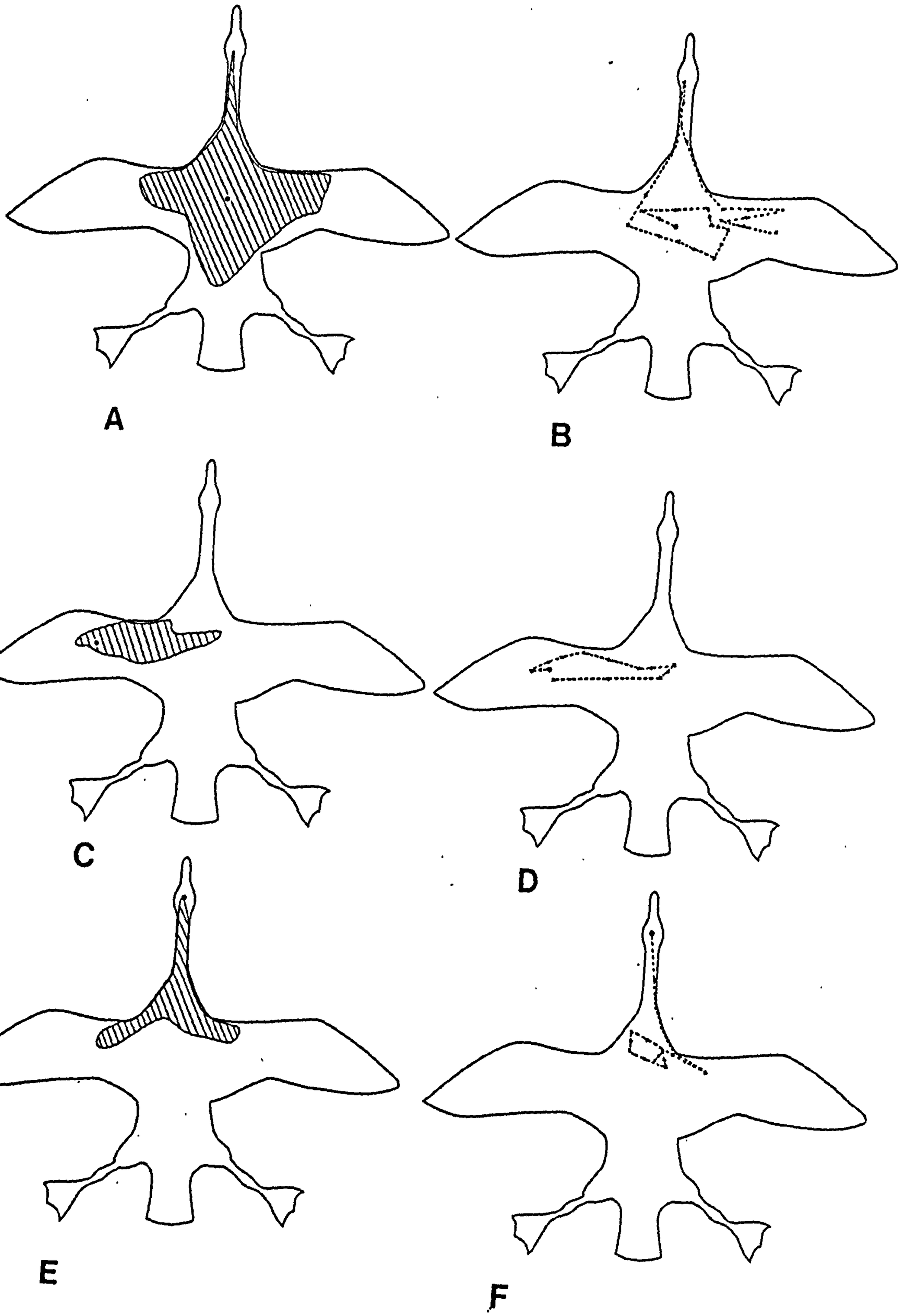
C. Range of movement of four lice placed on the wing.

D. An individual map of movement of one louse over a 12 hour period (placed on the wing).

E. Range of movement of five lice placed on the head.

F. An individual map of movement of one louse over a 12 hour period (placed on the head)

● indicates starting location of lice.


A
B
C
D
E
F

3.4.5.2 Locations of lice related to surface temperature of swans

Temperatures were measured at the base of feathers in different regions of swans. Wing temperatures were measured whilst the wings were folded and are therefore recorded on the ventral side temperature map. The results of measurements from swans are shown in Fig. 34. Surface temperatures were variable in different regions and are represented as a coded number in the illustrations. Highest temperatures (42-44°C) were recorded under the wing in an area with only a few downy feathers, and lowest temperatures were recorded in the cloacal region (24-26°C).

All lice moved to and from regions of the swan that would remain, for the most part, above water level. By comparing the extent of movement (shown in Figs.33A, C, E.) with the temperature map of the dorsal surface, it can be seen that lice were always in regions with temperatures of 33-38°C (code numbers 4-5).

3.4.6 Feeding times of lice

It was suggested (p.103) that the feeding activities of *T. anserinum* may be related to the periodicity of *S. eurycerca* microfilariae and to investigate this hypothesis, the locations and movements of lice were observed during the day and night time.

3.4.6.1 Distances moved by *T. anserinum* between 12.00-24.00 hours and 15.30-06.30 hours

Ten lice were observed from morning to midnight and ten were observed from afternoon until 06.30 hours the following morning (Table 26).

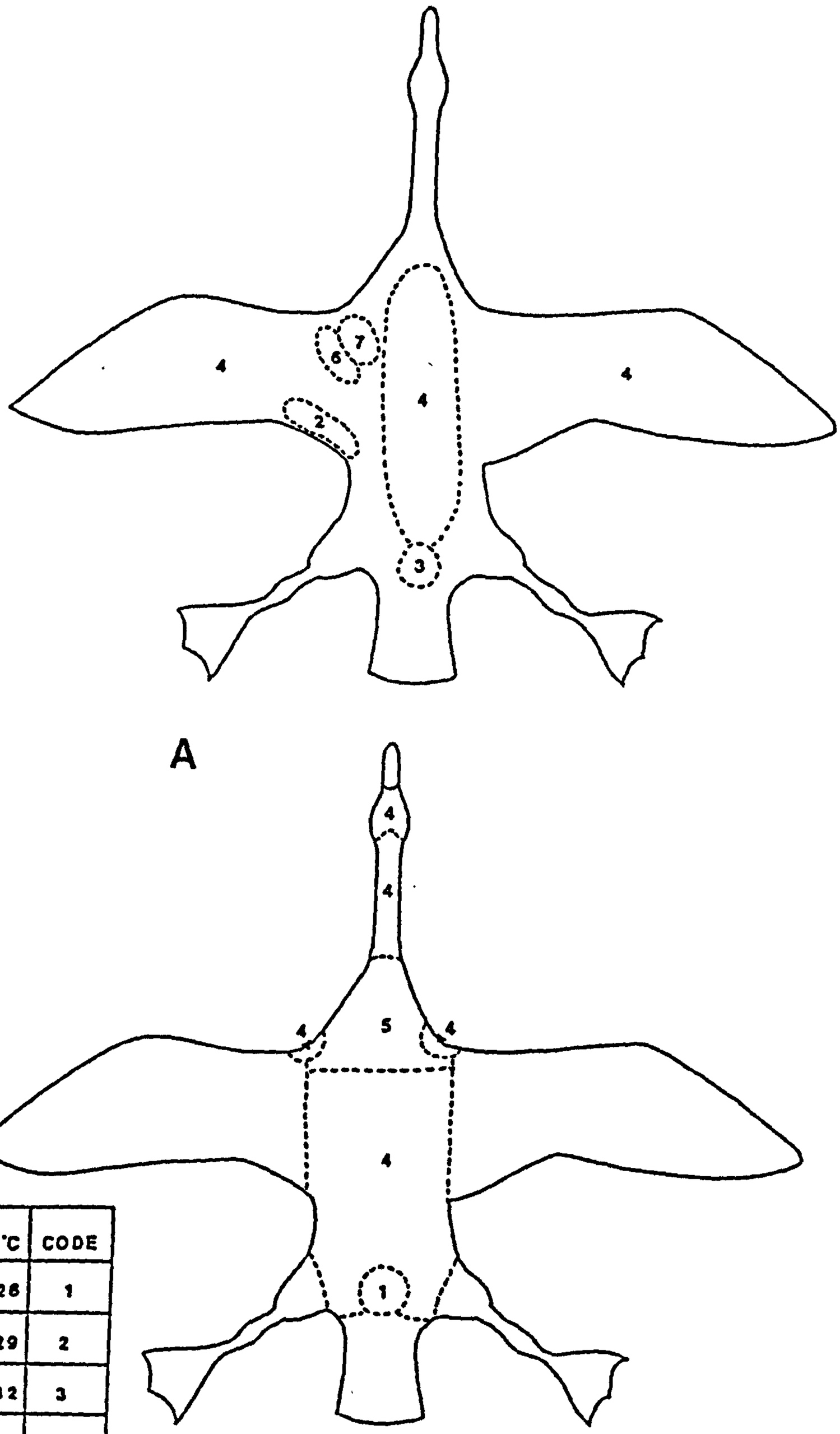
For seven lice observed from 12.00-24.00 hours the furthest mean distance moved was in the first half-hour (Fig.35A). Movement was recorded from 12.00 until 22.00 apart from three half-hour periods at 16.00, 19.30 and 20.30 hours. Three lice observed over 15.30-24.00 hours moved on the bird until 19.30 (Fig.35B). From this time until 24.00 no movement was observed.

The observations made overnight show that lice are mobile during the night as well as during the day. Four lice observed over 15.30-06.30 hours showed a pattern of intermittent movement until 01.30, after which time no movement was observed (Fig.35C). Six lice were observed over 18.30-06.30, but intermittent periods of movement were observed throughout the

FIGURE 34 : Skin surface temperatures of Mute Swans.

A. Ventral surface temperatures of swan (numbers refer to temperature ranges given in key).

B. Dorsal surface temperatures of swan.



TEMP. °C	CODE
24-26	1
27-29	2
30-32	3
33-35	4
36-38	5
39-41	6
42-44	7

B

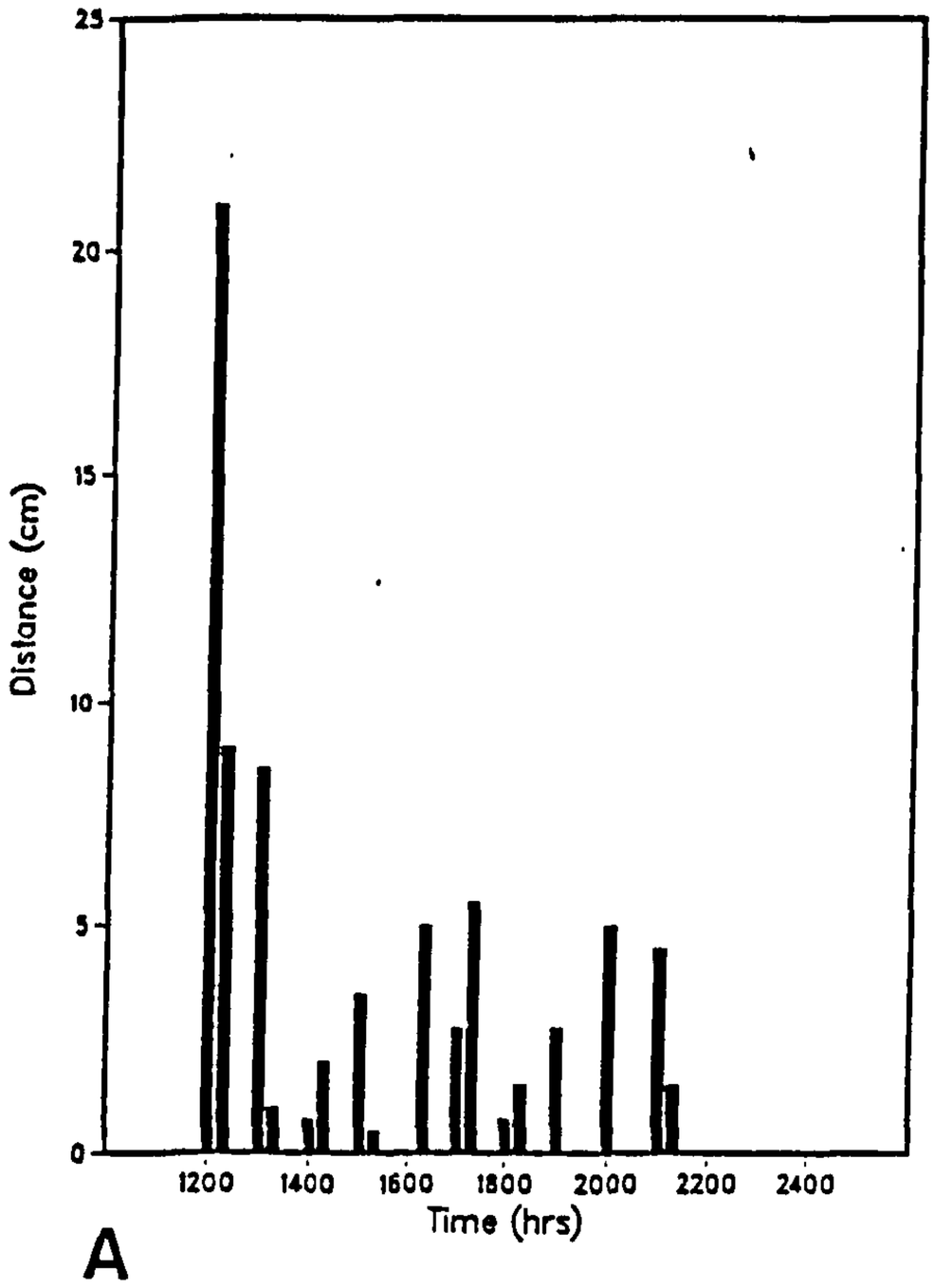
FIGURE 35 : Distances moved by *T. anserinum* (N = 20) on Mute Swans (cm).

A. Distances moved by *T. anserinum* during period 1200 - 2400 hours (N = 7).

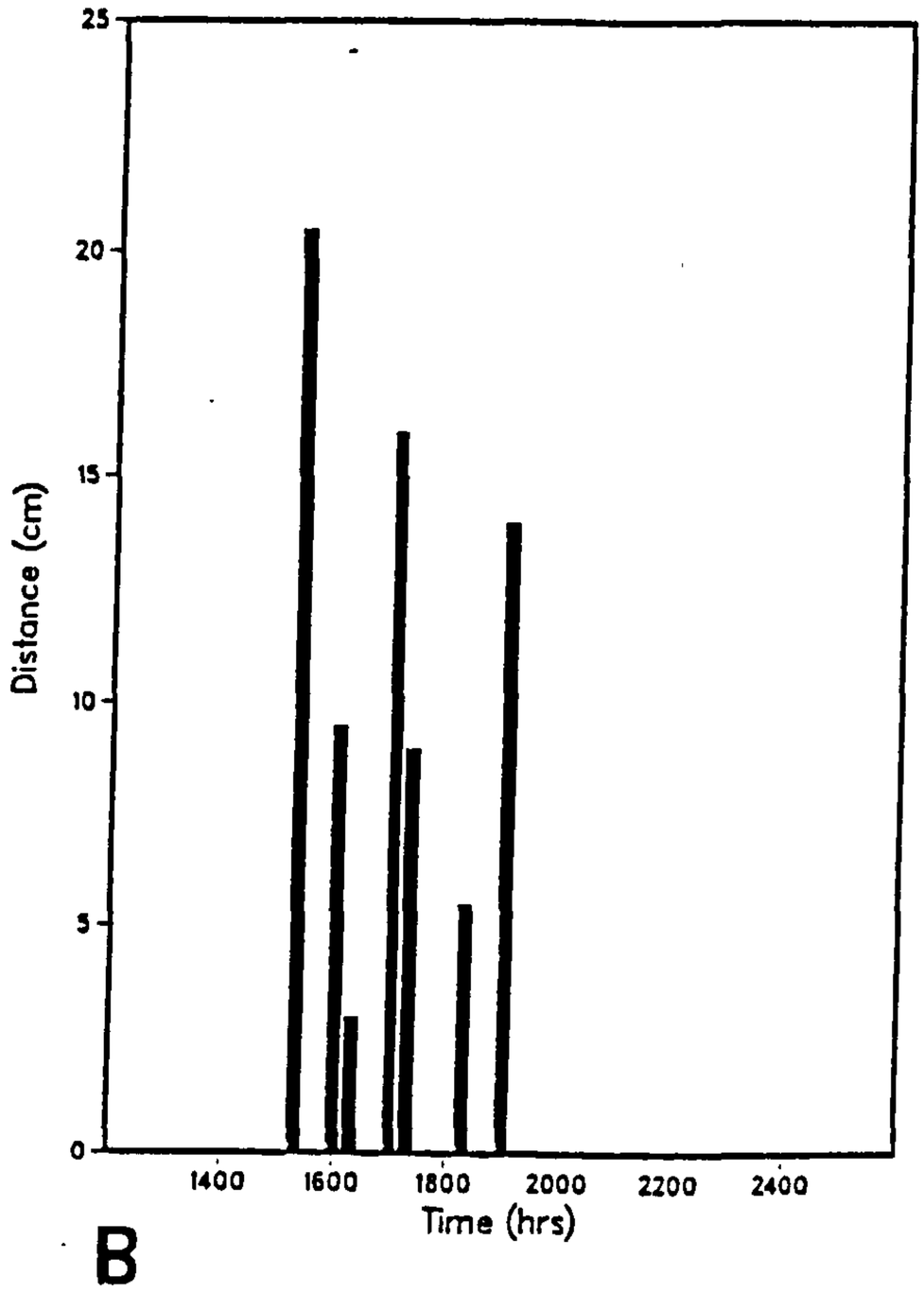
B. Distance moved by *T. anserinum* during period 1530 - 2400 hours (N = 3).

C. Distances moved by *T. anserinum* during period 1530 - 0630 hours (N = 4).

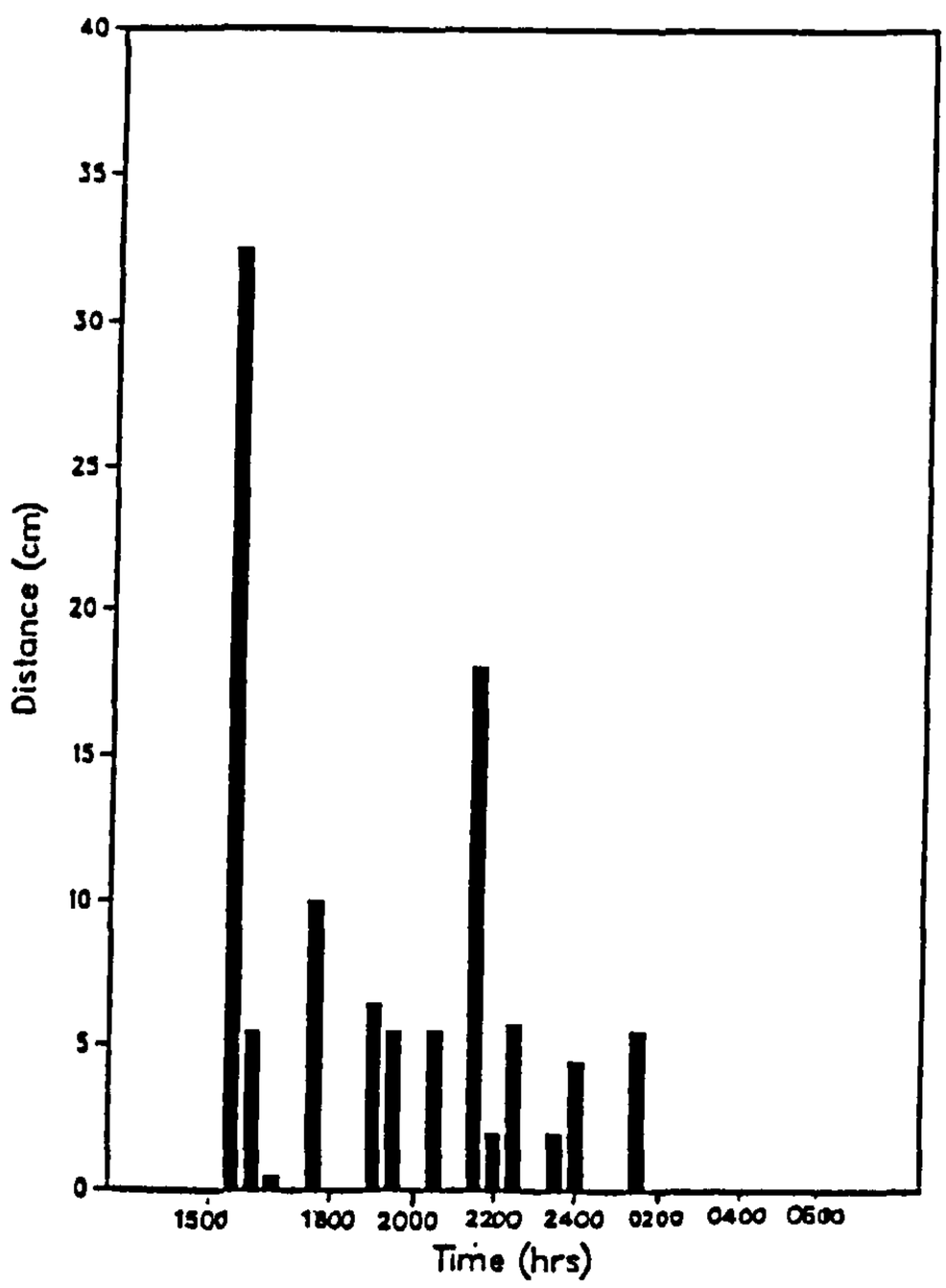
D. Distances moved by *T. anserinum* during period 1830 - 0630 hours (N = 6).


25
20
15
10
5
0
Distance (cm)
1200
1400
1600
1800
2000
2200
2400
Time (hrs)

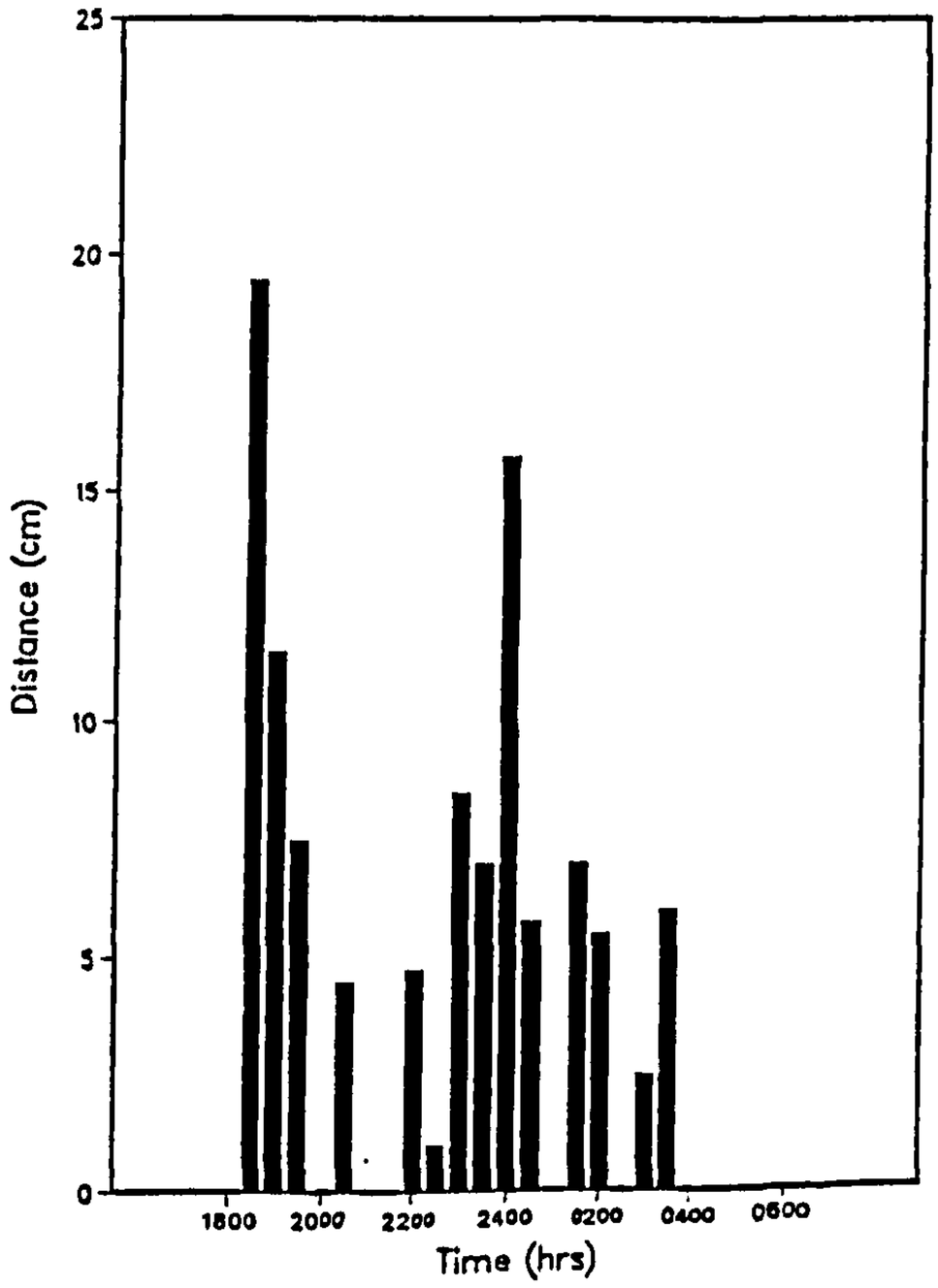
A


25
20
15
10
5
0
Distance (cm)
1400
1600
1800
2000
2200
2400
Time (hrs)

B


40
35
30
25
20
15
10
5
0
Distance (cm)
1500
1800
2000
2200
2400
0200
0400
0600
Time (hrs)

C


25
20
15
10
5
0
Distance (cm)
1800
2000
2200
2400
0200
0400
0600
Time (hrs)

D

experiment until 04.00 hours. Between 04.00-06.30 no movements were recorded. (Fig.35D).

Overall, lice moved throughout the periods of observation from 12.00 until 04.00 hours. There were three particular patterns of movement exhibited by all lice. First, the furthest movements were recorded in the first half-hour of being placed on the swan whether it was 12.00, 15.30 or 18.30 hours. Second, no movements were recorded in the last 2-4 hours of observation. This occurred whether it was 19.30, 22.00, 01.30 or 04.00 hours. Third, movement was intermittent. This was observed in individual profiles of movement, or from pooled observations.

3.4.6.2 Location of *T. anserinum* in the period 12.00-06.30 hours

It was necessary to establish the location of lice at specific times of day to try to determine the preferred sites for feeding.

For each of the three starting positions, back, wings and head, the proportion of lice in different locations on the bird were calculated for each half-hour period and results shown in Figs.36A, B, C.

For lice starting on the back (Fig.36A) between 12.00-15.00 hours, 75 per cent of lice had moved towards the scapular region and the wing. Between 15.00-19.00 hours, most lice were found on the scapular area and a few were on the wing and back. After 19.00 hours however, almost 80 per cent of lice had moved to the wing and by 24.00 hours, no lice were observed on the back but were all on the wing or scapular region.

For lice placed on the wing (Fig.36C) all remained on the wing from 12.00 hours until 16.30 hours. Between 17.00-23.00 hours lice were distributed between the wing and scapular region until 23.30 hours they moved from the scapular region completely onto the wing and the back. All lice remained on the wing and back until 03.30 hours when they then moved to the wing where they remained until the end of the observations.

Lice placed on the head remained in situ from 12.00 hours until 16.00 hours. Some (25%) moved onto the wing but most still remained on the head until 18.30 hours. Between 19.00-23.00 hours over half of the lice (60%) still remained on the head but some moved onto the scapular region (40%) for some of the time, and then onto the back and wings. At 24.00 hours, the lice were distributed evenly between the wing and scapular region until the experiments ended at 06.30 hours. (Fig.36B).

Figure 36 : Location of *T. anserinum* on Mute Swans between the period 1200 - 0630 hours.

A Location of lice starting from the back of the swan

B Location of lice starting from the head

C Location of lice starting from the wings

Lice were placed at three starting locations on swans - back, head and wings. The position of each of the 20 lice studied was determined every 30 minutes (between 1200 - 0630 hours) and is represented as a percentage on the graph.

For example, for lice placed on the back, if at 1200, 3 lice were on the back and 1 louse was on the scapular region, this would be represented on Fig.36A as 75% ■ and 25% □

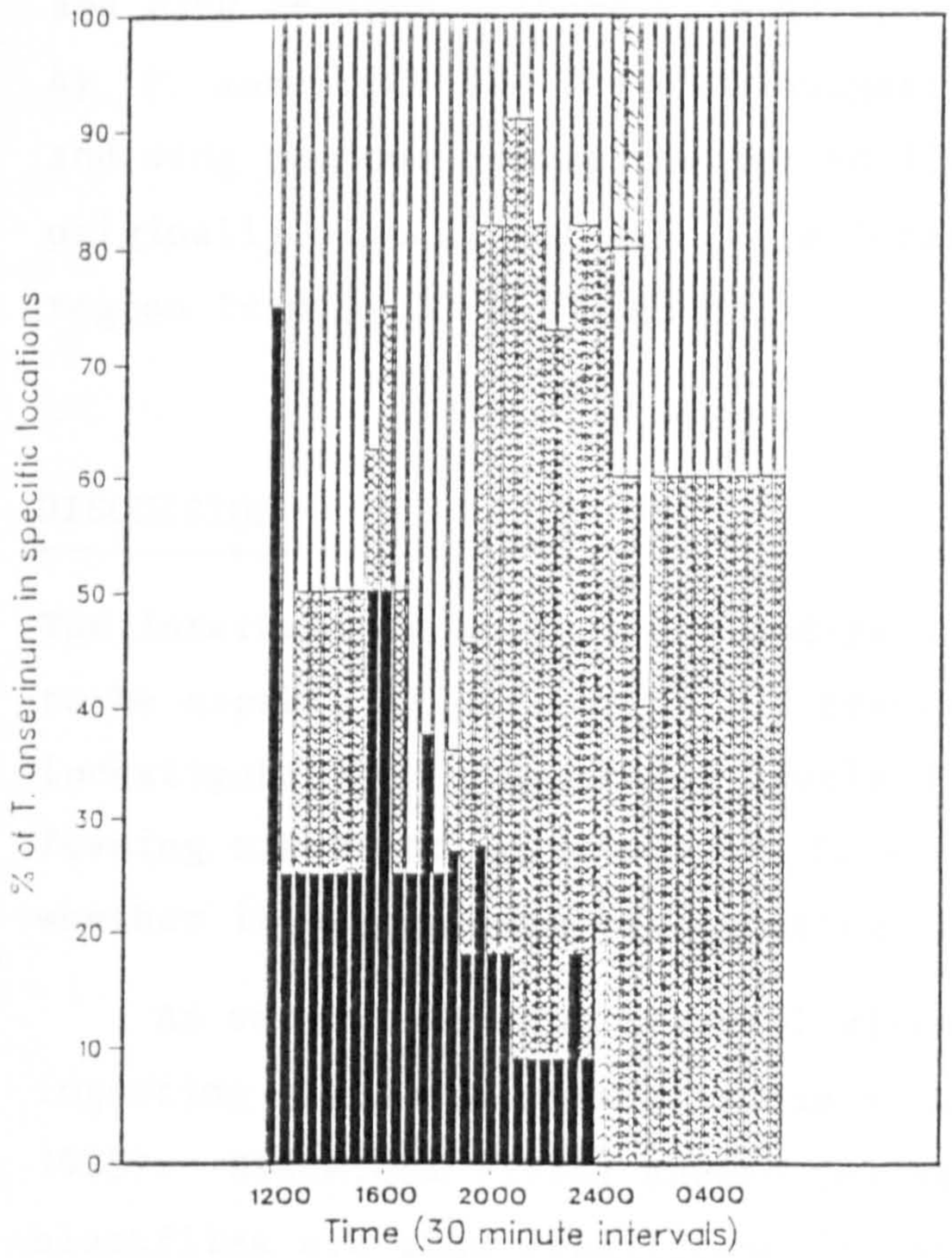
■ back

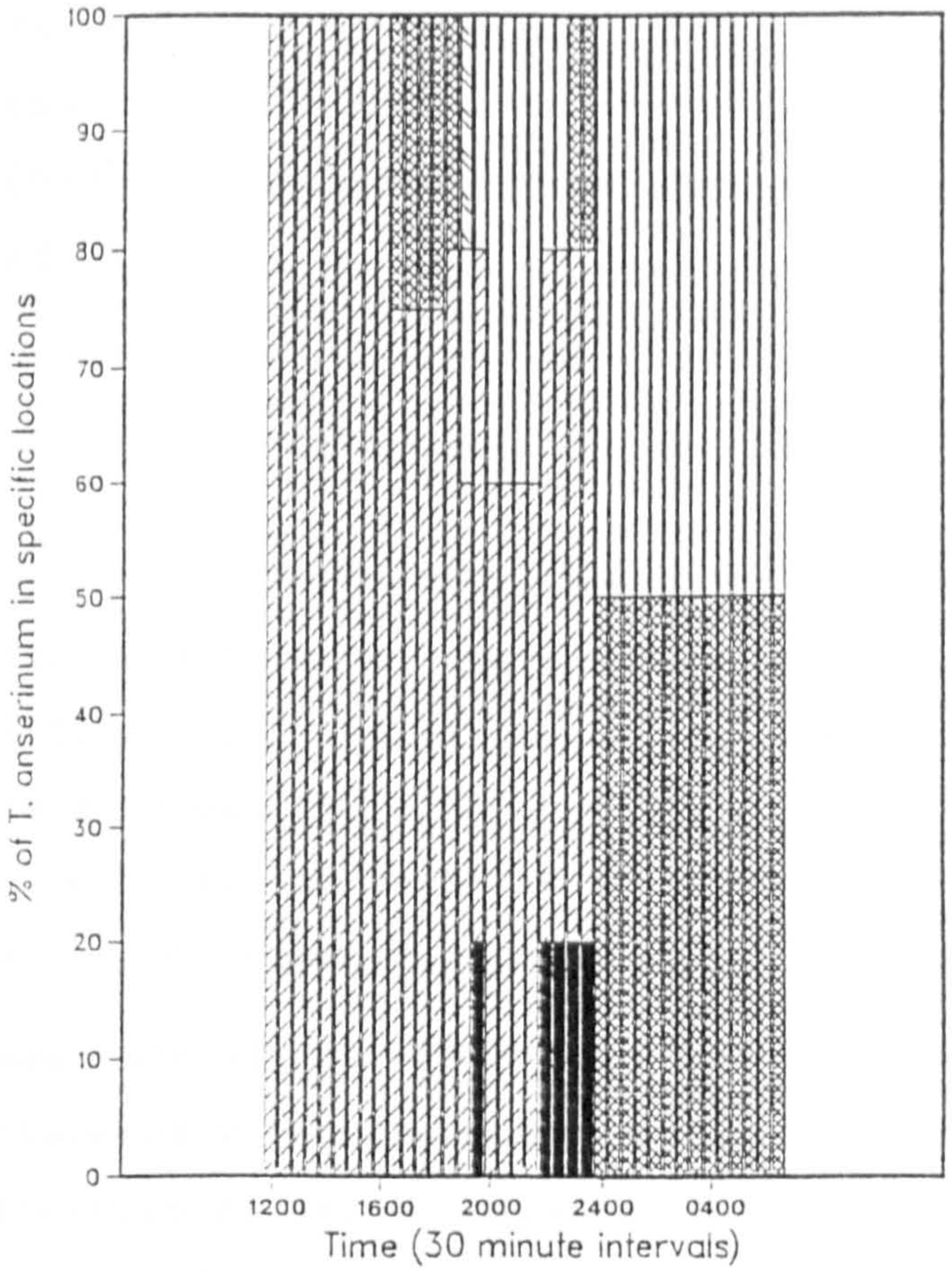
▨ wing

▨ head

▨ neck

□ scapular


100
90
80
70
60
50
40
30
20
10
0
% of T. anserinum in specific locations
1200
1600
2000
2400
0400
Time (30 minute intervals)


100
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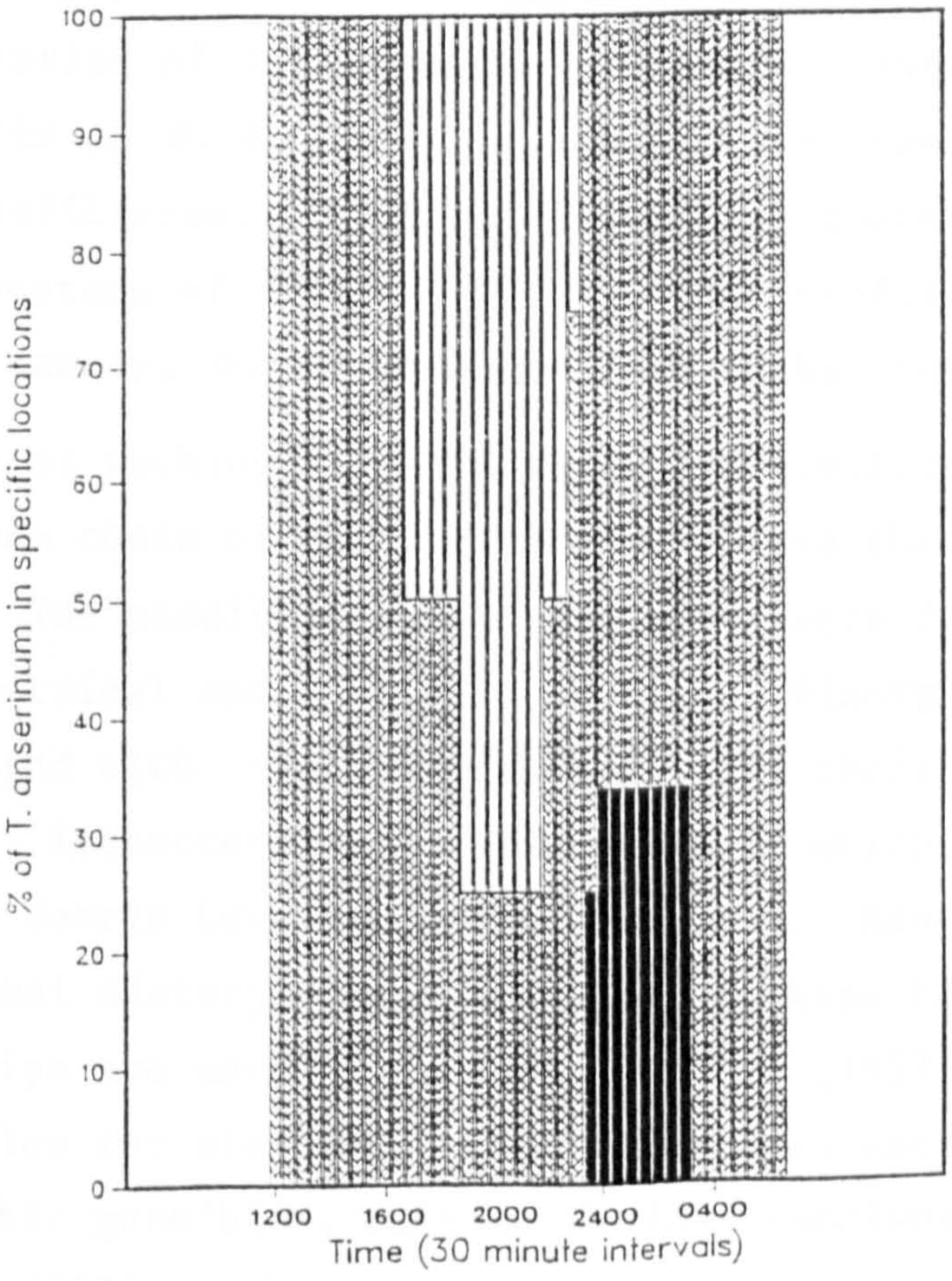
% of T. anserinum in specific locations
1200
1600
2000
2400
0400
Time (30 minute intervals)

A

B


100
90
80
70
60
50
40
30
20
10
0
% of T. anserinum in specific locations
1200
1600
2000
2400
0400
Time (30 minute intervals)

C

In summary, *T. anserinum* was located in five areas of the swan during the period 12.00-06.30 hours: on the neck, scapular, wing, head and back regions. There were no specific patterns of movement exhibited by *T. anserinum* but the data suggest that the lice move onto the scapular and wing regions during the period 12.00-24.00 hours. Wherever they were originally placed, all lice were located on the wing, back or scapular region from 01.30-06.30 hours.

DISCUSSION

The intermediate host of a blood-parasite *S. eurycerca* , must imbibe blood to be capable of developing and transmitting the filarial infection. By investigating the structures involved in feeding, the crop contents, feeding times and locations of *S. eurycerca*, it was possible to establish whether it was capable of ingesting and transmitting *S. eurycerca*.

As vectors of many filarial diseases, mosquitoes are capable of ingesting blood and microfilariae simultaneously (Gordon and Lumsden, 1939). Blacklock (1926) showed the structures of the mouthparts of blackflies are also significant in ingestion of microfilariae. *S. damnosum* has a short scarifying proboscis which makes it a good vector of the skin-inhabiting microfilariae of *O. volvulus*. Crosskey (1962) showed that the rasping proboscis of *S. damnosum* is adapted for ingestion of both skin and blood microfilariae. It is not surprising therefore that simulids are also vectors of filarial worms with microfilariae in the blood of wildfowl, namely, *O. fallisensis* from ducks (Anderson, 1956).

The mouthparts of sucking flies (mosquitoes, simulids) are structurally different from those of chewing lice which use their mandibles to penetrate tissues. The mandibles of *T. anserinum* were found to be dimorphic or asymmetrical and have pointed tips. Dimorphic mandibles are usually associated with insects which masticate their food (Snodgrass, 1935). Ischnoceran lice, for example, which feed exclusively on feather and skin debris have dimorphic mandibles. Most Amblyceran lice, whose additional dietary component is blood, also have dimorphic mandibles but the tips are more pointed which Keler (1957) thought was probably an adaptation for piercing. Lice which feed exclusively on blood have monomorphic mandibles, such as *Ricinus carolynae* and *R. sittae* (Nelson, 1972). *T. anserinum* could represent an intermediary stage between feather-feeding Ischoceran lice and specialised blood-

feeders like *Ricinus* . The pointed tips of the mandibles of *T. anserinum*, are probably used to pierce tissues to obtain a blood-meal.

Also associated with the mouthparts is the hypopharynx which was described on p. 87 . Associated with the hypopharynx are two structures, the sitophore sclerite and the lingual sclerite. Both structures are peculiar to Mallophaga and the closely-related Pscoptera. Their function is not entirely understood in mallophaga (Snodgrass, 1944) but it has been suggested that they are used to crush fungus filaments in Pscoptera (Weber, 1936). What is of specific interest to the present study is the typical structure of the sclerites is modified in three particular mallophagan genera - namely, *Trinoton, Dennysus* and *Heterodoxus* (Cumming 1916). *Dennysus hirundis* and *Heterodoxus spiniger* are the only two lice that are presently known to be intermediate hosts of filarial nematodes. *T. anserinum* has been proposed as a third example. It is possible that there is some connection between the sitophore and lingual sclerites and the ingestion of microfilariae. This at least warrants further investigation.

The results of this section show conclusively that *T. anserinum* feeds on blood. Over half (54.4%) of the dissected specimens had fed on blood which correlates well with Seegar's (1977) findings of 66 per cent of *T. anserinum* with blood from Whistling Swans. *T. anserinum* is a rapid blood-feeder taking on average 15 seconds to complete a blood-meal. This is a faster feeding rate than the human body louse (*Pediculus humanus corporis*) which can take several minutes to feed (Balashov, 1984). The mechanism of blood-feeding was observed and recorded uniquely on film during this study and it is believed that the mechanism is conducive to ingesting microfilariae with a blood meal. Indeed, on several occasions live microfilariae were observed to be drawn in through the mouthparts during this process. During feeding, the mandibles were maintained in an "open" position allowing a constant flow of blood to enter the pharynx.

Observations of the movements of radiolabelled lice indicated the neck, head, scapular regions, back and wings to be the preferred sites on Mute Swans. It was not possible to determine the specific times or locations when *T. anserinum* fed from the swan although lice were located on wing and scapular areas during the period of maximum microfilaremia of *S. eurycerca* (established by studying the periodicity of this nematode p. 36 .)

4. DEVELOPMENT OF *S. eurycerca* LARVAE WITHIN *T. anserinum*

An intermediate host must provide a suitable environment in which larval parasites can develop. Indeed, in arthropods which are unacceptable hosts, larvae usually die in the gut or fail to develop elsewhere (Robinson, 1955). Even in insects which are excellent hosts it cannot be assumed that once microfilariae have been ingested they inevitably develop into third stage infective larvae. Some factors can prevent larval development in apparently suitable host environments. For example, in most haemotaphagous insects, a peritrophic membrane is secreted around the blood in the midgut which traps some of the microfilariae and prevents them from penetrating the haemocoel (Lewis, 1953). Conversely, some mosquitoes produce salivary secretions containing anticoagulants which facilitate migration of the microfilariae by allowing them to escape from the blood meal in the midgut (Kartman, 1953).

Within the Family Onchocercidae, 50 of the 300 species of nematodes have known intermediate hosts. Altogether 29 species develop in mosquitoes, 6 in midges, 4 in simulids, 2 in tabanids, 1 in a hippoboscid, 2 in fleas, 2 in lice (including *Dip. reconditum* listed under fleas), 1 in a mite and 5 in ticks (Nelson, 1964). The morphological changes which occur during the development of filariae in their intermediate hosts are common to all members of the Onchocercidae.

During the early stages of development, filarial larvae are quiescent. The development proceeds by the larvae shortening and fattening (referred to as "sausage stage"), followed by an ecdysis into second stage larvae. These are also sluggish but grow rapidly and after a second ecdysis the third stage or infective larvae emerge. In mosquitoes third stage larvae invariably escape from the proboscis, head first and enter the final host through a puncture made by the vector. In fleas, infective stage larvae of *D. immitis* escape from the mouthparts or from injured areas of the exoskeleton and enter the host through wounds made during feeding. The length of time for complete larval development varies according to species and environmental conditions. *D. immitis* took 180 hours (7.5 days) to develop from microfilariae to mature infective larvae in six flea species (Steuben, 1954). Development of *O. fallisensis* microfilariae in species of blackfly required 7-14 days depending on temperature (Anderson, 1956).

Many researchers have implicated specific insects as intermediate hosts by observing the development of microfilariae within them. An

essential prerequisite in all these studies has been the availability of large numbers of experimental insects. The reasons for this are twofold: first, where insects are experimentally infected with microfilariae, mortality rates are very high (Kartman, 1953; Bradley, 1953); and second, observations of filarial development can extend over 20 days or more during which time at least 10 insects need to be dissected daily to produce statistically viable results. Consequently, Kartman (1953) dissected more than 1500 colony-reared mosquitoes to observe the development of *D. immitis* and Steuben (1954) observed the same nematode species develop in 110 laboratory-reared fleas. Anderson (1956) dissected 198 simulids to observe the morphological changes occurring during the development of *Ornithofilaria fallisensis* . Seegar (1977) did not observe the ongoing development of *S. eurycerca* in *T. anserinum* but dissected 189 lice and recovered 450 larvae of *S. eurycerca* from them. It is essential then in a study of filarial development in lice to have a large number of available insects.

This section seeks to determine whether *T. anserinum* provides a suitable environment where microfilariae of *S. eurycerca* can develop into third stage larvae by:

1) attempting to establish a laboratory colony of *T. anserinum;*
2) observing the development of naturally occurring larval stages within *T. anserinum.*

4.1 MATERIALS AND METHODS

4.1.1 Laboratory rearing of *T. anserinum*

During the course of this study, a series of experiments was conducted to establish the conditions required to maintain a laboratory population of *T. anserinum* . Live lice were removed from Mute Swans and placed into plastic pill boxes (diameter = 5 cm). Each pill box contained five layers of moist filter paper, downy feathers, and contour feathers from the swan's back were packed closely together.

4.1.1.1 Preliminary experiments

Initial attempts to maintain lice in vitro were performed at temperatures of 32-34°C in a water bath incubator, where high humidities could be achieved. Newly-emerged, blood-filled feathers were placed into pill

boxes every day. Out of eight lice (6 adults, 1 nymph 2 and nymph 1) maintained in these conditions, seven survived for two days and one for three days. On examination of the dead lice, all appeared to be dehydrated because the normal rounded appearance of the abdomen and appendages were concave and sunken. These preliminary experiments indicated that not only was a high humidity required but *T. anserinum* also needed a more regular intake of fluids.

4.1.1.2 Maintenance of *T. anserinum* by regular blood-feeding

Once the technique of feeding lice with blood had been established (p.92) lice were fed twice daily and maintained in pill boxes in different conditions of temperature and humidity. Temperatures of 22°C and 60-70% relative humidity were achieved by placing pill boxes over a tray of water. Relative humidities were calculated using a wet-dry hydrometer. Higher temperatures (30-35°C) and humidities of 80% were obtained in an incubator containing a solution of sulphuric acid (25%) (Buxton and Mellanby, 1934).

Twenty-two lice were maintained at 22°C, 65% RH; ten lice were kept at 30-31°C, 80% RH; thirteen lice at 31-32°C, 80% RH and three lice at 35°C, 80% RH.

4.1.2 RESULTS

Large numbers of lice were not available for in vitro experiments. A total of 48 lice collected from swans, were maintained at four different conditions of temperature and humidity. The age profile of experimental lice was 39 adults, 5 third instar, 3 second instar and 1 first instar.

The combined survival results for 48 lice are summarised (Table 27). The longest survival time was 9 days. Most lice (86%) survived for 1 day and 58% survived for 2 days. After this time the number surviving decreased more rapidly.

The survival results of lice at each set of temperature and humidity conditions are summarised (Table 28). At 22°C, 65% RH five control lice were maintained with no regular blood meals and survived only for 3 days. Under the same conditions the longest survival time of 9 days was achieved. At 30-31°C and 80% RH, the longest survival time was 7 days, although, on a separate occasion only 2 adult lice survived for 3 days in the same conditions. At 31-32°C and the same humidity, 4 out of 13 lice survived for 4 days. At a higher temperature of 35°C, 2 out of 3 lice survived for only 3 days.

Table 27: Percentage survival for all lice under different conditions of temperature and humidity

DAY	TOTAL LICE ALIVE (blood-fed)	% SURVIVAL	CONTROL LICE ALIVE (unfed)	% SURVIVAL
t1	43	100	5	100
t2	37	86	5	100
t3	25	58	5	100
t4	15	35	0	0
t5	7	16		
t6	5	11		
t7	3	7		
t8	2	5		
t9	2	5		

Table 28 : Percentage survival of lice at specific conditions of temperature and humidity

DAY	TOTAL LICE ALIVE (blood-fed)	% SURVIVAL	CONTROL LICE (unfed)	DAY	TOTAL LICE ALIVE (blood-fed)	% SURVIVAL
CONDITIONS: 22°C / 65% RH				30-31°C / 80% RH		
t1	22	100	5	t1	8	100
t2	22	100	5	t1	8	100
t3	17	77	5	t3	4	50
t4	8	36	0	t4	3	37
t5	5	23		t5	2	25
t6	4	19		t6	1	12
t7	2	9		t7	1	12
t8	2	9		t8	0	0
t9	2	9				
t10	0	0				
CONDITIONS: 31°C / 80% RH				31-32°C / 80% RH		
t1	2	100		t1	13	100
t2	2	100		t2	7	54
t3	2	100		t3	5	38
t4	0	0		t4	4	31
				t5	0	0
CONDITIONS: 35°C / 80% RH						
t1	3	100				
t2	3	100				
t3	2	67				
t4	0	0				

DISCUSSION

Large numbers of experimental insects are essential for in vitro studies of larval development. It was not possible to obtain more than 5-6 live specimens of *T. anserinum* at one time. In an attempt to establish a laboratory population of *T. anserinum* it was soon evident that regular blood meals were required for their survival. Without feeding, lice survived for only three days. With a blood meal twice a day, lice survived from three to nine days. The longest survival rate was achieved under conditions of 22°C and 65 per cent relative humidity. At higher temperatures and humidity (35°C, 80% RH) lice survived for only three days. Although 22°C is a much lower temperature than would be found on a swan, it possibly reduced the activity of the lice and therefore maintained the body fluid balance for a longer period. At higher temperatures it was likely that lice required a more regular intake of blood than was provided. The small number of experimental lice hindered the attempts to establish a laboratory population and it was therefore not possible to observe the ongoing development of *S. eurycerca* larvae in experimentally infected lice.

4.2 Dissection of *T. anserinum* to observe developing larval stages of *S. eurycerca*

It was not possible to acquire and maintain sufficient numbers of live lice for a long enough duration to observe the ongoing development of *S. eurycerca* larvae. However, lice were collected throughout the study and were preserved in 70 per cent alcohol. The stages in the development of the larvae (L_1, L_2, L_3) were determined from dissection of the preserved material.

4.2.1 MATERIALS AND METHODS

T. anserinum (N = 127) were dissected on a microscope slide as described on p. 88. The developing stages were photographed and measured using a binocular microscope (x100).

4.2.2 RESULTS

Of 127 preserved specimens of *T. anserinum*, 23 (18%) had developing stages of *S. eurycerca*. Fifteen of the 23 (83%) infected lice had blood in their crops, and 20 (87%) had been removed from swans with microfilariae in the blood (determined by the sedimentation technique p. 18). The results of the dissections (Table 27) show 34 developing larvae were removed from 23 *T. anserinum*. Twelve larvae were removed from the head, thirteen from the thorax and eight from the abdomen. One larva was inbetween the thorax and the head. Measurements of the larvae indicate their stage of development (Table 29). (Fig.37)

Table 29 : Measurements of lengths of *S. eurycerca* larvae dissected from *T. anserinum* infesting Mute Swans

LENGTH (μm)	LOCATION AND NUMBER OF LARVAE		
	HEAD	THORAX	ABDOMEN
200 - 399	0	2	5
400 - 599	0	0	3
600 - 799	2	4	0
800 - 999	5	4	0
1000 - 1199	2	0	0
1200 - 1399	1 (1)*	1	0
1400 - 1599	2	1	0
1600 - 1799	0	0	0
1800 - 1999	0	1	0
TOTAL LARVAE	12 (1)*	13	8

(* Located inbetween head and thorax)

FIGURE 37 : Developing stages of *S. eurycerca* larvae dissected from *T. anserinum*.

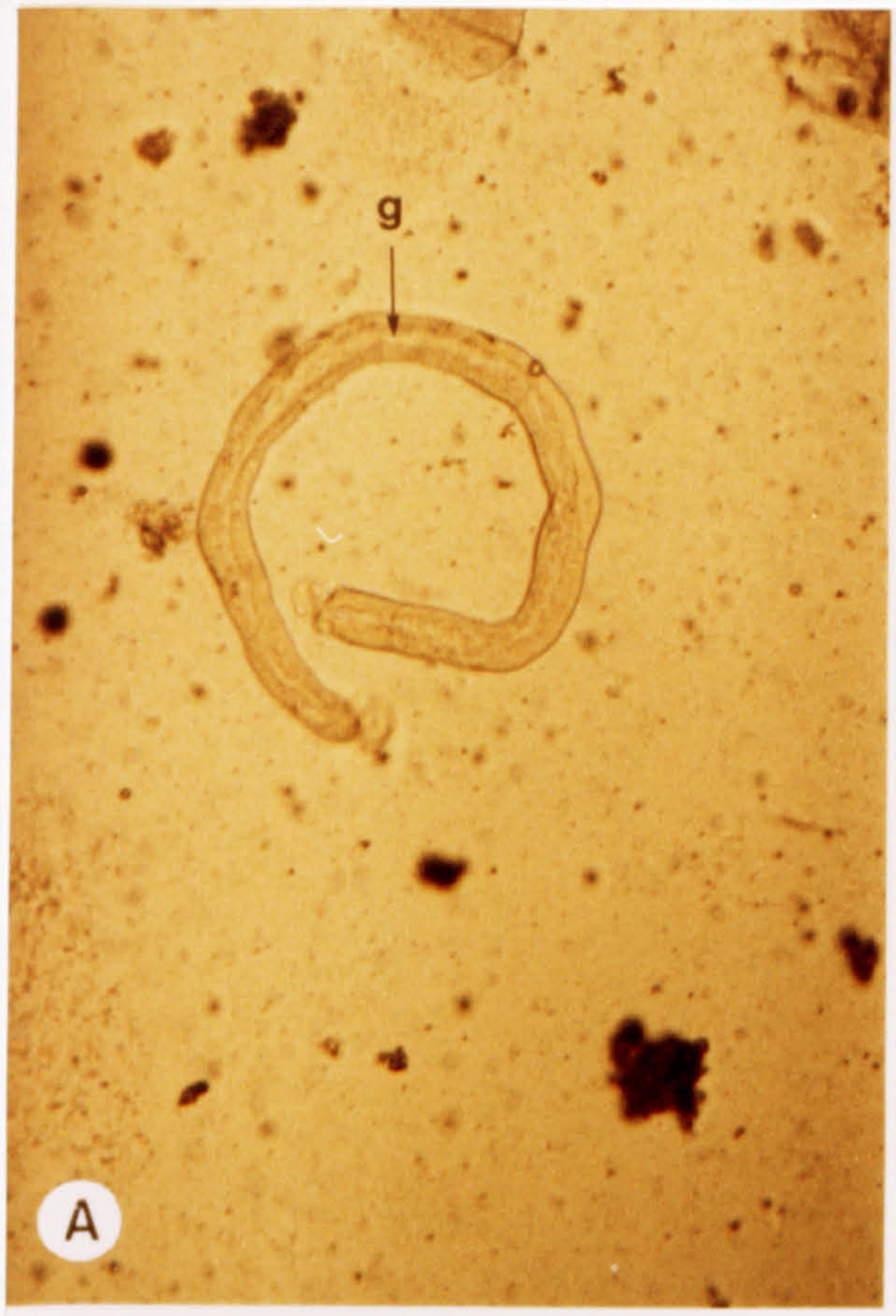
A. First stage larva dissected from abdomen of louse.

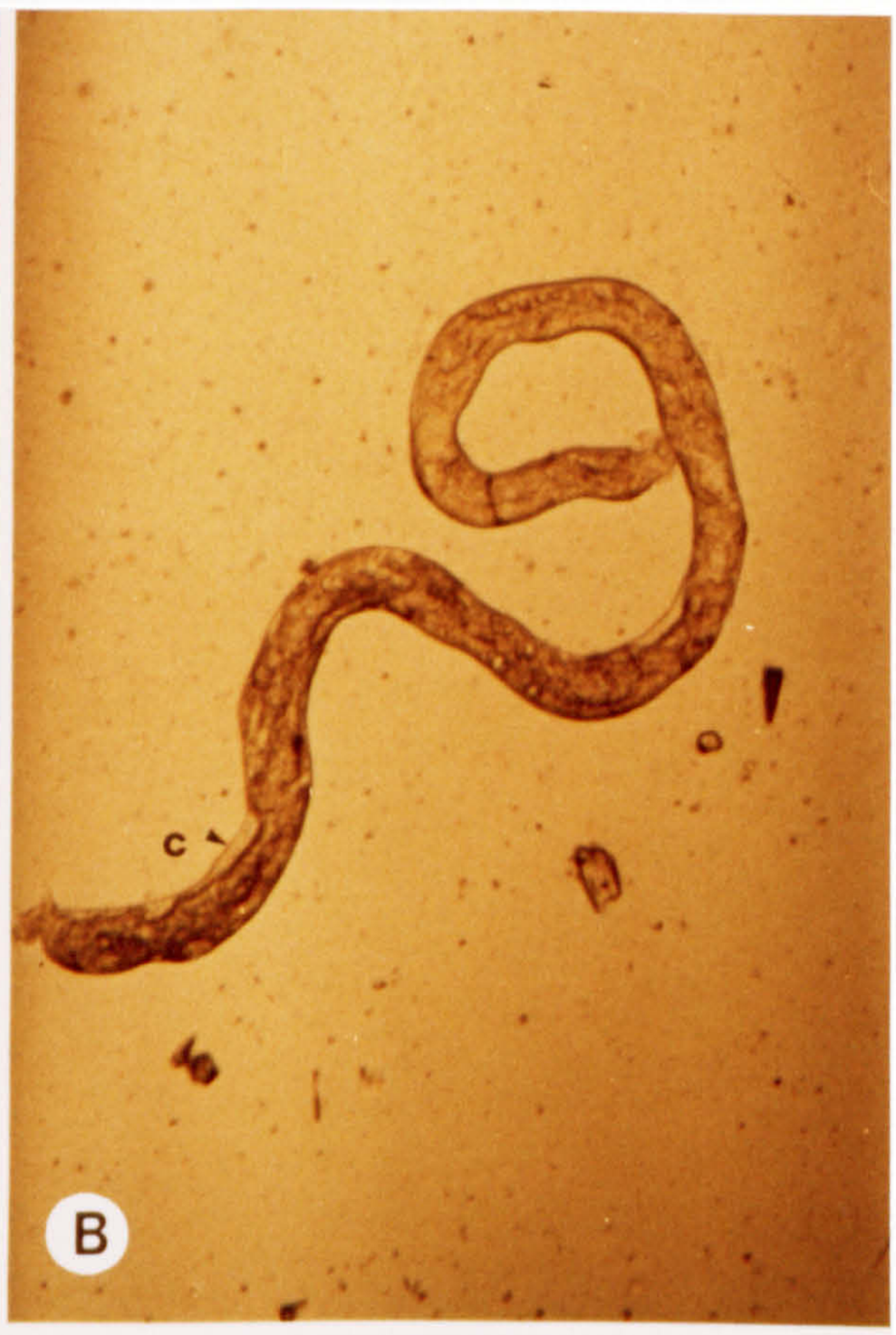
B. Second stage larva dissected from thorax of louse.

C. Third stage larva dissected from head of louse.

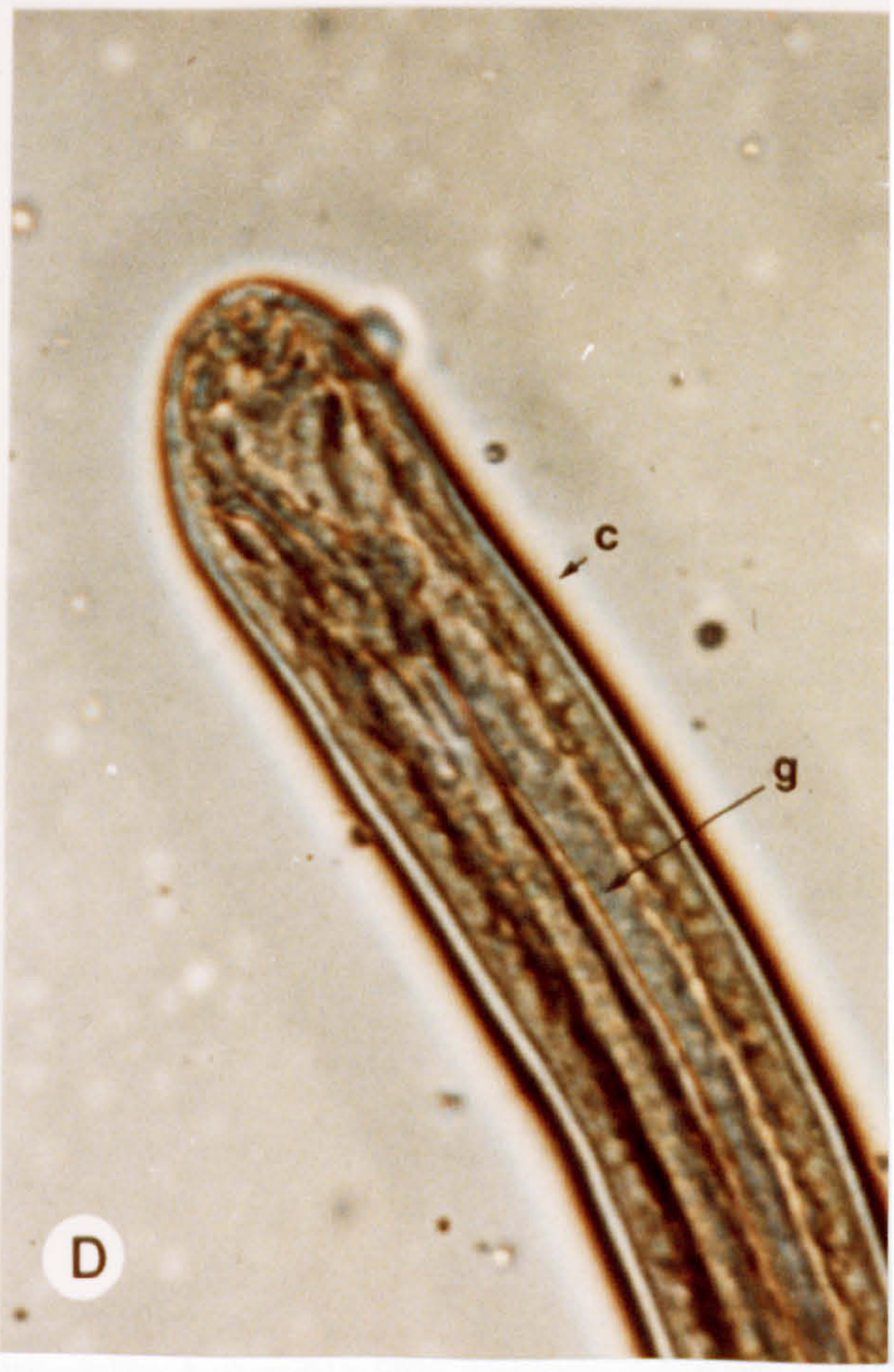
D. Detail of anterior end of third stage larva.

c cuticle
g gut


g
A


c
B


C


c
g
D

DISCUSSION

The original aim of this section was to observe the ongoing development of *S. eurycerca* larvae in *T. anserinum*. All the studies which have successfully observed sequential larval development have experimented with insects which can be reared in vitro in large numbers. The developmental periods for larvae have been determined for *Ornithofilaria fallisensis* in blackflies (Anderson, 1956), for *D. immitis* in mosquitoes (Kartman, 1953) and *D. immitis* in fleas (Steuben, 1954).

Dissection of insects is also a widely used and acceptable method of searching for developing stages of filariae. Of 125 dissected specimens of *Heterodoxus spiniger* , 21 contained infective larvae of *Dipetalonema reconditum* ; an infection level of 16.8% (Nelson, 1962). Blacklock (1926) dissected 780 *S. damnosum* and determined the infection level of *O. volvulus* to be 2.6%. Newton and Wright (1956) recovered developing stages of *D. immitis* from more than 24% of dissected fleas. Suguri (1985) and McGreevy et al (1982) both dissected mosquitoes to obtain *W. bancrofti* larvae.

The dissection method was applied in this study to observe the developmental stages of *S. eurycerca* in *T. anserinum*. The development of *S. eurycerca* microfilariae was found to be similar to the development of other filarial nematodes in their intermediate hosts.
All stages of *S. eurycerca* were found in *T. anserinum* . Most "sausage stage" and second stage larvae were located in the abdomen and thorax. During the development of microfilariae to "sausage stage" there is a decrease in length and "sausage stage" larvae were sometimes shorter (200-250 μm) than microfilariae (260 μm). The majority of third stage larvae were dissected from the head. Similar patterns of development were observed in *S. eurycerca* by Seegar (1977) and in *O. fallisensis* by Anderson (1956).

From 127 specimens of *T. anserinum* , 23 (18.0%) were found to harbour 34 developing larvae of *S. eurycerca*. These results compare with Seegar's (1977) dissection of 81 lice of which 42 (51%) harboured 450 developing larvae. The disparities could be explained by the observation that the mean microfilaremia of swans in this study was much lower (8 microfilariae per 0.25 cm^3 blood) than in Whistling Swans in Seegar's research (82 microfilariae per 0.25 cm^3 blood) (p. 29). In addition, most lice in this study were preserved in 70 per cent alcohol which may

not have preserved the delicate larvae. More of Seegar's dissections were performed on fresh lice.

In summary, three developmental stages of *S. eurycerca* were found in specimens of *T. anserinum*. Even though the ongoing development of larvae was not observed it is believed that *T. anserinum* provides a suitable environment for their development.

5. TRANSMISSION OF FILARIAL PARASITES BY *T. anserinum*

Dispersion away from an infected definitive host to colonise new hosts is necessary for continuation of filarial species. The probability of finding a new host may be very low and the dispersal stage in the life-cycle of free-living parasites (for example, *Nematodirus spathiger*, a sheep nematode) results in high mortality. To compensate for this, parasite fecundity is usually very high.

The inclusion of an intermediate host in the life-cycle may therefore increase the probability of a parasite locating a new definitive host. In turn, the activities of the intermediate hosts themselves may increase the likelihood of their being acquired by a new definitive host. It is precisely these activities that an intermediate host of *S. eurycerca* must exhibit on swans. *T. anserinum* must be capable of transferring from one swan to another and must be shown to be mobile enough to accomplish this dispersal stage.

The aim of this section is to determine if *T. anserinum* is active enough to transfer between hosts and therefore if it is capable of transmitting *S. eurycerca* .

5.1 MATERIALS AND METHODS

A radioactive label was used to observe the activities of *T. anserinum* on swans. The method of labelling is described on page 106 . During the course of the experiments, 20 individual lice were observed and only one louse was monitored on one swan at a time. Observations were made over periods from 12.00 (mid-day) to 24.00 hours, 15.30 to 24.00 hours, 15.30 to 06.30 hours, and 18.30 to 06.30 hours. Periods of observation were determined by the availability of the isotope.

Throughout the study observations have been made on the behaviour

of *T. anserinum* whilst handling swans. In addition, notes were taken of observations made by other workers on the activities of *T. anserinum*.

5.2 RESULTS

5.2.1 General activities of *T. anserinum*

The general activity of *T. anserinum* was determined over the observation periods irrespective of the actual time of day when the experiments were conducted. Active/inactive scores were obtained for each louse by marking it as active if it had moved during the half-hour between observations, and inactive if it had remained in the same position. The scores were combined for all lice over a time scale where P_1 corresponded to the first interval between observations. The periods of activity/inactivity for all 20 lice are illustrated (Fig.38A).

All 20 lice were observed up to P17. Fifteen lice were active in the first half-hour (P1) and the number of active lice then decreased to 11 (P2) and 8 (P3). Between 2 and 7 lice were always active up to P17. Even though 8 lice were not observed after P17, the remaining 12 lice still showed some activity up to P21. Beyond this time however (P22-P24) no lice were active.

Apart from the last 90 minutes, activity was observed throughout the whole observational period.

5.2.2 Activity from different starting locations

Overall, there appeared to be more activity when lice were placed on the back. Activity was observed in almost all of the observational period. Nine out of 11 lice placed on the back were active in the first half-hour (P1) and gradually fewer lice were active. During P6-P21 between 1 - 5 lice were active (Fig.38B).

Fewer lice were observed on the wings (N = 4) and only two of these were active at any one time (Fig.38D). There were periods when no activity was observed at all (P3-P6; P8-P9; P21-P24).

A similar intermittent profile was recorded for lice placed on the head (Fig.38C). However, 4 out of 5 lice were active in P1. Periods with no activity were observed (P4-P7; P12-P13; P15-P17; P19-P24).

The data suggested that there was more activity when lice were placed on the back, than on the head and wings.

FIGURE 38 : Activity patterns of *T. anserinum* on Mute Swans.

For each louse an active/inactive score was obtained in each half hour period of observation (irrespective of the actual time of day).

A. Activity patterns of 20 lice
(in time period of 1, 15 out of 20 lice were active).

B. Activity patterns of lice placed on the back as a starting location.
(N = 11)

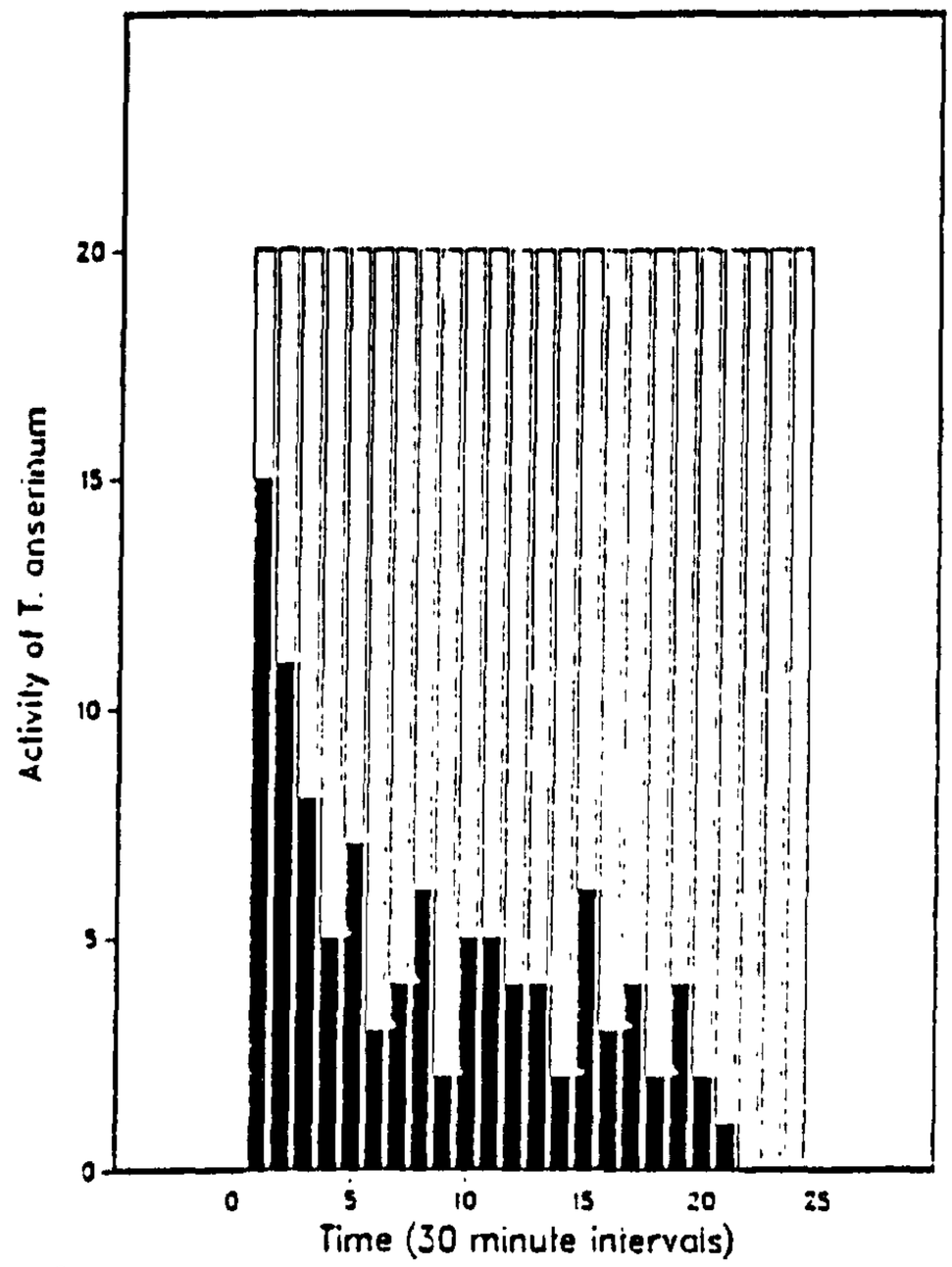
C. Activity patterns of lice placed on the head.
(N = 5)

D. Activity patterns of lice placed on the wings.
(N = 4)

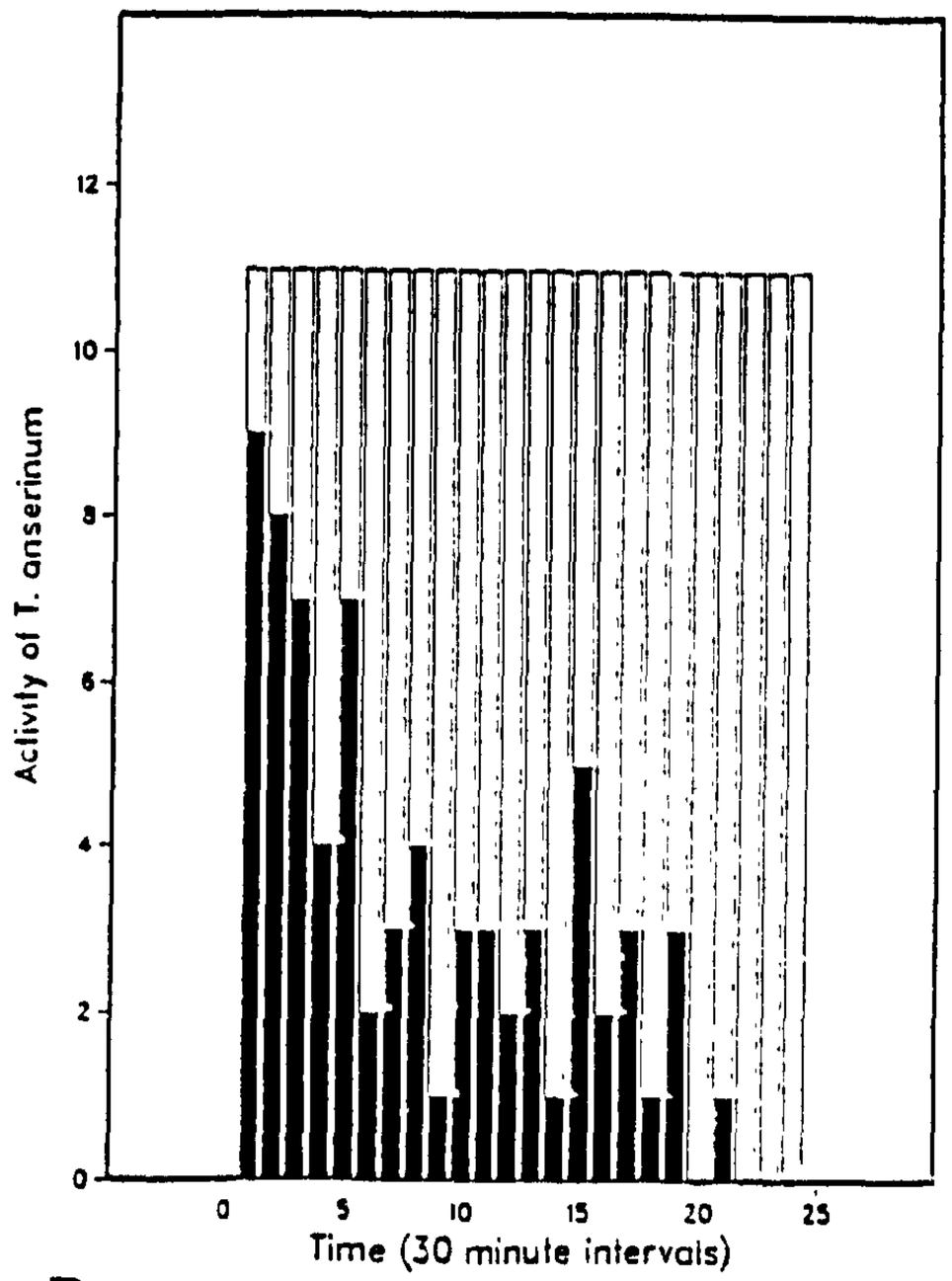
(note, time period 1 is the first 30 minute interval of observation).

Active lice ■

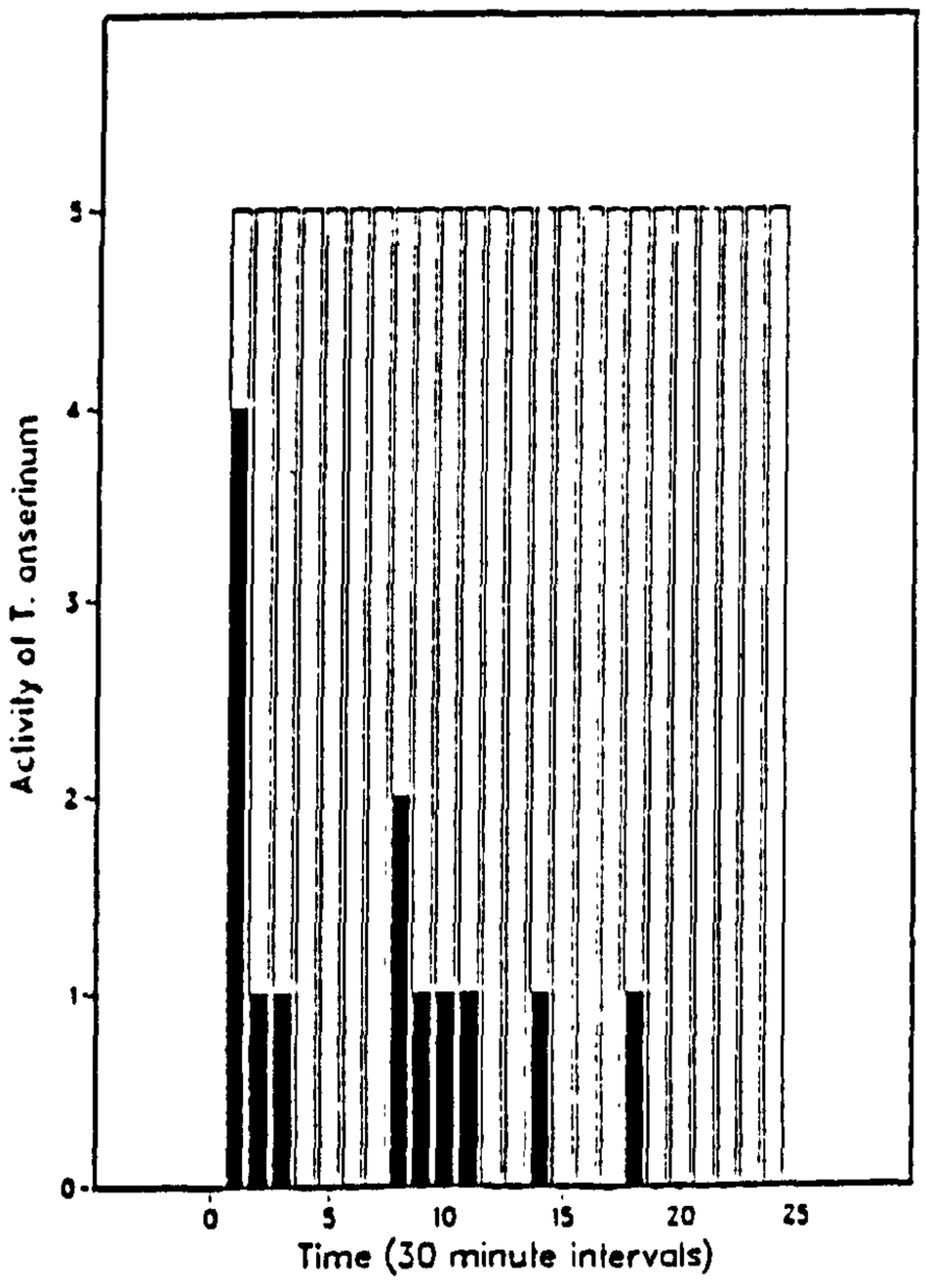
Inactive lice □


Activity of T. anserinum
0
5
10
15
20
Time (30 minute intervals)
0
5
10
15
20
25

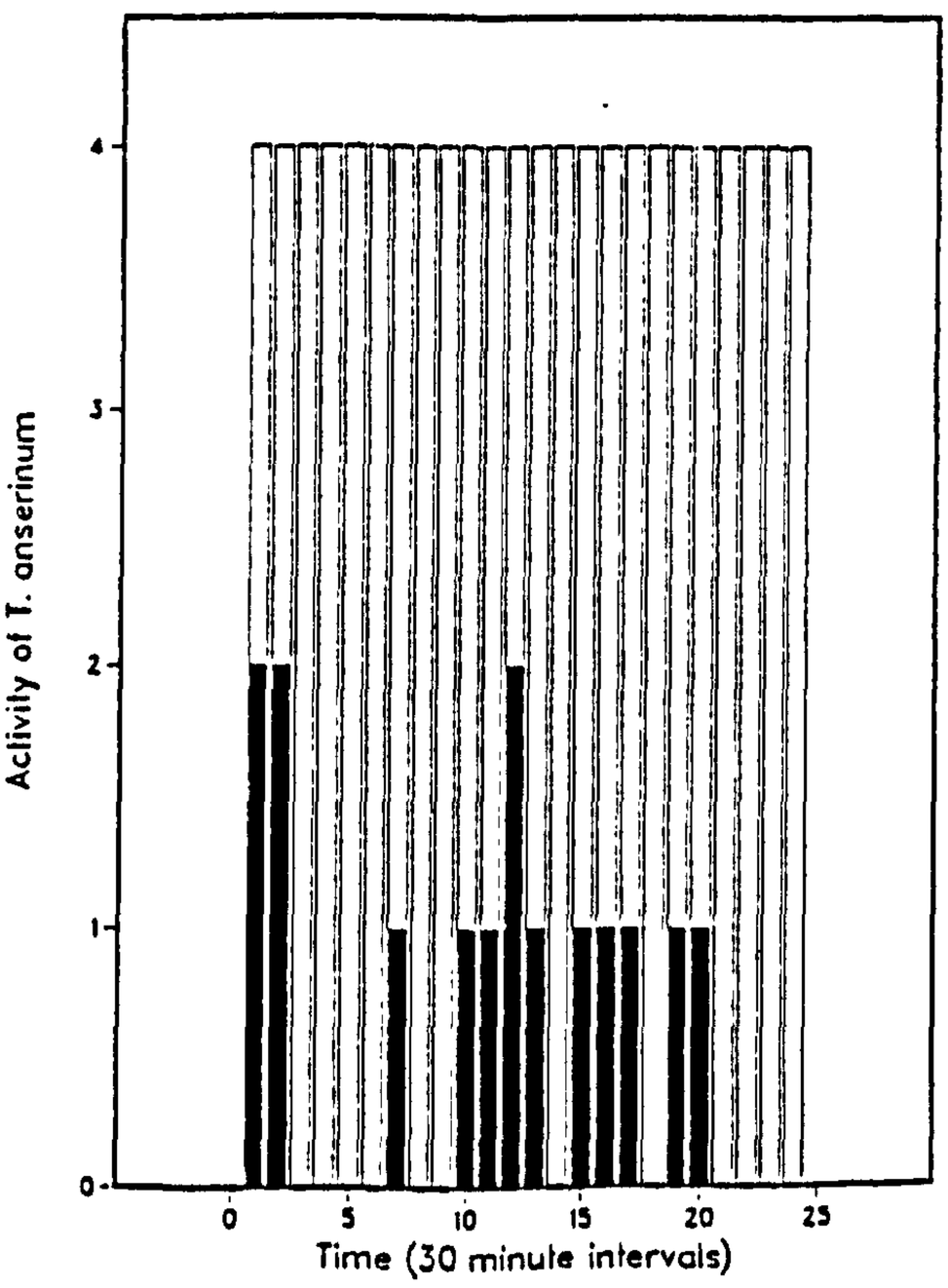
A


Activity of T. anserinum
0
2
4
6
8
10
12
Time (30 minute intervals)
0
5
10
15
20
25

B


Activity of T. anserinum
0
1
2
3
4
5
Time (30 minute intervals)
0
5
10
15
20
25

C


Activity of T. anserinum
0
1
2
3
4
Time (30 minute intervals)
0
5
10
15
20
25

D

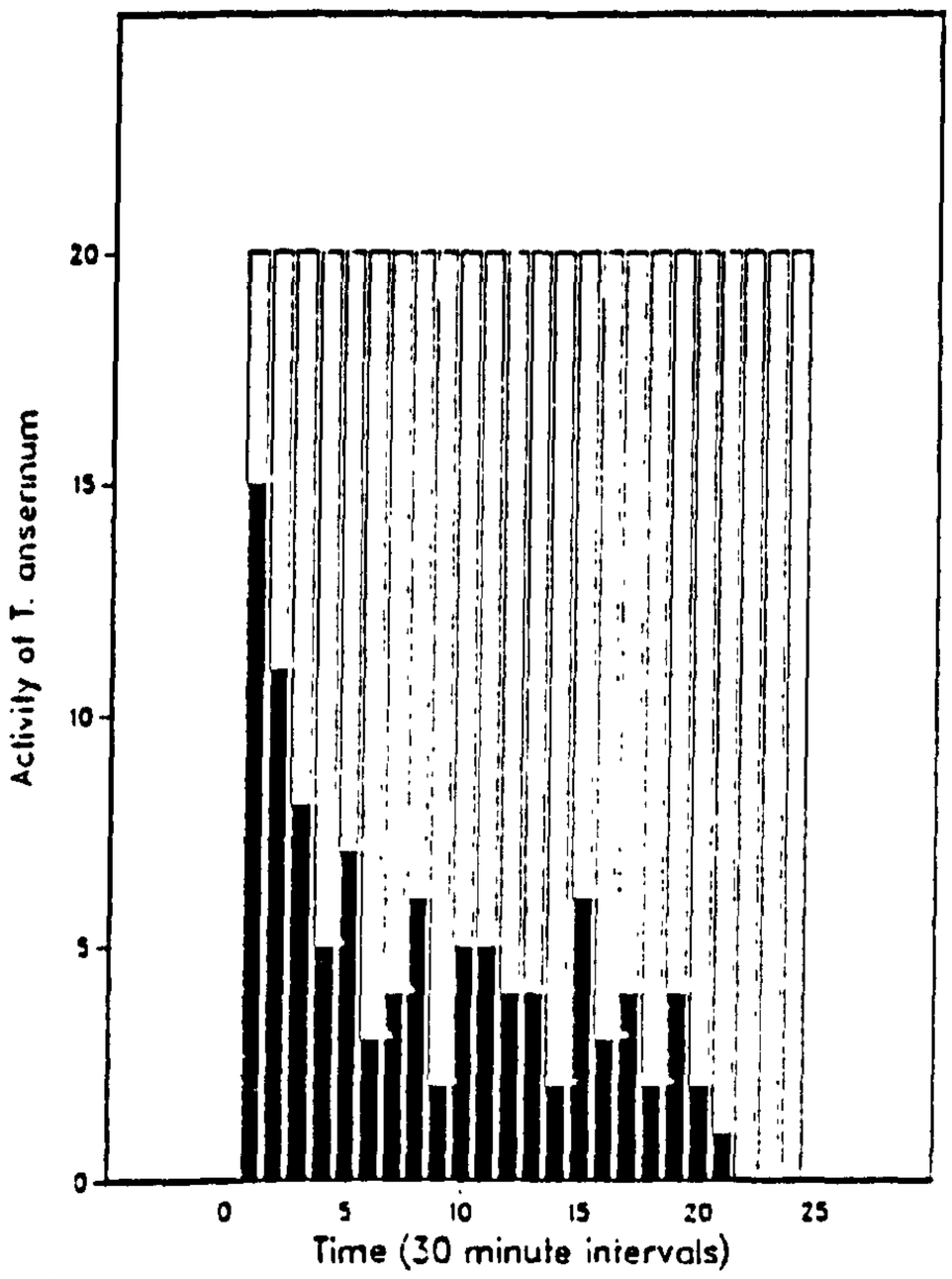
FIGURE 39 : Mean distances moved by *T. anserinum* from three starting locations.

(because not all 20 lice were observed over the same time periods, the minimum observational time common to all lice was 17 30 minute periods)

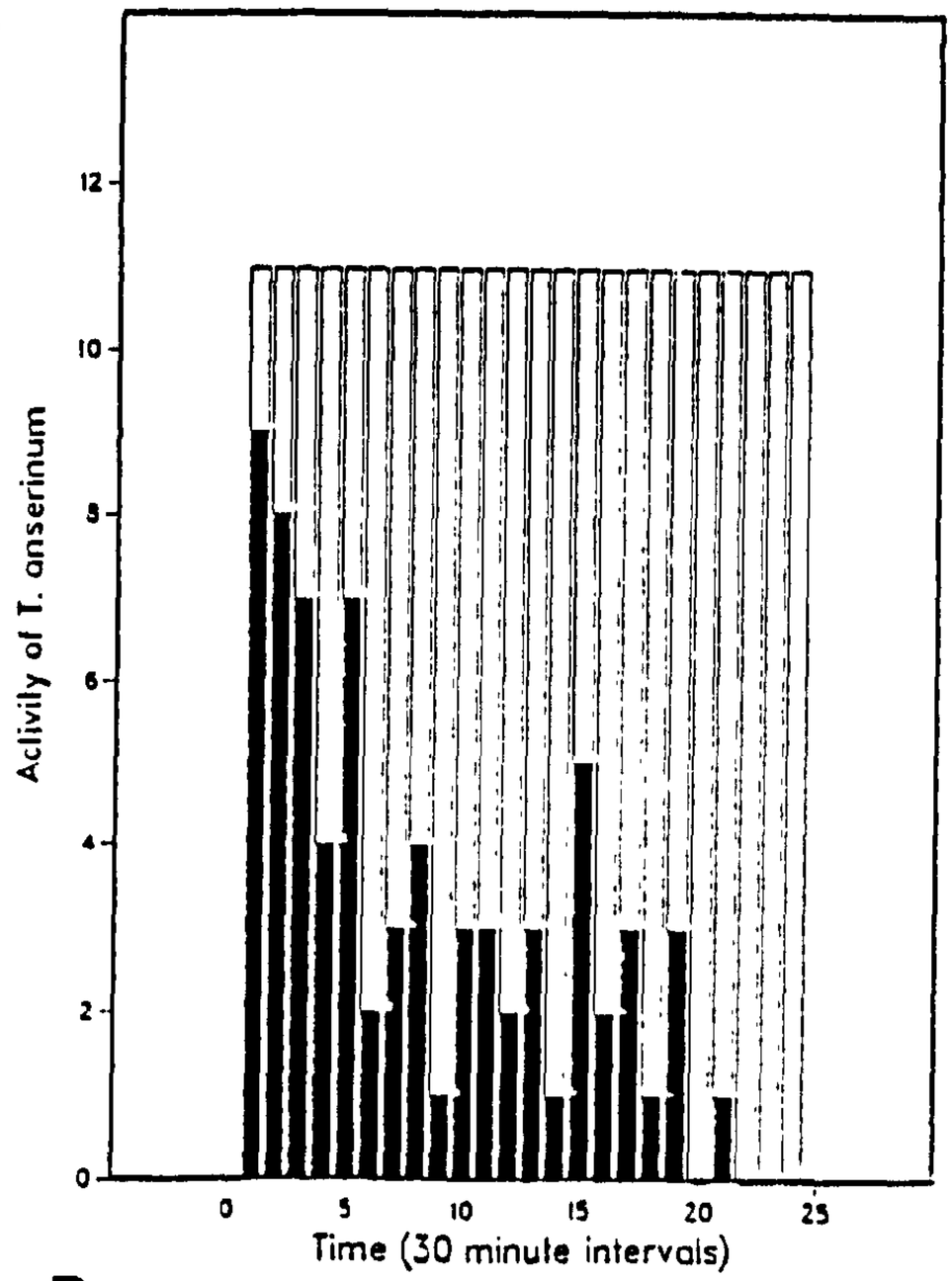
A. Mean distances moved by lice placed on the back as a starting location (cm) (N = 11)

B. Mean distances moved by lice placed on the head as a starting location (cm) (N = 5)

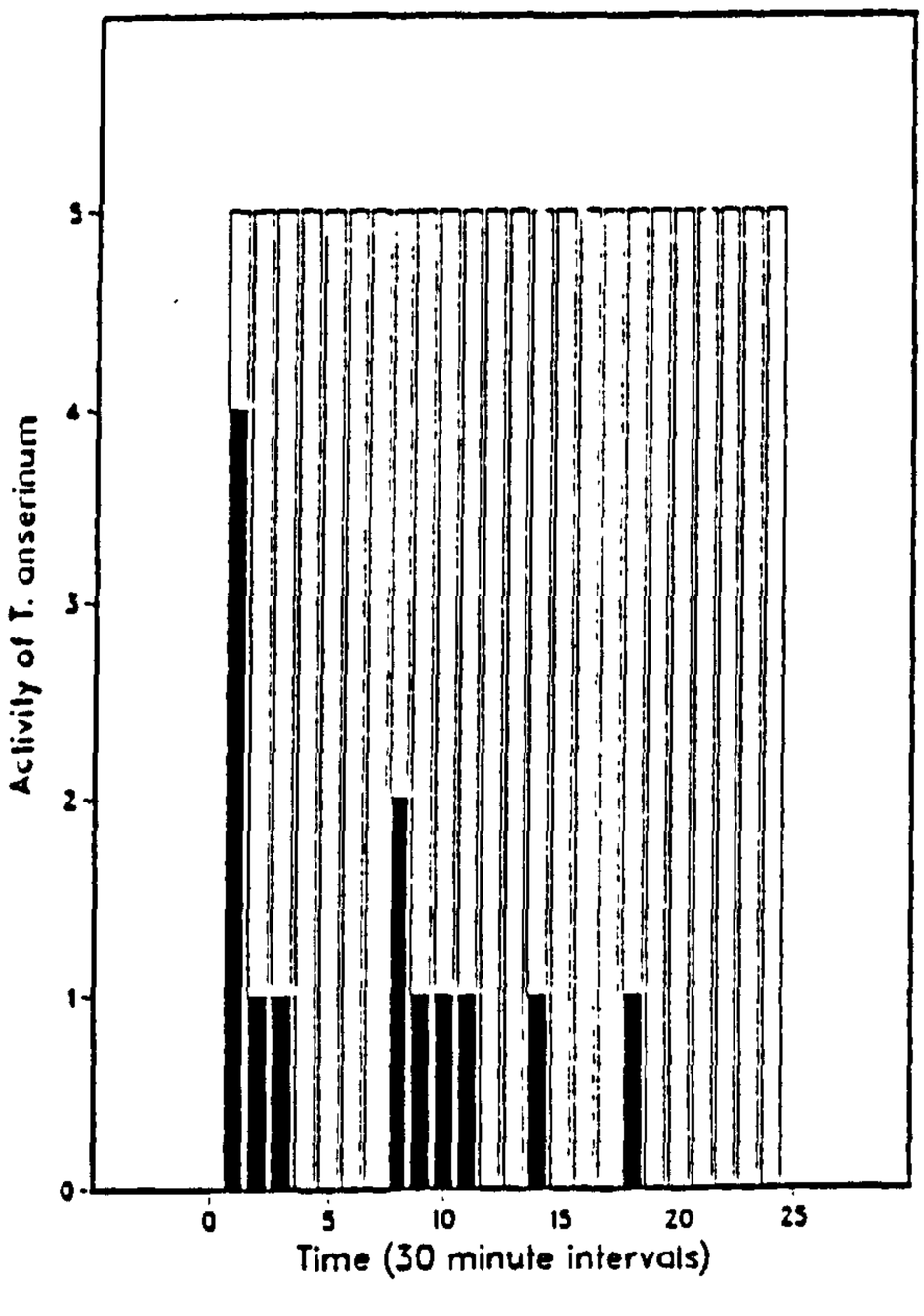
C. Mean distances moved by lice placed on the wing as a starting location (cm) (N = 4)


Activity of T. anserinum
0
5
10
15
20
Time (30 minute intervals)
0
5
10
15
20
25

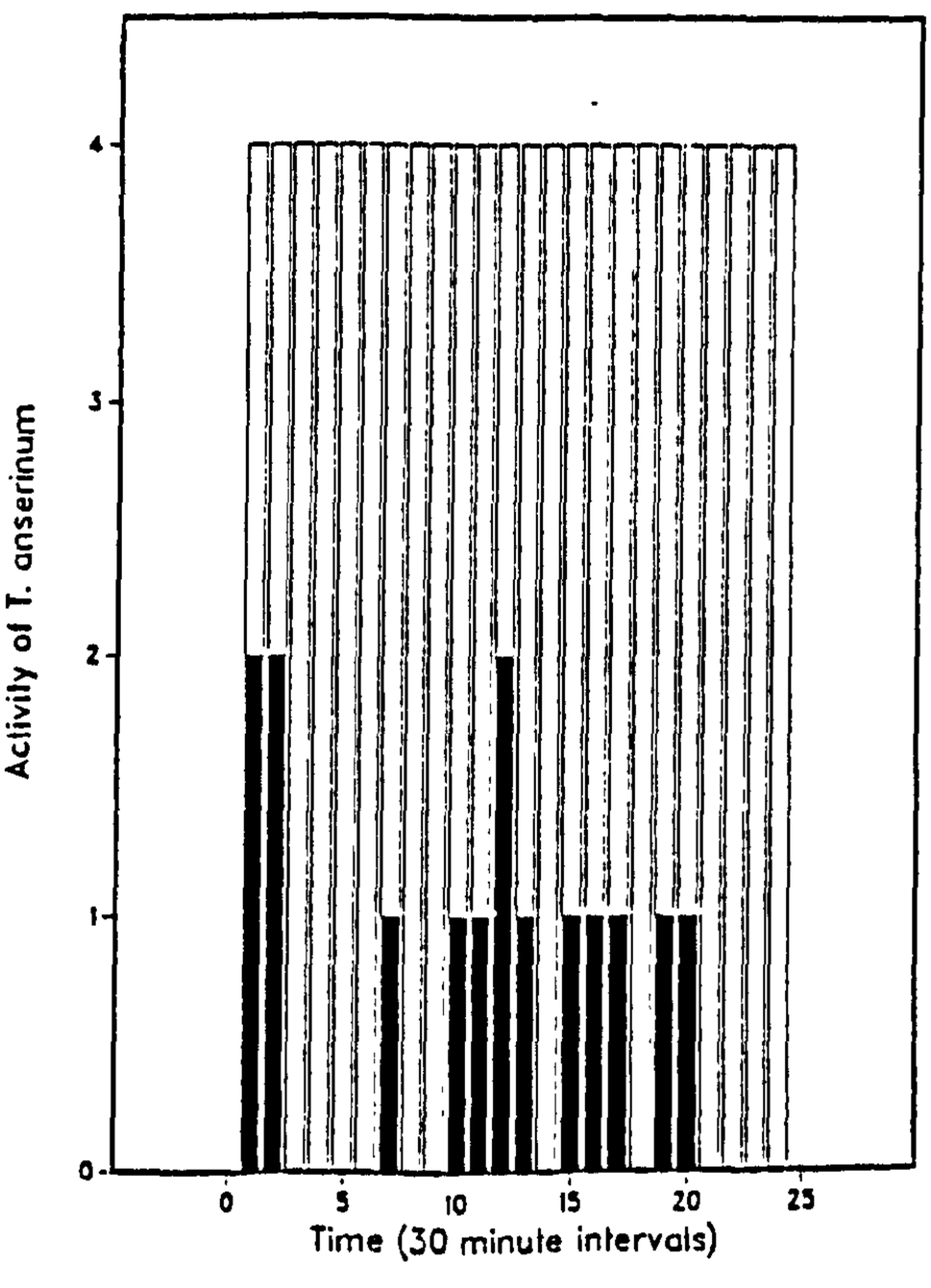
A


Activity of T. anserinum
0
2
4
6
8
10
12
Time (30 minute intervals)
0
5
10
15
20
25

B


Activity of T. anserinum
0
1
2
3
4
5
Time (30 minute intervals)
0
5
10
15
20
25

C


Activity of T. anserinum
0
1
2
3
4
Time (30 minute intervals)
0
5
10
15
20
25

D

5.2.3 Distances moved by *T. anserinum*

The distances moved by lice in different starting locations were measured (Fig.39A,B,C).To make the total distances comparable they were calculated for all lice up to P17 (the minimum observational time).

The mean distance moved by lice on the back was 121 cm compared to mean distances of 44 cm and 35 cm for lice placed on head and wings respectively. The range of individual distances covered were largest for lice on the back (59.2 - 320.4 cm) when compared to the lice on the head (0.0 - 133.4 cm) and wings (20.0 - 71.0 cm). These quantitative data support the suggestion that there is more activity when lice are placed on the back than on the head and wings.

5.2.4 Observations of activity of *T. anserinum* whilst handling swans

Members of the Wildfowl Trust and Swan Study Groups handle swans throughout the year. All of them have remarked on the agility and high degree of activity exhibited by *T. anserinum* . After handling 5 - 20 swans in a day, most swan handlers expect to have acquired a number of *T. anserinum* which mostly are found in their hair. Some regular handlers of sick swans, which tend to have higher numbers of lice, use a "louse-comb" with fine teeth to remove lice at the end of the day.

DISCUSSION

The observations made of *T. anserinum* show it to be very active and to cover relatively long distances on the host. Casual observations by swan handlers reveal that after a day spent catching swans they invariably had to remove *T. anserinum* from their hair and clothing.

It seems *T. anserinum* easily transfers onto man and other swans. The situations where such transfer could take place outlined by Hopkins (1949) would apply equally to lice on swans. In addition, the gregarious nature of swans in moulting, immature and wintering flocks would facilitate further transfer of lice.

T. anserinum appears active enough to successfully disperse *S. eurycerca* from swan to swan.

CHAPTER DISCUSSION

The four basic requirements of an intermediate host outlined in the introduction to this chapter have been investigated and *T. anserinum* has been found to satisfy all of them.

As an obligate ectoparasite, *T. anserinum* has a constant association with the swan unlike most dipteran vectors which only occasionally overlap spatially and temporally with their hosts. *T. anserinum* has therefore continuous opportunity to effect filarial transmission.

T. anserinum has been shown to be capable of ingesting blood and microfilariae and probably requires several blood meals every day for survival. As with all filarial species, there is no multiplication of filariae in *T. anserinum* and when larvae mature and leave, the louse is no longer infected. The maintenance of transmission therefore requires frequent contact between the louse and the source of infection, - the swan. This contact has to occur at a time when adequate numbers of microfilariae are present in peripheral blood. Experiments in this study have shown the timing of feeding of *T. anserinum* to be variable, but this is not so critical for the uptake of *S.eurycerca* because microfilariae are present in the peripheral vessels all the time.

The lice must be receptive to the development of the nematodes from microfilariæ to mature third stage larvae. All stages of developing larvae were demonstrated in *T. anserinum* . What still remains to be determined, and what Seegar (1977) also failed to demonstrate, is the longevity of each larval stage within the intermediate host.

CHAPTER IV

INTRODUCTION

The genus, *Sarconema* , was first applied to nematodes from the cardiac muscle of Whistling Swans (Wehr 1939) and was recorded for the first time in Bewick's Swans by Ryzhikov (1958). Sultanov (1963) described nematodes from the cardiac muscle of *Anser anser* and *A. fabalis* as *Sarconema anseris* , but the apparent differences between *S. anseris* and *S. eurycerca* were not considered to be sufficient to classify them as two species (Sonin and Borgarenko, 1965).

S. eurycerca has been recorded on four swan species (Whistling, Trumpeter, Bewick's and Mute Swans) and in four species of geese (Canada, Whitefronted, Domestic and Bean geese). It has been reported in swans from the USA and Canada, USSR (Yakutia and Chukotka) and England and it is likely that its present distribution reflects the limited research that has been performed on this species.

The pathogenicity of *S. eurycerca* is somewhat controversial. Some authors have cited this parasite as a cause of death in swans (Table 30), but it can also be found in apparently healthy birds.

To date, there is no systematic record of the occurrence of *S. eurycerca* in swan hearts in Britain and there is therefore no real understanding of its significance on swan mortality. Little published data is available on the heamatology and clinical chemistry of swan blood associated with this nematode.

This chapter seeks to broaden the current understanding of the pathogenicity of *S. eurycerca* by:

1) examining the detailed morphology of adult nematodes and their microfilariae;
2) exploring the pathological effects of *S. eurycerca* on swans; and
3) comparing the haematology and clinical chemistry of both infected and non-infected groups of swans.

Table 30: References to studies recording *S. eurycerca* in swans

DATE	AUTHOR	FINDINGS
1938	COBURN, D.R. (cited in QUORTRUP and HOLT, 1940)	Whistling Swan: moderate infection worms directly under epicardium and embedded in myocardium; primary cause of death lead poisoning.
1939	WEHR, E.	Whistling Swan: classification of *S. eurycerca*.
1940	QUORTRUP, E.R. & HOLT, A.L.	Whistling Swans: (1937-38) 2 out of 22 infected (9%) Utah. Whistling Swans: (1939) 10 out of 42 infected (23.8%) Utah. Whistling Swan: primary cause of death *S.eurycerca* - mature worms in epicardium, heavy larval infection in endocardium, Idaho.
1946	COWAN, I. McT.	Trumpeter Swans: 2 infected, pericardiumcontained 25 worms, heart pale and flabby. Blood smears from endocardium had large nos. microfilariae, British Columbia.
1958	RYZHIKOV	Bewick's Swans: 4 out of 6 infected, Yakutsk USSR.
1965	BOUGHTON, E.	Mute Swan: heart considerably enlarged, myocardium fibrous, 11 female, 5 male nematodes, microfilariae in endocardium. Henly-on-Thames.
1967	KLUGE, J.P.	Whistling Swan: cardiac failure due to lesions produced by nematode, Washington, D.C.
1968	HOLDEN, B.L. & SLADEN, J.L.	Whistling Swan: overall incidence 16%, seven hearts examined, 2.36 nematodes per host in myocardium. Chesapeake Bay, Maryland.
1974	McDONALD, M.E.	Whistling Swan: (1957-63) prevalance 16.2%; 4-26 worms per heart, mean number 12.2, Utah.
1975	MACNEILL, A.C.	Whistling Swan: 52 nematodes recorded in a single heart. Blood vessels of heart congested, muscle pale and flabby, 69 Trumpeter and 43 Whistling Swans autopsied - none infected, British Columbia.
1975	IRWIN, J.C.	Whistling Swans: 23 autopsied, 12 infected. Worms in epicardium, myocardium and associated with widespread tissue damage. Lake St. Clair, Ontario.
1978	MACNEILL, A.C. & BARNARD, T.	Whistling Swans: 1 infected out of 52; Trumpeter Swans - 3 infected out of 83, British Columbia.
1979c	SEEGAR, W.S.	Whistling Swans: overall prevalance 20% - higher in juveniles (24%) than adult swans (19%). Maryland, N. Carolina and North Slope, Alaska.
1979a	SEEGAR, W.S.	Mute Swans: overall prevalance 17.1% - Birmingham 17%, Abbotsbury, Dorset 9.6%, Christchurch 9.6% (N = 426). Bewick's Swans, Slimbridge 3.6% (N = 164).

DATE	AUTHOR	FINDINGS
1980	McKELVEY, R.W. & MACNEILL, A.C.	Results from necropsies of 52 Whistling and 114 Trumpeter Swans from 1965-1979. Myocardial path. usually associated with *S. eurycerca* incidence of which appears to be increasing from 2.9% (1965-1976) to 7.8% (1965-1979). More frequently found in Trumpeter Swans than Whistling Swans.

1. TAXONOMY AND MORPHOLOGY OF *S. eurycerca*

1.1 Taxonomy of *S. eurycerca*

Within the phylum Nematoda, *S. eurycerca* belongs to the class Phasmidia and order Spirurida which are as adults, parasites of vertebrates and require intermediate hosts (usually arthropods) in the larval stage (Chitwood, 1974). *S. eurycerca* is further classified within the suborder Spirurina, the superfamily Filarioidea and family Onchocercidae (Nelson, 1964) which includes nematodes with microfilarial larvae.

1.2 Morphology of *S. eurycerca*

The following descriptions of the morphology of *S. eurycerca* summarise the findings of Wehr (1939), Ryzhikov (1958), Sultanov (1963), Sonin (1966), and Seegar (1977).

Adult females are approximately three times as long as males (Wehr, 1939). The maximum width is located slightly behind the connection of the oesophagus with the intestine; the posterior end is rounded in both sexes. The cuticle of *S. eurycerca* has transverse, spiral thickenings and the mouth is round with four pairs of cephalic papillae surrounding it. The oesophagus is short and the intestine narrow and is filled with a granular mass (Ryzhikov, 1958).

Females are 3-4 x 10^4 µm long with a maximum width of 0.31 - 1.02 x 10^3 µm (Table 31). The vulva is situated anteriorly slightly before the connection between the oesophagus and intestine. The vagina has thick walls and the body cavity is filled with twisted genitalia. In mature specimens, these loops extend anteriorly to the oesophagus and posteriorly nearly to the anus (Ryzhikov, 1958).

Male specimens are 9.0 - 13.0 x 10^3 µm long and have a maximum width of 0.27 - 0.51 x 10^3 µm. The spicules are nearly equal in length with widening proximal ends and conical, tapering blunt distal ends. There are seven pairs of caudal papillae arranged laterally and a single papilla at the margin of the tail end.

Microfilariae of *S. eurycerca* are about 260 µm long with the posterior part of the body gradually narrowing to a bluntly pointed tip (Wehr, 1939). The cuticle is distinctly serrated on the entire body (Sonin, 1966). Seegar (1977) recorded the length to range from 270 - 340 µm. Descriptions of microfilariae of *S. eurycerca* from Canada geese were

Table 31: Measurements of *S. eurycerca* from different hosts

CHARACTER	FROM SWANS (after Wehr, 1939; Ryzhikov, 1958; Sonin, 1966)		FROM SWANS (after Seegar 1971)		FROM GEESE (after Sultanov, 1963)	
	(measurements in x 10^3 µm)					
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Length	9-13	25-40	11-13	30-35	9-11	29-34
Width	0.35-0.51	0.69-1.02	0.33	0.75	0.27-0.36	0.31-0.42
Oesophagus Length	0.27-0.42	0.34-0.48	-	-	0.27	0.36
Oesophagus Width	0.035-0.06	0.063-0.084	-	-	0.041-0.045	0.072
Distance to Nerve Ring	0.108-0.155	0.126-0.168	0.11	0.12	0.081	0.099
Anal Pore From Post.	-	-	-	0.105	-	-
Length of Spicule I	0.012-0.14	-	-	-	0.108	-
Length of Spicule II	0.126-0.145	-	-	-	-	-
Distance to Vulva	-	0.17-0.34	-	-	-	0.324

first given by Levine and Hanson (1953). They reported the lengths to range from 323 - 341 μm.

1.2.1 MATERIALS AND METHODS

1.2.1.1 Examination of adult nematode morphology

Mute and Whooper Swans hearts (N = 72) were obtained from post-mortem examinations in Scotland, the Midlands and Norfolk. Each heart was dissected for adult nematodes and the precise location of each nematode noted. Nematodes were preserved in formalin (2% solution).

The morphology of adult *S. eurycerca* specimens was examined, photographed and drawn using a binocular microscope (x10 - 60) and a photomicroscope. The external morphology of *S. eurycerca* was examined using a Scanning Electron Microscope. The rapid fixation technique (Eisenback, 1985) was used to prepare *S. eurycerca* for the Scanning Electron Microscope and the method was applied as in Appendix 6 .

1.2.1.2 Examination of microfilariae morphology

A blood smear was prepared using a volume of infected blood (0.02 cm^3) and allowed to dry before staining with Giemsa using a technique developed by Denham (personal comm.).

Buffered distilled water (pH 7.2, 15.0 cm^3) and concentrated Giemsa (3.0 cm^3) were drawn into a hypodermic syringe (20.0 cm^3) and thoroughly mixed by inverting the syringe several times. The prepared blood smear was placed downwards in a plastic petri dish and the stain injected underneath. In this way there was minimum contact of the stain with the air and this reduced the amount of deposit formed.

The blood film remained in contact with the stain for 15 minutes after which the slide was washed under a gentle flow of tap water for about 1 second, left to drain and dried in an upright position.

Nuclei of the microfilariae stained dark purple and the cuticle a pinkish shade. Slides were examined and photographed using light microscopy (x 40).

FIGURE 40A : Anterior section of adult female nematode (*S. eurycerca*) drawn using a light microscope

oro oral opening

vlv vulva

nr nerve ring

u_1, u_2 uteri

m muscle of body wall

c cuticle with striae

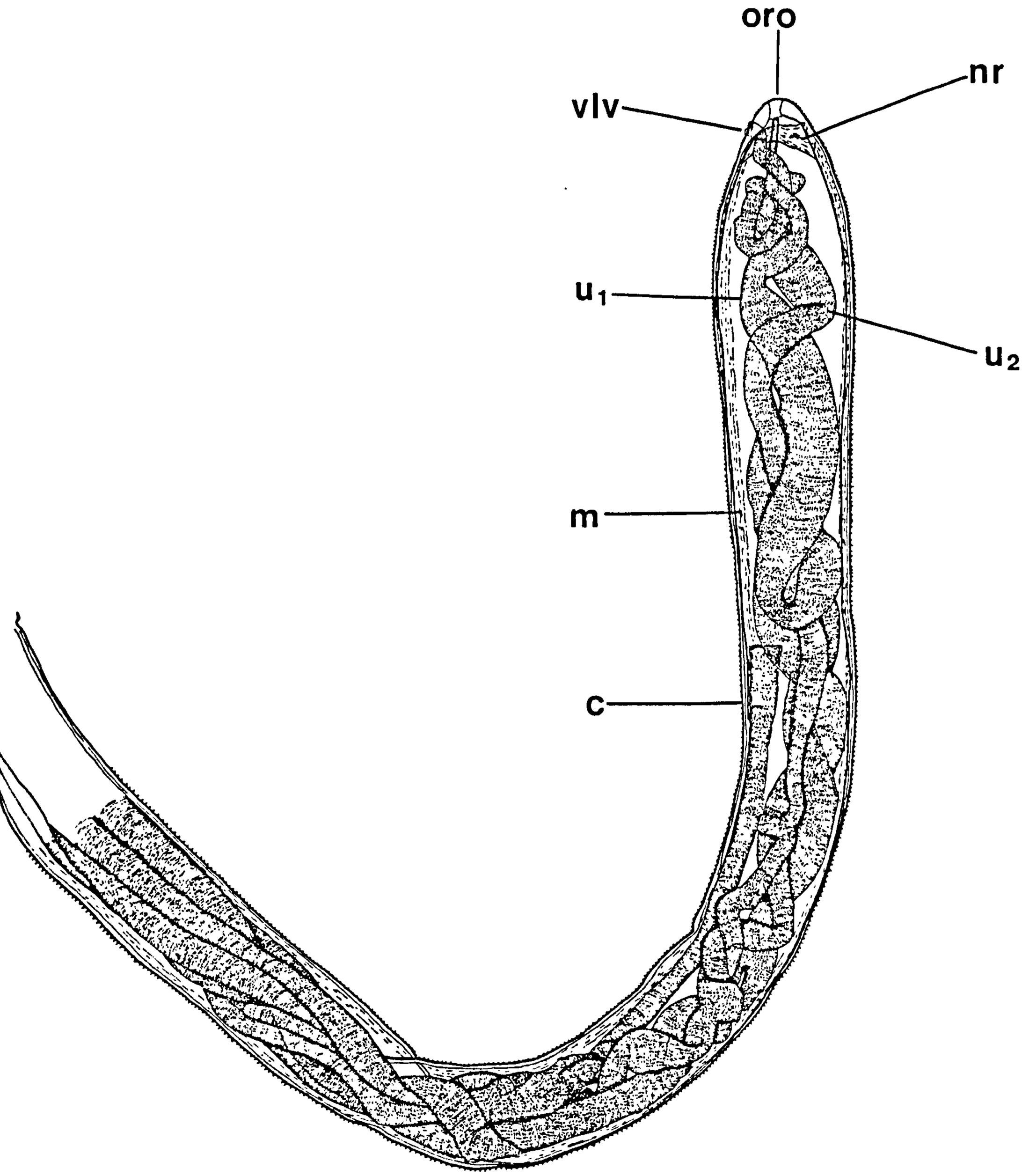

oro
nr
vlv
u1
u2
m
c

FIGURE 40B : Posterior section of adult female nematode (*S. eurycerca*) drawn using a light microscope

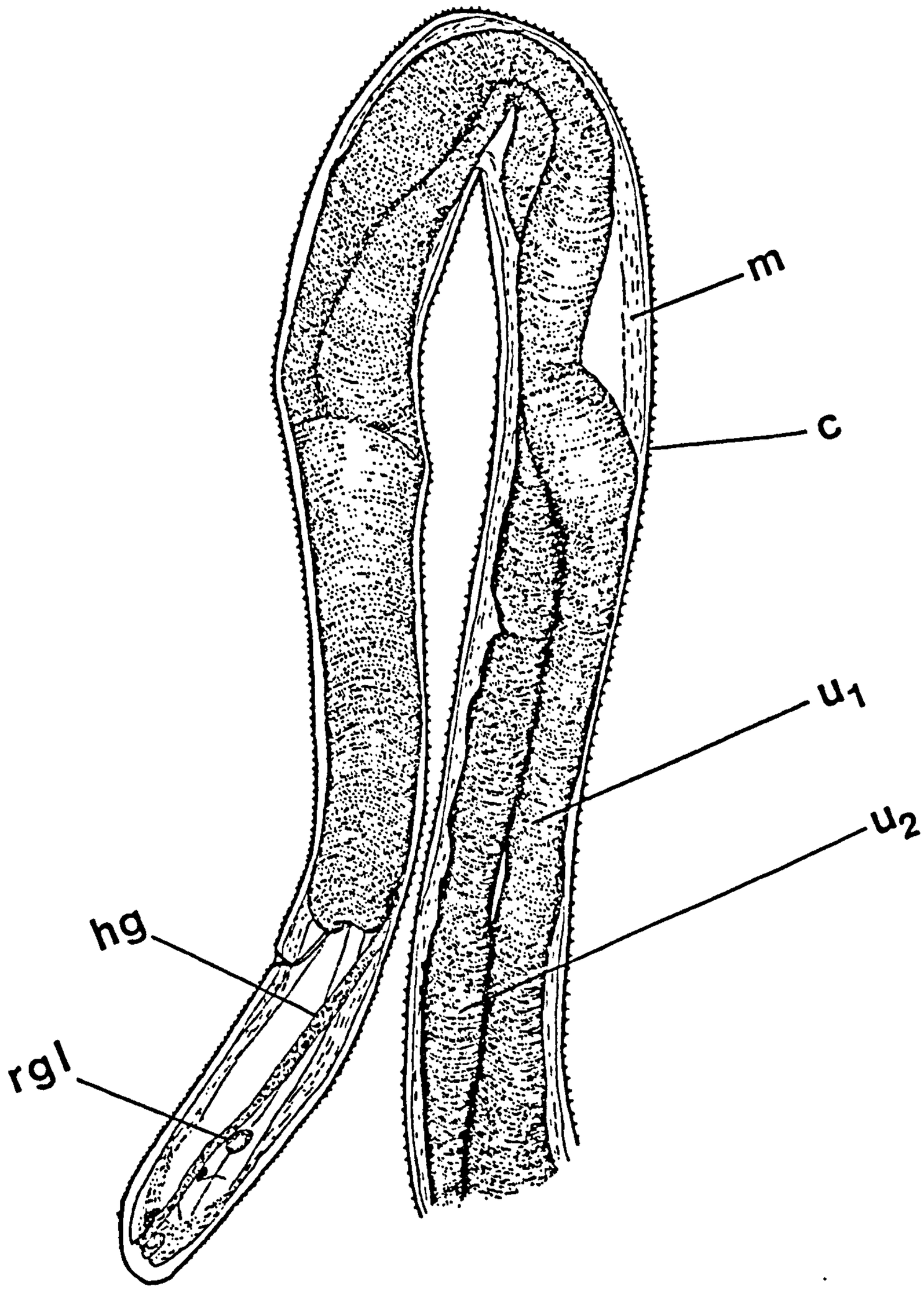
m muscle of body wall

c cuticle with striae

u_1, u_2 uteri

hg hind gut

rgl rectal gland


m
c
u1
u2
hg
rgl

1.3 RESULTS

1.2.1 Morphology of *S. eurycerca* adults

Female nematodes (N = 27) were dissected from eight swan hearts but no male specimens were collected.

The internal morphology of female nematodes was examined using light microscopy. From these preparations, the oral opening is visible but there are no lips present. The nerve ring obscures the stoma (mouth cavity and pharynx), but part of the oesophagus is visible (Fig.40A). The rectum lies posteriorly alongside the rectal gland. The vulva is situated anteriorly and from this position in the body two uteri extend posteriorly almost to the anus. The uteri occupy most of the body cavity; they are convoluted and twisted in appearance and eggs are visible in them through the cuticle. On dissecting the uteri further, different stages of development of microfilariae were visible in the eggs (Figs.41A-D, 49A).

The length of female nematodes ranged from 26.0 - 28.9 x 10^3 μm and maximum width was 0.74 x 10^3 μm.

The external morphology, studied on the Scanning Electron Microscope, shows the anterior end of the nematode to be more rounded and wider (Fig.42A) than the posterior end (Fig.42B). The oral opening is visible (Fig.44A,B) and is bordered by indentations in the cuticle. There are four pairs of indentations which could be interpreted as the positions of the four pairs of cephalic papillae described by Ryzhikov (1958). The vulva is situated anteriorly and its surface morphology shows it to be eliptical and surrounded by a raised dome of non-striated cuticle (Fig.43AB).

Striation and annulation of the cuticle commonly occurs in parasitic nematodes including the Filarioids. The cuticle of *S. eurycerca* is marked by regularly arranged transverse grooves (striae) inbetween which is the interstrial region. The pattern of striae varies in different regions along the length of the nematode. Anteriorly, the striae are regularly spaced and uniform in depth (Fig.45A). Still at the anterior end but more dorsal (taking the vulva to be ventrally located (Chitwood, 1974), deeper striae occur at intervals. These are termed annulations and the intervals between them, annules (Fig.45B). Approximately one-third of the length from the anterior, the cuticular pattern is still ridged but the striae join together in irregular shapes (Fig.45C). At the posterior end, the striae are arranged in a regular pattern but are shallower and spaced more openly than the anterior striae (Fig.45E). The striae form an eliptical shape at the terminal end (Fig.45F).

FIGURE 41 : Developmental stages of microfilariae within uteri of *S. eurycerca* female viewed through a light microscope.

A. Oval shaped eggs in early developmental stage (note, stained centre is nucleated material)

25µm

B. Eggs more rounded with nuclei more elongated in shape.

25µm

C. Coiled microfilariae within eggs.

25µm

D. Fully developed microfilariae hatched from eggs, still within uteri.

250µm

(note, ◭ highlight hatched microfilariae of *S. eurycerca*)

dmf developing microfilariae


A


B

dmf
C

D

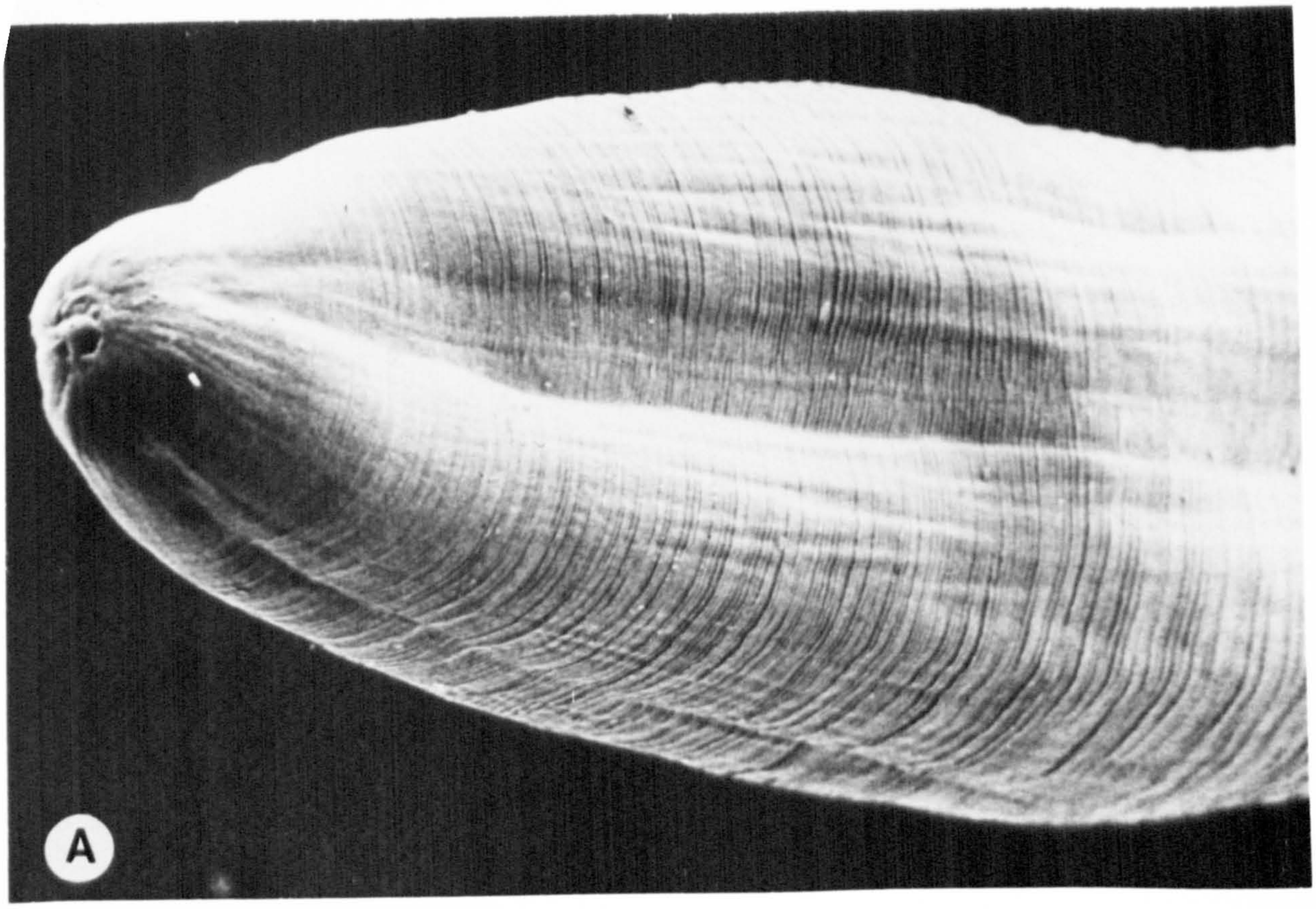
FIGURE 42 : Electron micrograph of external morphology of adult *S. eurycerca*.

A. Anterior section.
(note, striated pattern on cuticle)

0·07mm

B. Posterior section.

0·15mm


A

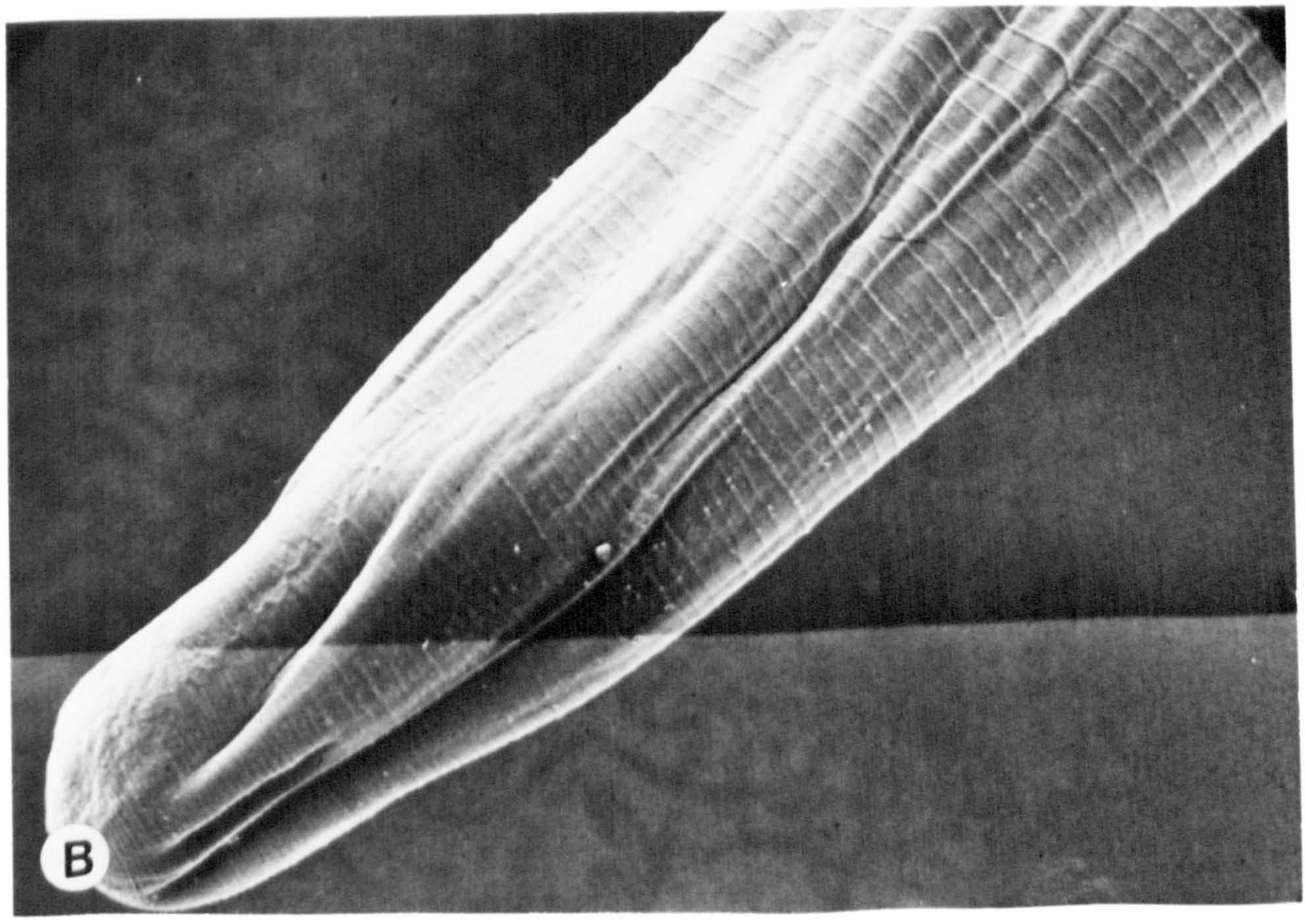

B

FIGURE 43 : Electron micrograph of vulva of adult female *S. eurycerca*.

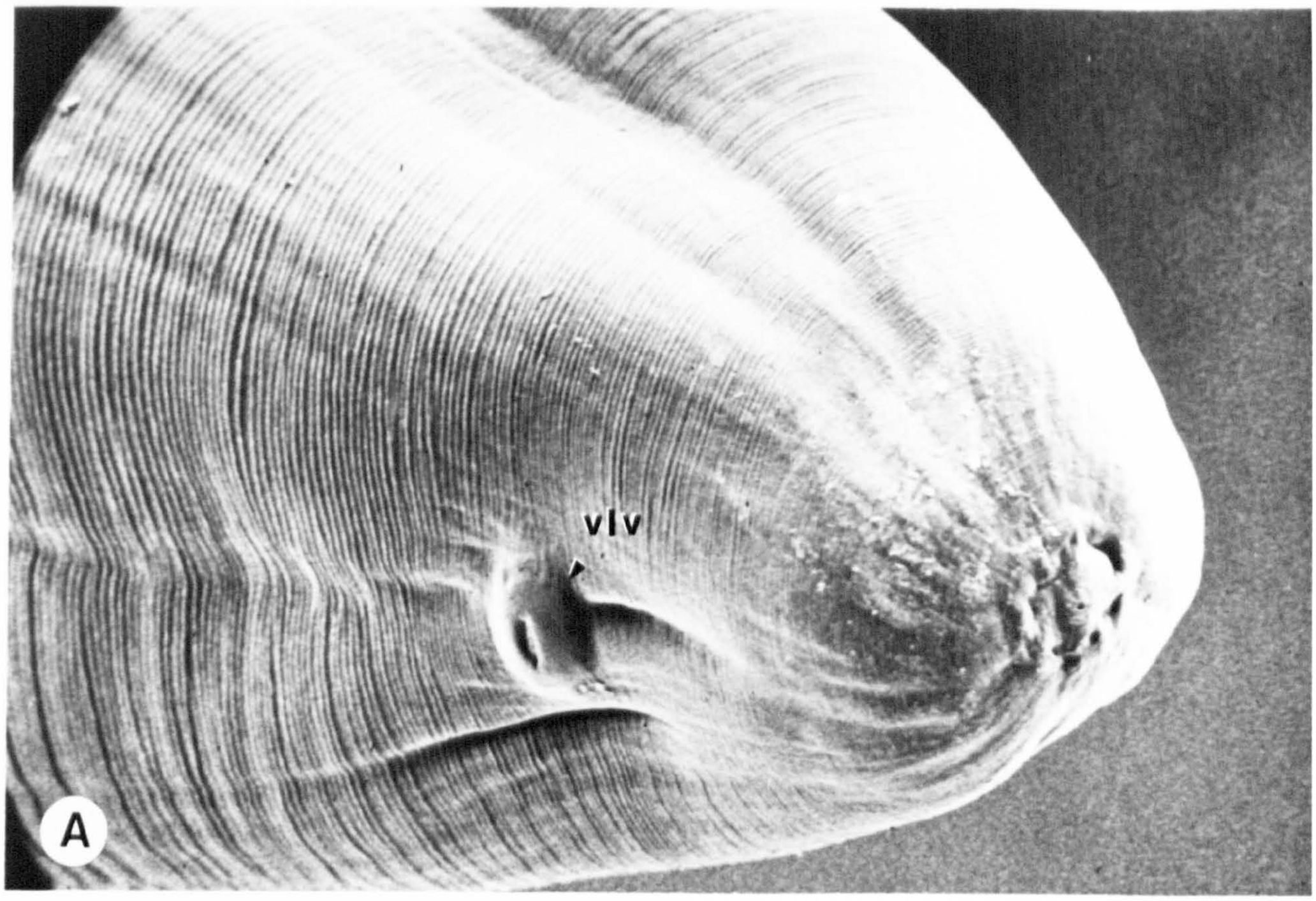
A. Anterior view showing oral opening and vulva.

200µm

B. Detailed view of vulva.
(note, raised dome of non-striated cuticle around vulva)

40µm

vlv vulva

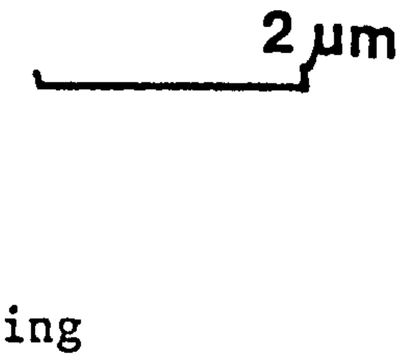

vlv
A

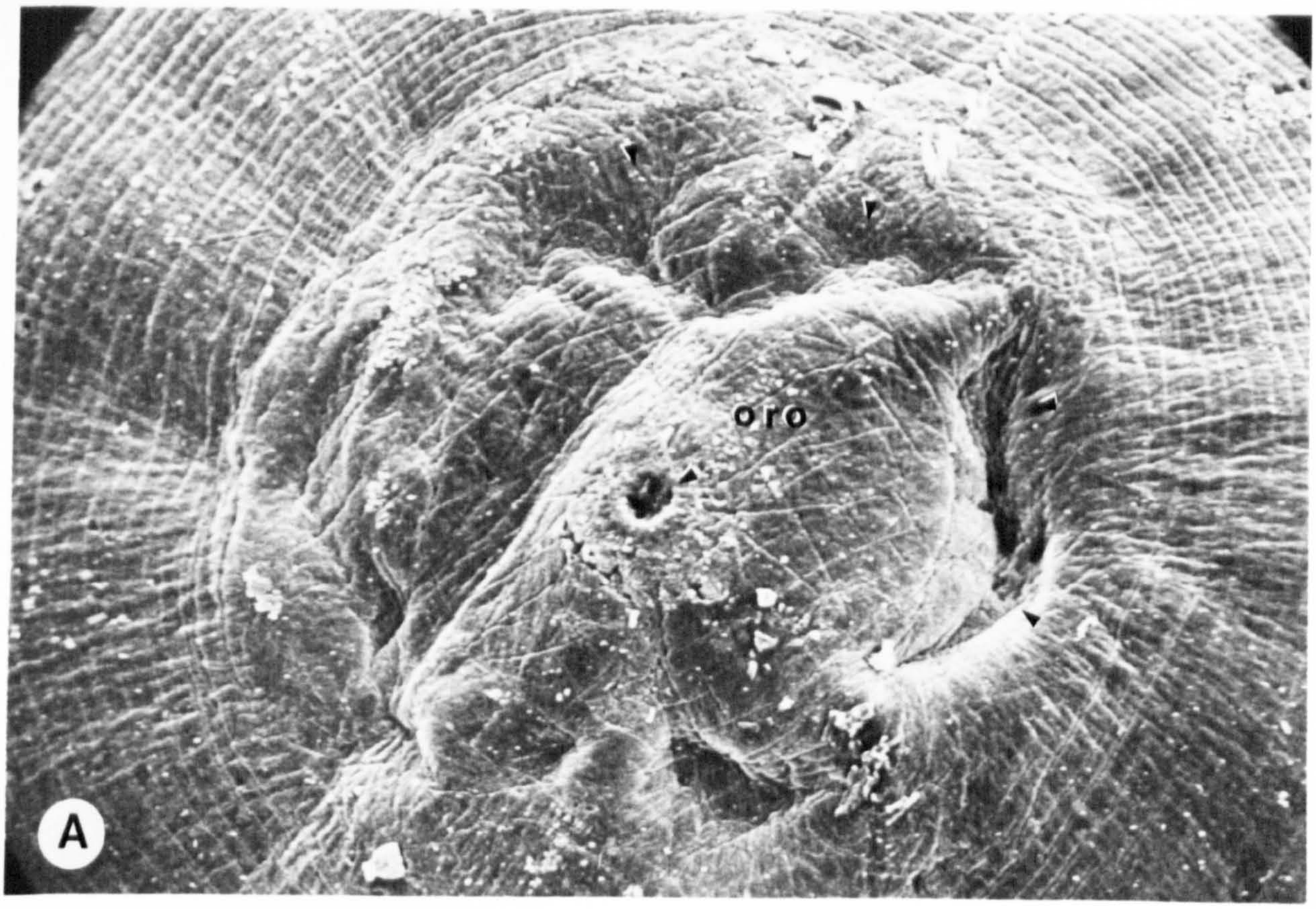

B

FIGURE 44 : Electron micrograph of oral opening of adult female *S. eurycerca*.

A. Anterior view of oral openings.
(note, ▲ indicate indentations in cuticle thought to be positions of two of the four pairs of cephalic papillae)

B. Detail of opening into alimentary canal of nematode.




oro
A

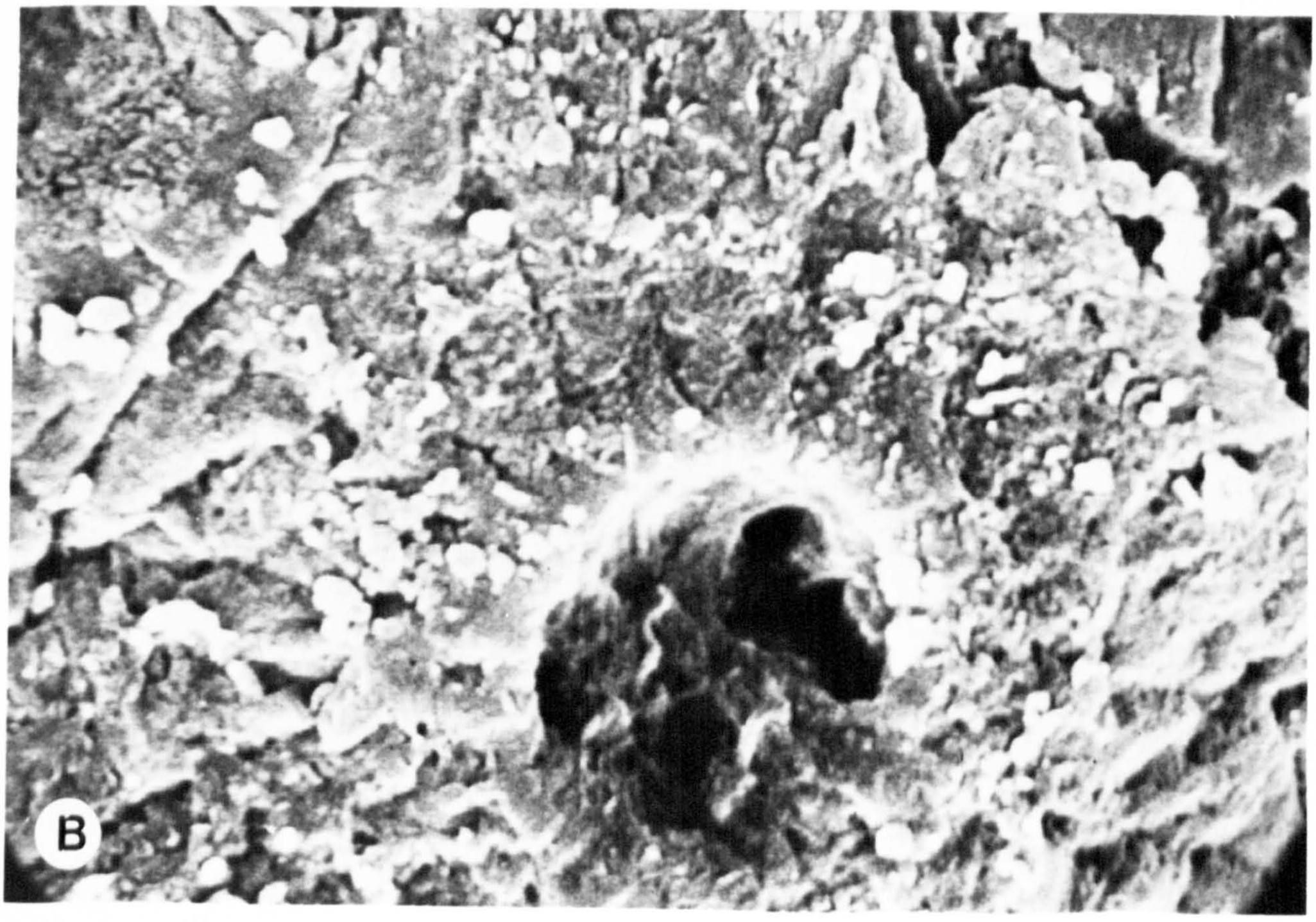

B

FIGURE 45 : Electron micrographs of cuticular striations and annulations of adult *S. eurycerca*.

A. Pattern of striae in anterior region of nematode (ventral position)
(note, regular spacing and uniformity of depth)

160µm

B. Pattern of striae in anterior region but dorsal to the vulva.
(note, striae are deeper)

5µm

C. Pattern of striae approximately one-third of the distance along from the anterior tip of nematode.
(note, irregular shape of striae)

75µm

D. Detailed view of raised string forming irregular patterns shown in 45C.

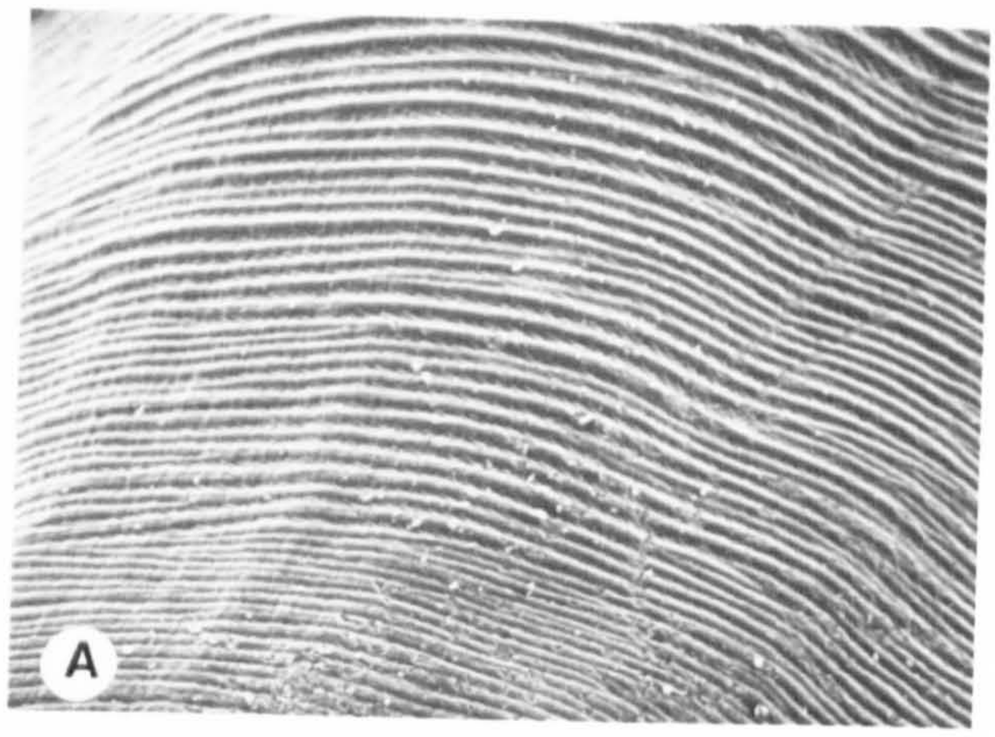
10µm

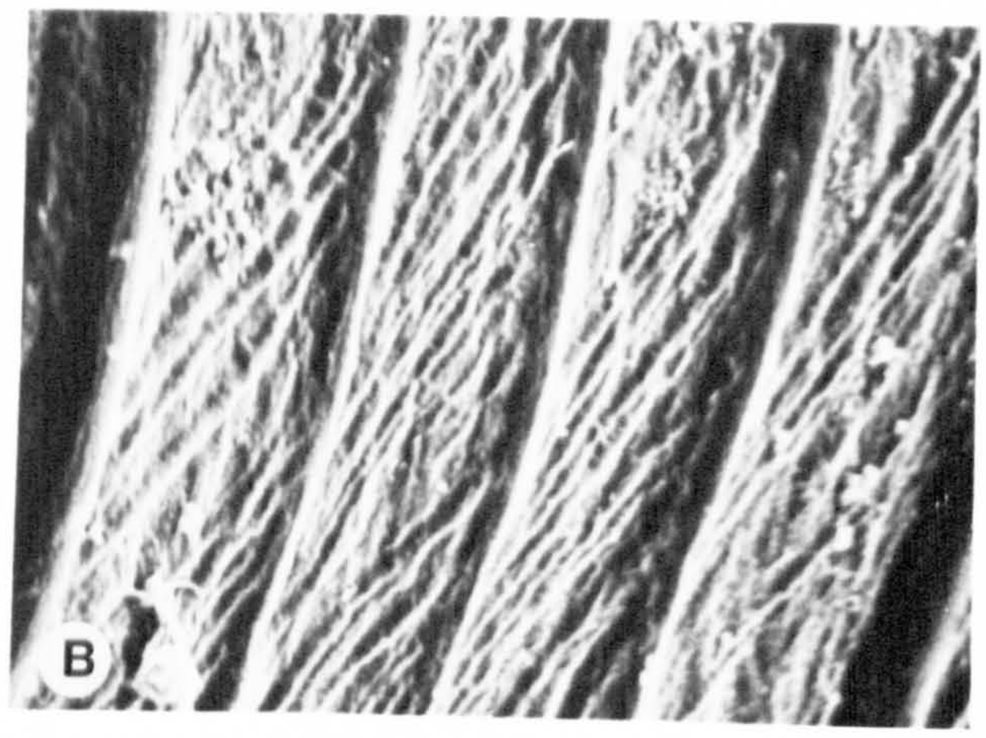
E. Pattern of striae at posterior region of nematode.
(note, grooves are shallower than at anterior end 45A)

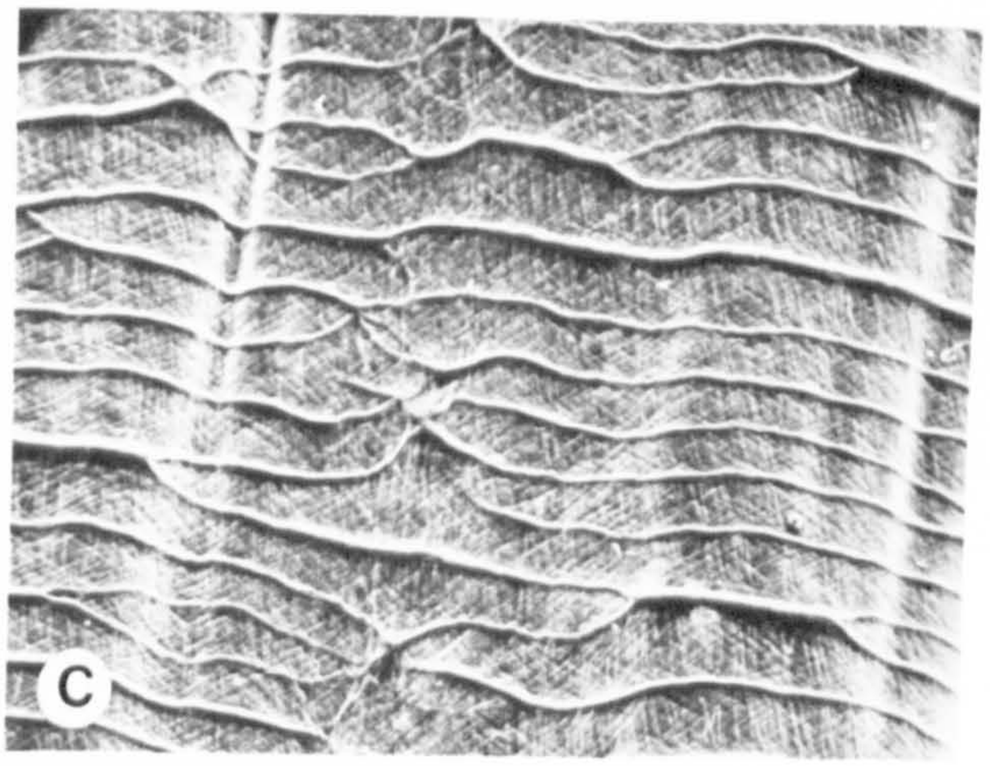
6µm

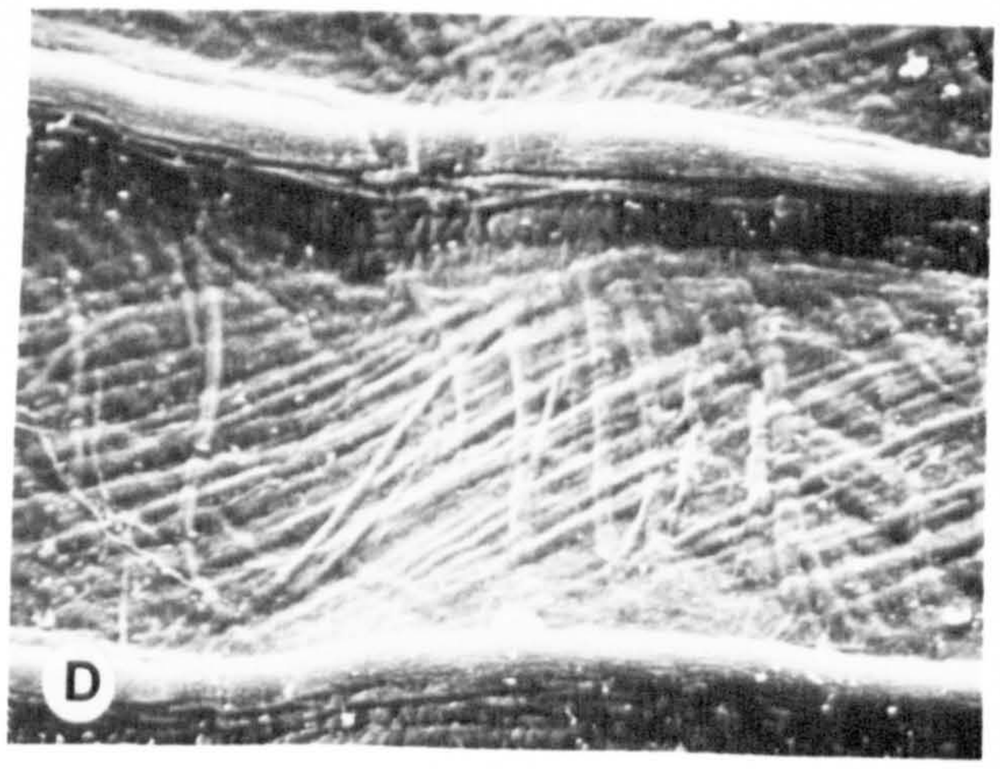
F. Terminal end of adult nematode.
(note, striae form eliptical patterns)

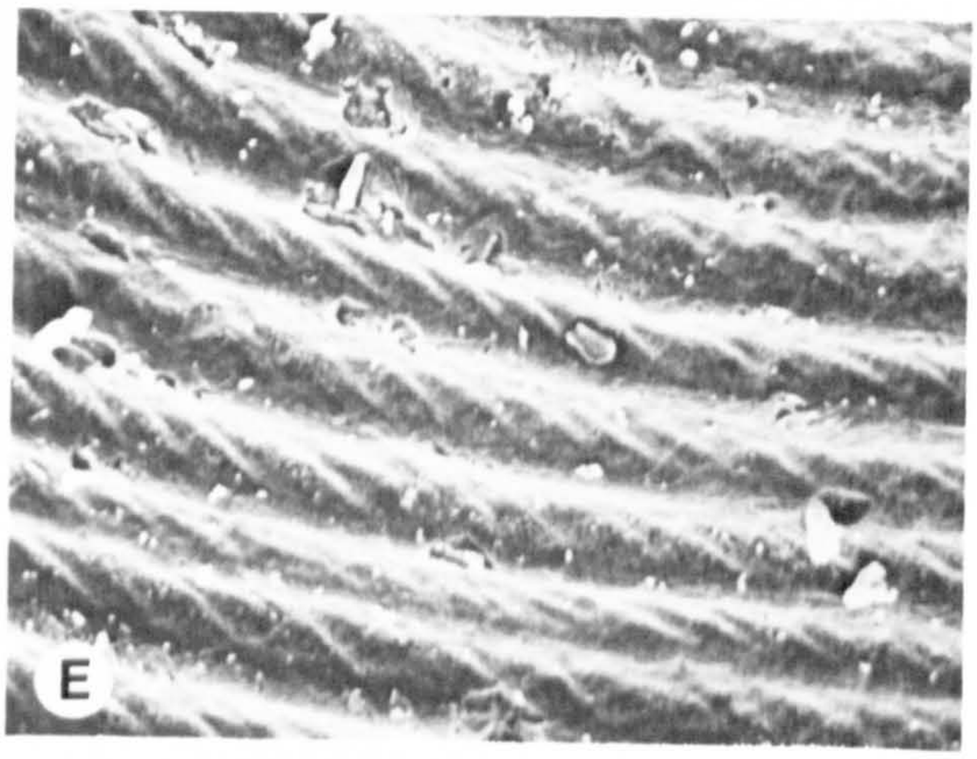
120µm


A


B


C


D


E

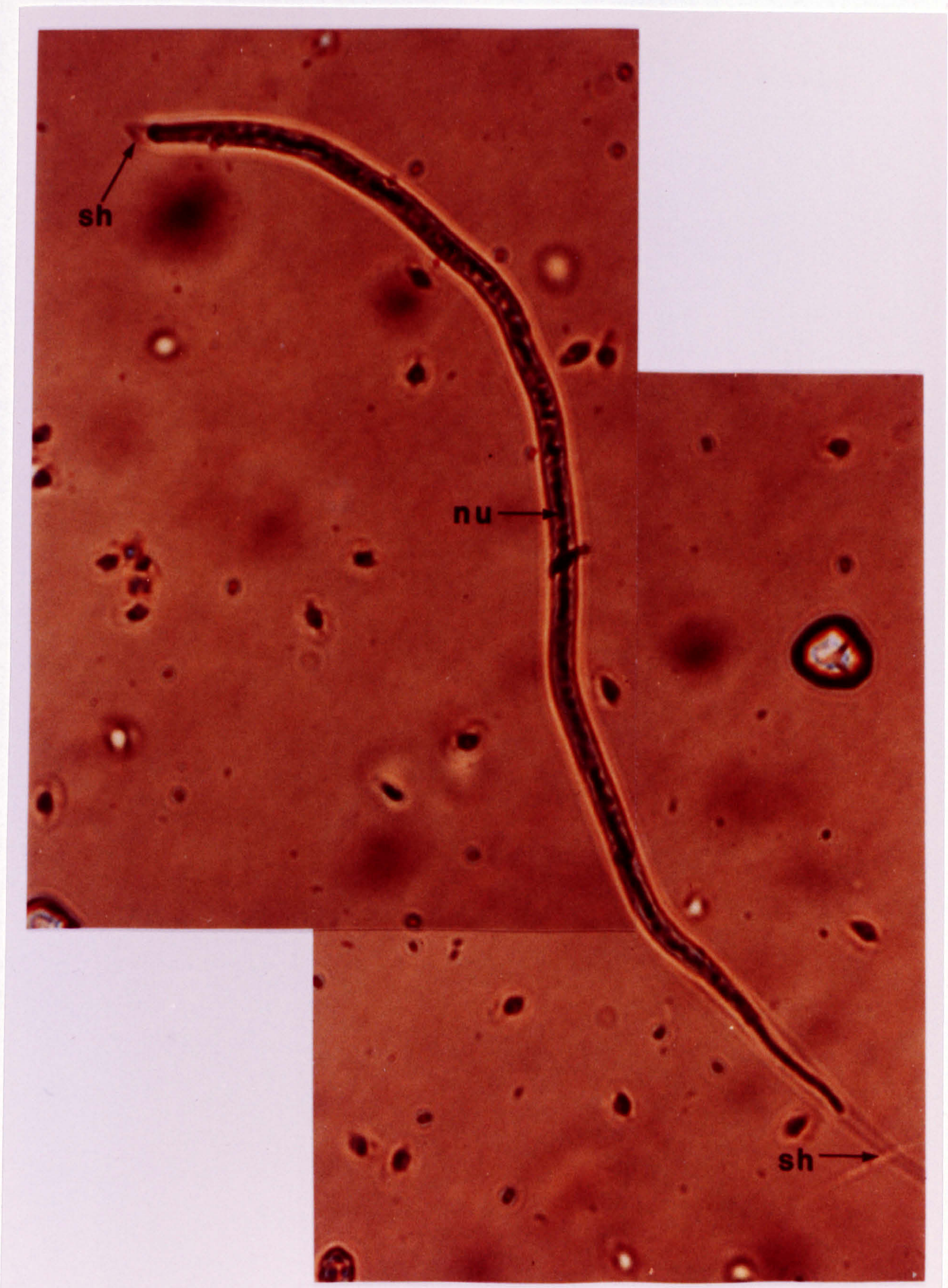

F

FIGURE 46 : Microfilaria of *S. eurycerca* viewed with a light microscope

(note, transparent sheath visible at both ends of microfilariae is characteristic of blood-dwelling nematode larvae)

0 30µm

sh sheath
nu nucleus


sh
nu
sh

1.3.2 Morphology of *S. eurycerca* microfilariae

Microfilariae of *S. eurycerca* consist of a long, narrow nucleated body surrounded by a sheath (Fig. 46). The mean length of microfilariae is 318 μm (Standard deviation = 27 μm) ranging from 263 μm to 382 μm (N = 88). The anterior end is blunt and rounded and the sheath extends a mean distance of 11.1 μm (N = 20) beyond the nucleated portion. The posterior end is narrow and pointed and the sheath extends 28.9 μm (N = 20). The nuclei are rounded and are positioned along the whole length of the body.

2. PATHOLOGICAL EFFECTS OF *S. eurycerca* ON SWANS

Previous reports of the pathological effects of *S. eurycerca* have been described mostly from Whistling Swans and from one Mute Swan (Boughton, 1965). *S. eurycerca* is thought to be pathogenic or contributory to the cause of death of the host as demonstrated by Quortrup and Holt (1940), Cowan (1946), Boughton (1965), Kluge (1967), Irwin (1975), MacNeill (1975), and MacNeill and Barnard (1978). Clinical examination of infected hearts show them to be considerably enlarged (Boughton, 1965; Irwin, 1975). Myocardial tissue is pale and flabby (Cowan, 1946; MacNeill, 1975) and fibrous (Boughton, 1965). In addition blood vessels are severely congested (MacNeill, 1975).

Histological examinations show infected hearts to have necrotic tracts, focal haemmorhages, epicardial and endocardial fibrosis and widespread inflammation and degeneration (Irwin, 1975).

Adults of *S. eurycerca* are not confined to specific locations in the heart and have been found in the epicardium (Coburn, 1938; Quortrup and Holt, 1940; Boughton, 1965; Irwin, 1975), in the myocardium (Coburn, 1938; Boughton, 1965; Holden and Sladen, 1968; Irwin, 1975), and in the endocardium of the heart (Quortrup and Holt, 1940; Boughton, 1965). Some nematodes are visible on the surface of the heart under the pericardial membrane (Cowan, 1946; Coburn, 1938).

In this section, the pathological effects of *S. eurycerca* are considered. First, the incidence of adult worms in swans is described; second, the macroscopic effects of the worms are outlined and third, details are given of the histological effects on heart tissue.

2.1 MATERIALS AND METHODS

2.1.1 Post-mortem examinations of swans

Dead swans were dissected to remove the heart and to determine the presence of *S. eurycerca*. Post-mortem examinations were performed at the Veterinary Investigation Centre, Sutton Bonington.

All precautions against transmitting disease were taken during post-mortem examinations. A laboratory coat was worn under a plastic apron and disposable gloves were used to protect the hands from direct contact with the carcass.

The swan was weighed and its external appearance noted, including its plumage condition, bone structure, lacerations and ectoparasitic load. The ventral surface of the bird was covered with iodine disinfectant. An incision was made along the mid-rib region from head to cloacal opening. Skin and fat were parted from the connective tissue surrounding the viscera. Breast muscle was removed from the rib-cage. The cartilaginous joints between the ribs and the bones at the wing and neck joints were cut, allowing complete removed of the rib-cage. Connective tissue around the heart was loosened and the organ removed by cutting arteries and veins. The heart was preserved in buffered formalin.

The cause of death of each swan was determined from this examination.

2.1.2 Dissection of swan hearts

A total of 75 swan hearts were dissected for adult nematodes of *S.eurycerca*. The appearance of tissues was noted and six morphological measurements taken: total length of heart, total width and the width of each of the auricular and ventricular walls.

All nematodes were dissected from the heart and preserved in formalin (10% buffered) for later examination.

2.1.3 Preparation of tissue sections for histological examination

Using the procedure described in Appendix 7, heart tissue sections were prepared from both infected and non-infected swans.

2.2 RESULTS

2.2.1 Incidence of adult *S. eurycerca* in swan hearts

A post-morten examination was performed on 75 swans. Each heart was dissected to determine the presence of *S. eurycerca* . Most hearts had come from Mute Swans (N = 71), but 3 were taken from Whooper Swans and one from a Black Swan. (Table 32).

Eight swan hearts were infected with adult *S. eurycerca* (Table 33). Seven were from Mute Swans and 1 from a Whooper Swan. The cause of death in six of the birds was lead poisoning; the remaining two were killed by other toxins and trauma. The weights of lead poisoned swans were severely reduced when compared to the average weights of healthy birds; (average weights of adult male Mute Swans = 12.2 kg; adult female Mute Swans = 8.9 kg; adult male Whooper Swans = 10.8 kg; adult female Whooper Swans = 8.1 kg (Scott, 1972)).

The number of nematodes per heart ranged from 1 - 13. There was no evidence to suggest that the overall size of infected hearts was larger than non-infected ones. The mean length of non-infected hearts (7.68 cm, N = 70) was larger than the mean for infected hearts (6.87 cm, N = 8), but the sample size of infected hearts was too small to test statistically. Also, 75 per cent of the infected swans were lead poisoned which causes severe atrophy of most body organs, including the heart.

2.2.2 Location of adult *S. eurycerca* in heart tissue

Three nematodes were located in the myocardium of the right auricle (Table 34). A further five were removed from the pericardium surrounding the right auricle; one of which was situated in the pericardial sac (Fig. 47). Six nematodes were insinuated in the myocardial tissue of the left ventricle and a further four in the pericardium around this chamber. Seven nematodes were embedded in the fatty tissue adjacent to the pericardium near the left auricular-ventricular junction. One nematode was dissected from the superficial tissues at the apex of the heart. Histological tissue sections also show the nematodes to be located in sub-endocardial tissue adjacent to Purkinje fibres (Fig.49B), in myocardial tissue (Fig. 48) and in the pericardium at the outer edge of the heart (Fig.49C).

2.2.3 Histopathology of *S. eurycerca* in swan hearts

Histological examination of the parasitised myocardium showed multifocal eosinophilic myocarditis characterised by a slight infiltration of eosinophils into the myocardium (Fig. 50A). The parasitised tissue was compared with non-parasitised myocardial tissue (Figs.50A & 50B).

Associated with the myocarditis were slight areas of vacuolar degeneration of muscle fibres and areas of fibrosis (Fig.50A).. The pericardium also showed a slight increase in cellularity recognised by an infiltration of chronic inflammatory cells (Fig. 50C). The sub-endocardial Purkinje fibres showed slight sub-endocardial haemmorhages characterised by invasion of erythrocytes indicating possible tissue migration of the parasite (Fig.50D).

Cross sections of the female nematode in heart tissue showed the nematode to be gravid and the uteri full of eggs (Figs.49A & 49B). There did not appear to be any cellular reaction from the host to live nematodes.

A pale eosinophilic deposit was seen on the outer surface of the cuticle of a dying worm (Figs.49C & 49D). One uterus was devoid of eggs and larvae, which indicated the nematode was degenerating (Fig.49D). The normal homogeneity of the cuticle wall tissue was clumped and vacuolated indicating cuticular damage which was attracting a cellular reaction from the host (Fig.49D).

Table 32: Swans dissected to determine the number of hearts containing adult nematodes

SWANS	N	NO. OF HEARTS INFECTED WITH NEMATODES	%	DESCRIPTION OF SPECIES OF SWANS
Adult males	32	4	12.5	31 Mute Swans; 1 Black Swan
Adult females	31	3	9.8	29 Mute Swans; 2 Whooper Swans
Juvenile males	8	1	12.5	7 Mute Swans; 1 Whooper Swan
Juvenile females	4	0	0.0	4 Mute Swans
Total	75	8	10.7	

Table 33: Description of swans and swan hearts infected with *S. eurycerca*

SITE OF DEAD SWAN*	AGE	SEX	WEIGHT (kg)	CAUSE OF DEATH	NUMBER OF NEMATODES IN HEART	BIOMETRICS OF HEART (cm)					
						LENGTH	WIDTH	RIGHT AURICLE WIDTH	RIGHT VENTRICLE WIDTH	LEFT AURICLE WIDTH	LEFT VENTRICLE WIDTH
Gunthorpe, Notts.	Ad	♂	6.0	Lead poisoning	1	6.5	4.2	0.1	0.5	0.2	1.2
Buckenham, Norfolk	Ad	♂	9.0	Toxin	2	7.0	5.0	0.2	0.5	0.2	1.2
Norwich, Norfolk	Ad	♂	5.1	Lead poisoning	1	6.4	5.1	0.2	0.5	0.2	1.6
Norwich, Norfolk	Ad	♂	6.5	Lead poisoning	1	8.0	5.4	0.1	0.4	0.2	1.2
Colwick, Notts.	Ad	♀	6.1	Lead poisoning	3	5.4	5.3	0.2	0.6	0.2	1.3
St. Neots, Cambs.	Ad	♀	7.0	Lead poisoning	3	7.2	5.1	0.1	0.6	0.2	1.4
Ranworth, Norfolk	Juv	♂	5.3	Lead poisoning	13	6.5	4.2	0.1	0.4	0.1	1.2
(Whooper Swan) Strathbeg Loch, Grampians	Ad	♀	10.0	Trauma	3	8.0	4.9	0.1	0.4	0.5	1.6

(* Note: All are Mute Swans except where otherwise stated)

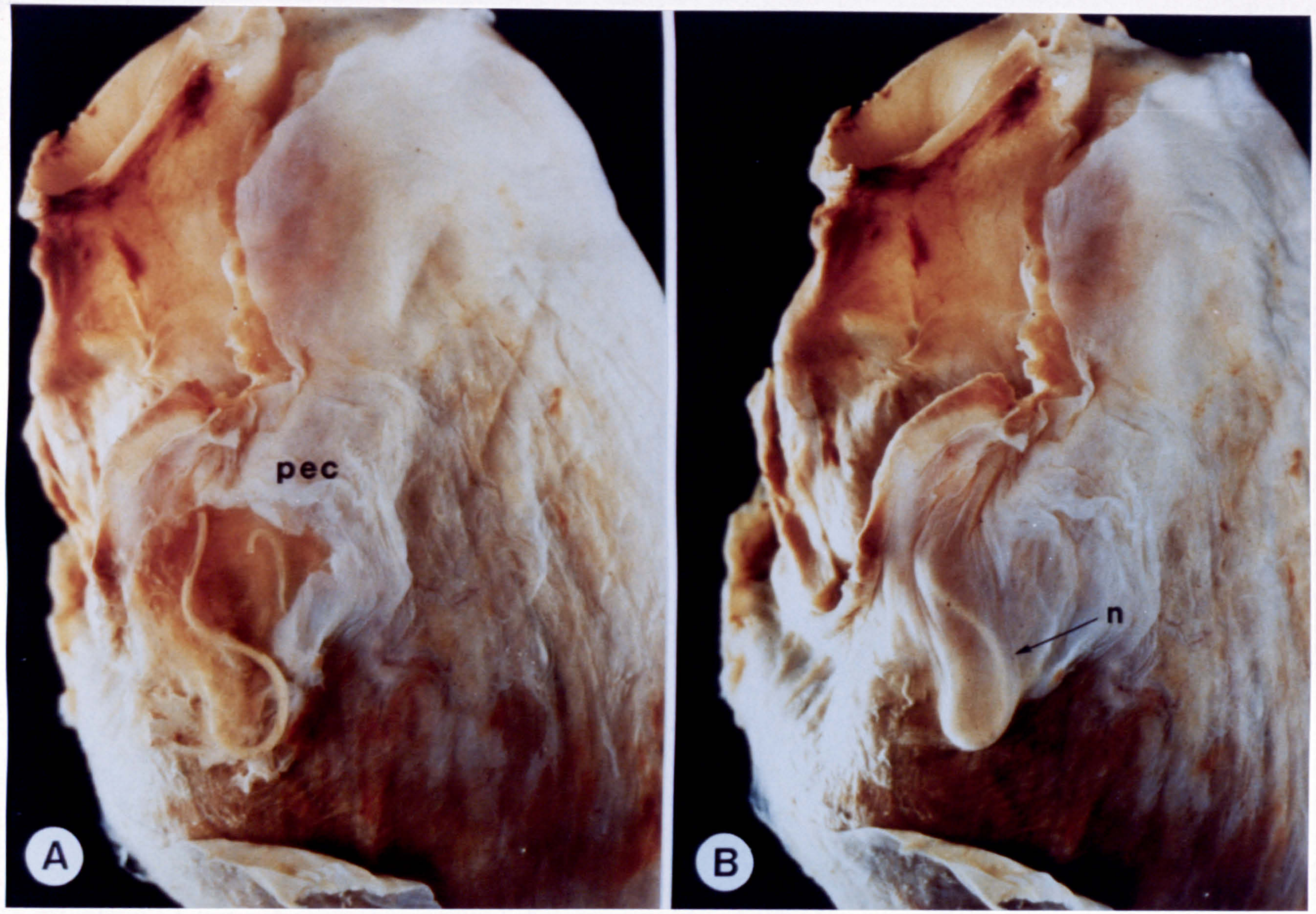
Table 34: Location of adult *S. eurycerca* in hearts

LOCATION	NUMBER OF NEMATODES	
	N	%
Right auricle myocardium	3	11.1
Right auricle pericardium	5	18.5
Left ventricle myocardium	6	22.2
Left ventricle pericardium	4	14.8
Left auricular-ventricular myocardium	0	0.0
Left auricular-ventricular fatty tissue and pericardium	7	26.0
Epicardium of heart apex	1	3.7
Unknown	1	3.7
Total	27	

FIGURE 47 : Mute Swan heart with adult female nematode *S. eurycerca* in pericardial sac.

A. Nematode dissected from pericardial sac.

B. Nematode *in situ* on surface of heart.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A
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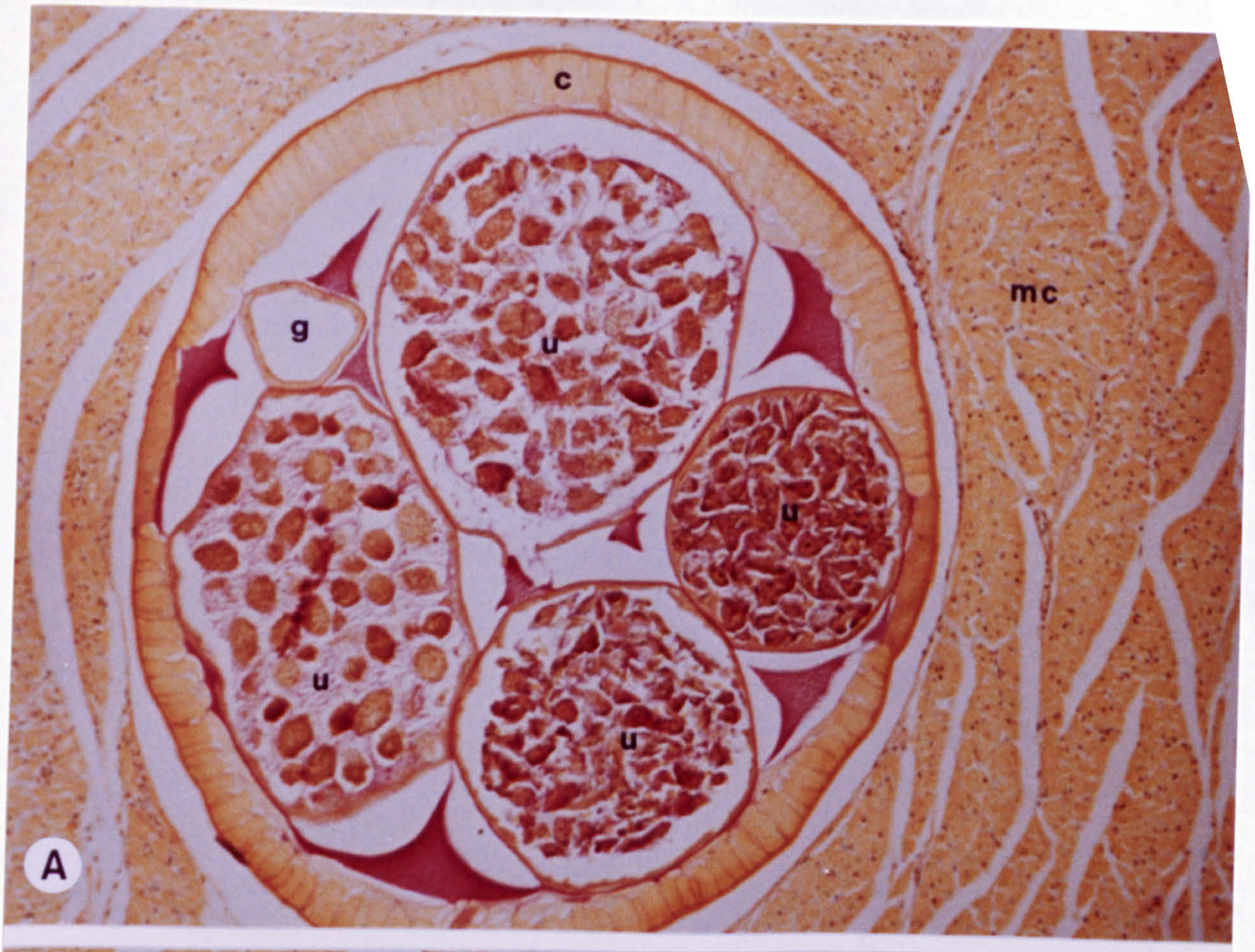
FIGURE 48 : Cross section of adult female nematode *S. eurycerca* in myocardial tissue of Mute Swan heart viewed through a light microscope.

A. Section shows most of the body of the nematode to contain uteri full of eggs.

B. Section shows uterus devoid of eggs (possibly of a dying nematode).

c cuticle
u uterus
mc myocardial tissue
g gut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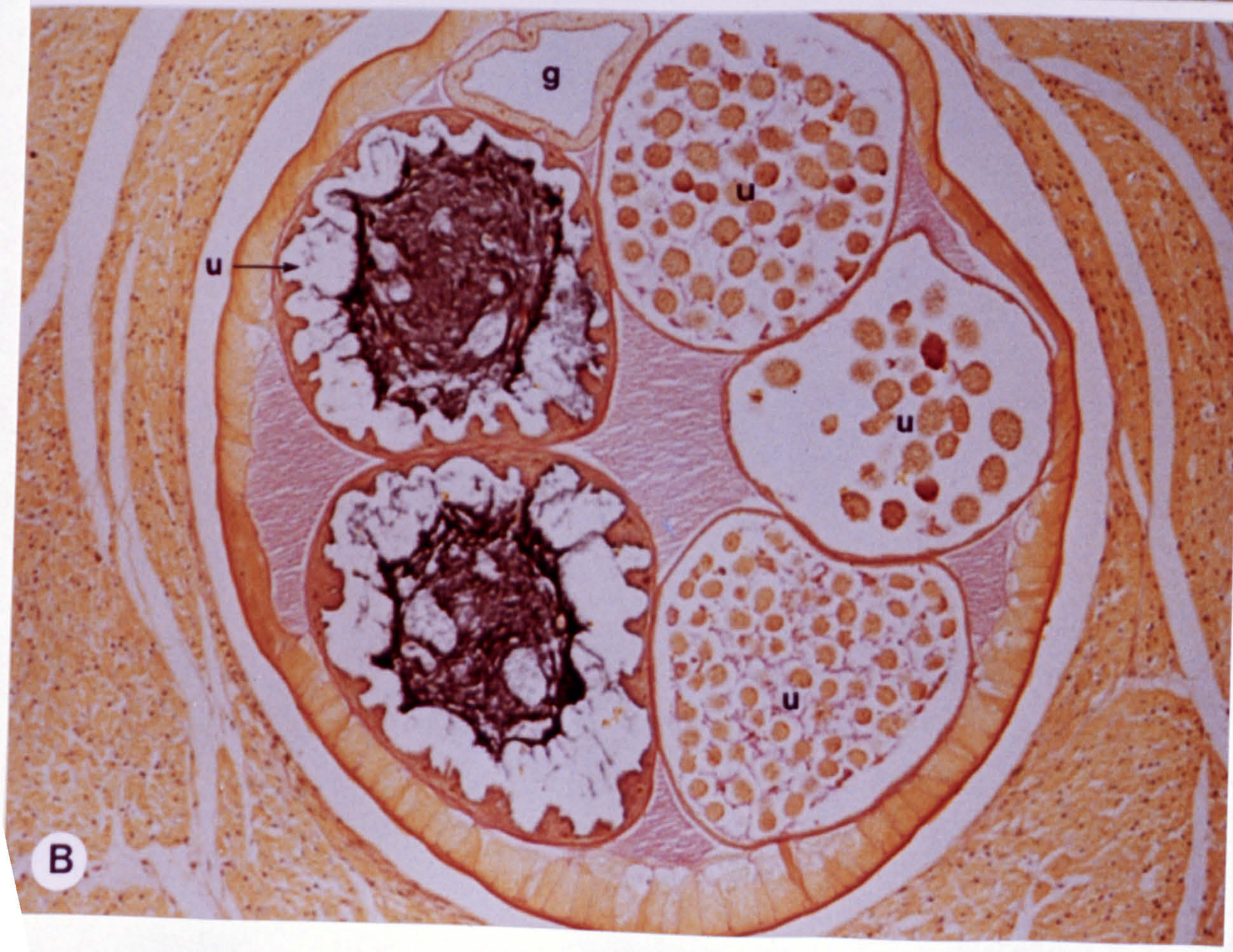
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FIGURE 49 : Histological tissue sections of Mute Swan heart parasitised by adult nematode *S.eurycerca*.

A. Uteri of nematode showing different stages of development of eggs.

▲ on left-hand side indicates uterus containing youngest eggs.

(Section stained with PAS and Alcian Blue)

40µm

B. Cross section of nematode situated in endothelial tissue adjacent to Purkinje fibres
(Section stained with PAS and Alcian Blue)

40µm

C. Oblique section of nematode in pericardial heart tissue.

▲ points to infiltration of eosinophils around edge of the nematode.

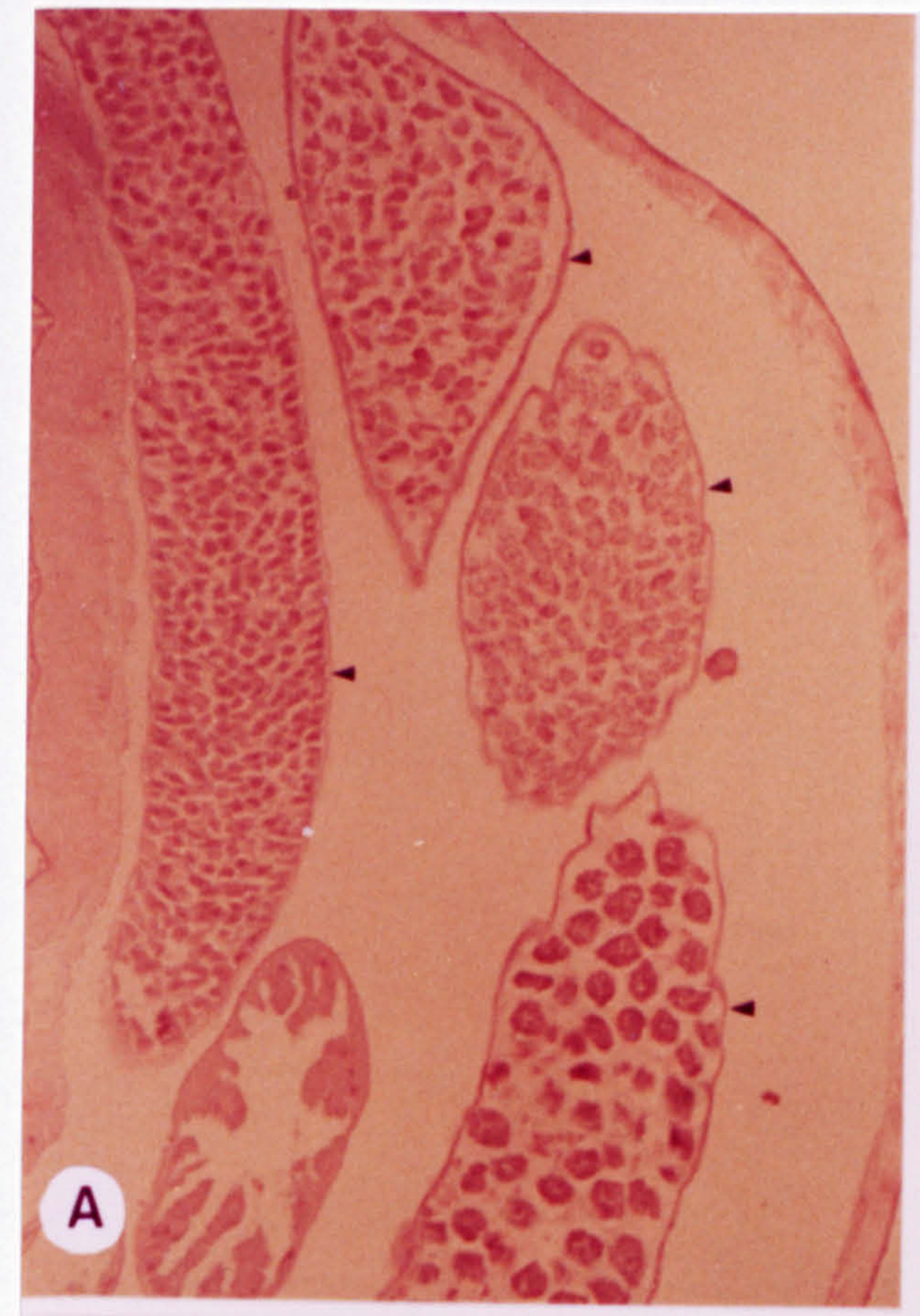
40µm

D. Detailed view of eosinophilic deposit on outer surface of cuticle.

(note, clumped and vacuolated appearance of cuticle wall of nematode
▲ indicating cuticular damage)

250µm

n nematode
c cuticle
pf Purkinje fibres
sec sub-endocardial tissue
pec pericardial tissue
eo eosinophils
u uterus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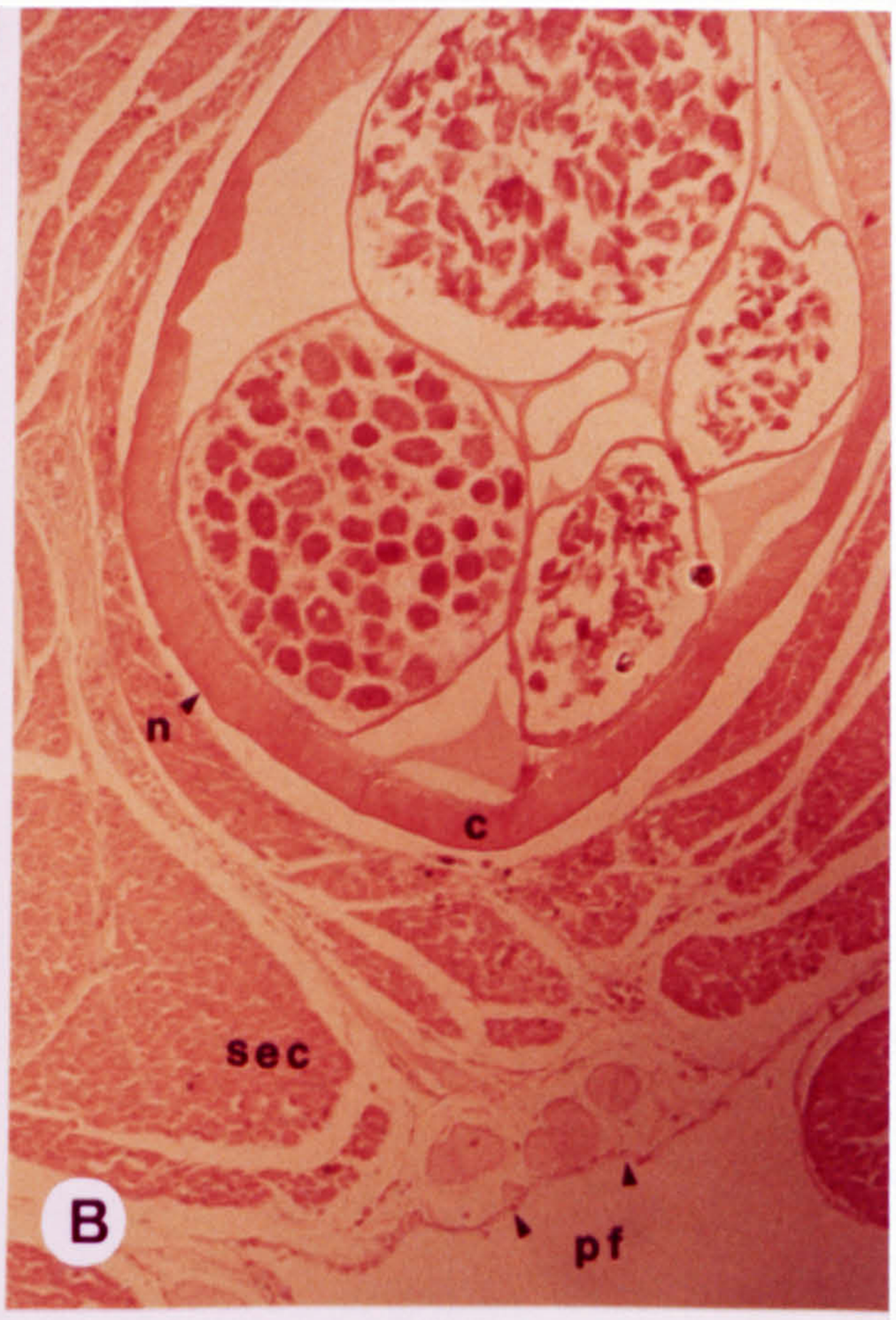
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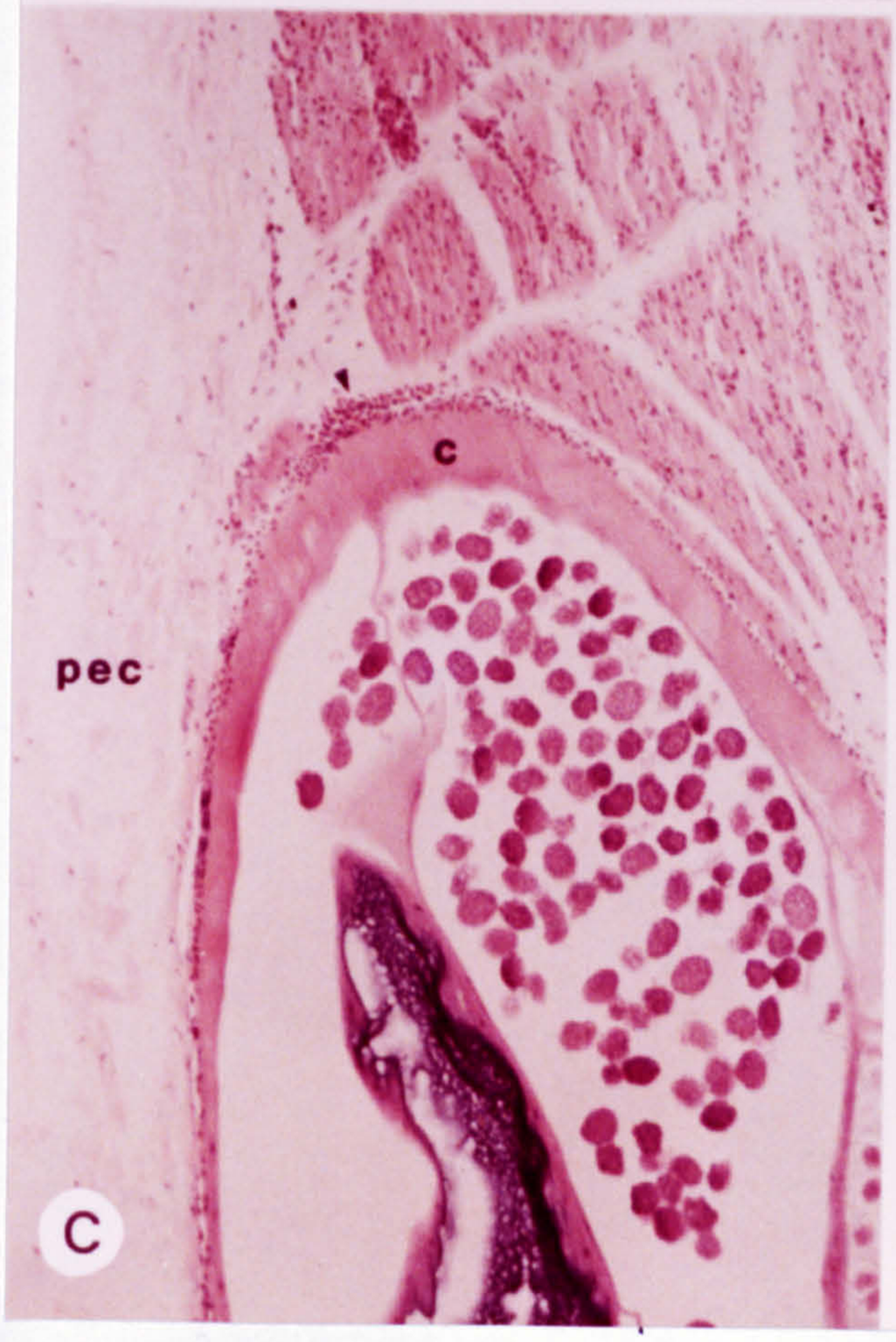
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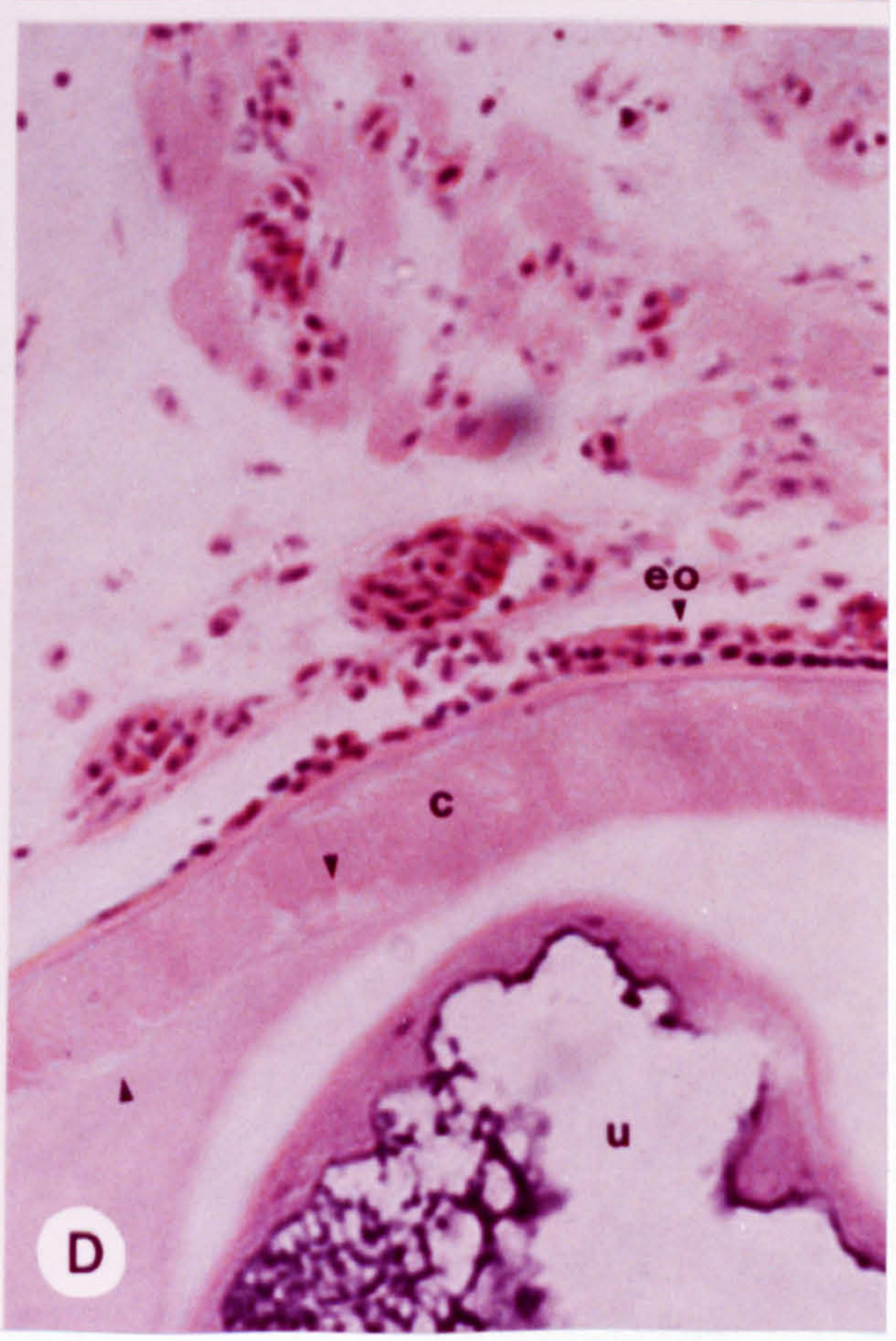
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D

FIGURE 50 : Comparison of parasitised with non-parasitised heart tissue.

A. Myocardial tissue of Mute Swan heart parasitised by *S. eurycerca*.
▲ indicate areas of infiltration by eosinophils.

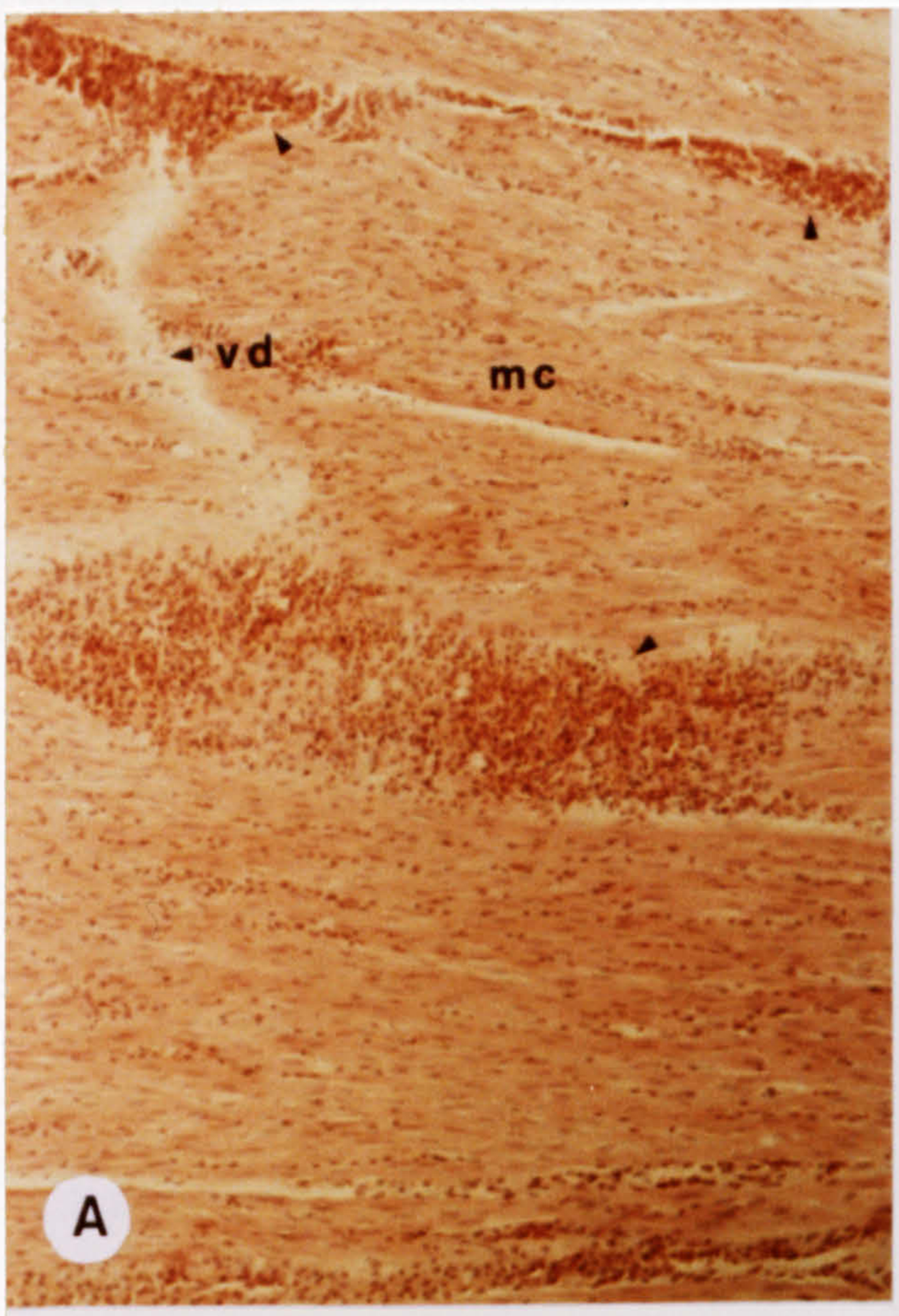
B. Myocardial tissue of a non-parasitised Mute Swan.

C. Pericardium and pericardial heart tissue parasitised by *S. eurycerca*.
(note, slight increase in cellularity underneath pericardium.)

D. Endocardial heart tissue parasitised by *S. eurycerca*.
(around Purkinje fibres there is evidence of slight sub-endocardial haemmorhaging indicating possible migration path of parasite from blood inside heart towards the heart muscle)

40µm

vd vacuolar degeneration
mc myocardium
pec pericardial tissue
hm haemmorhages
sec sub-endocardial tissue
pf Purkinje fibres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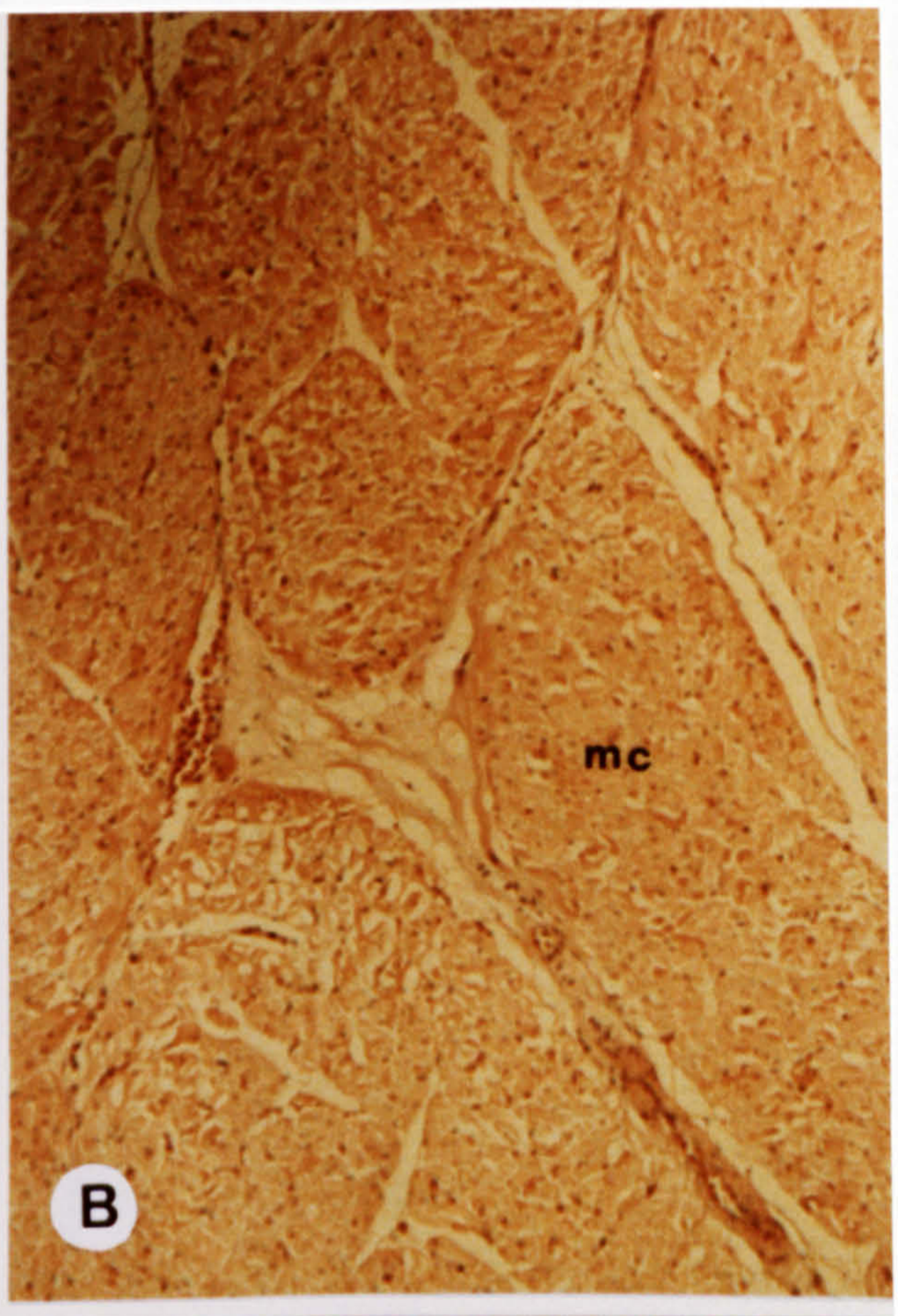
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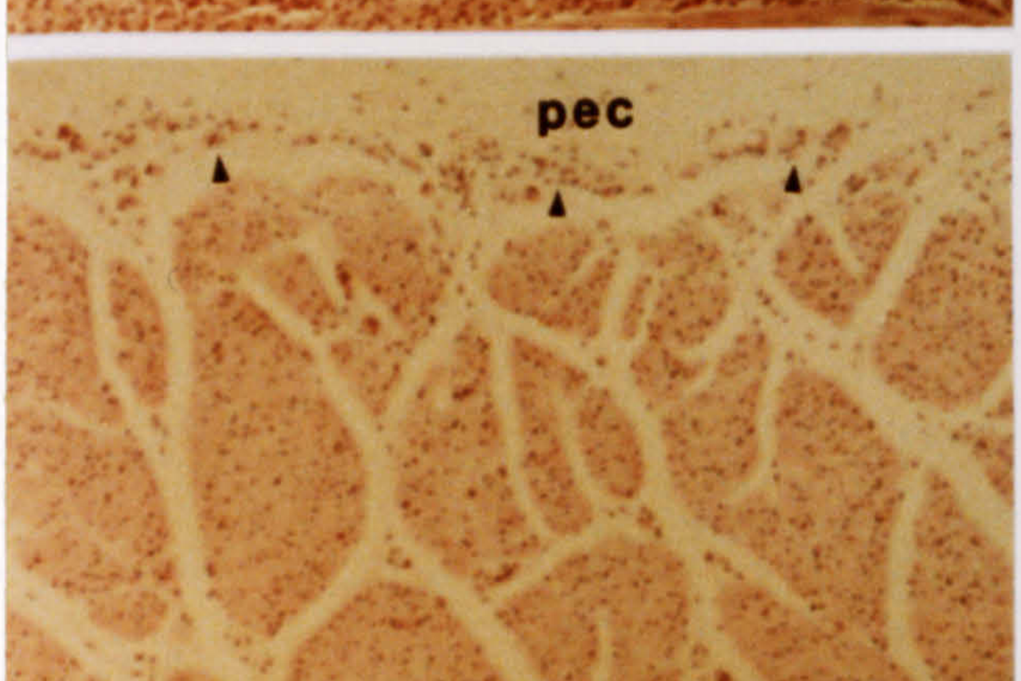
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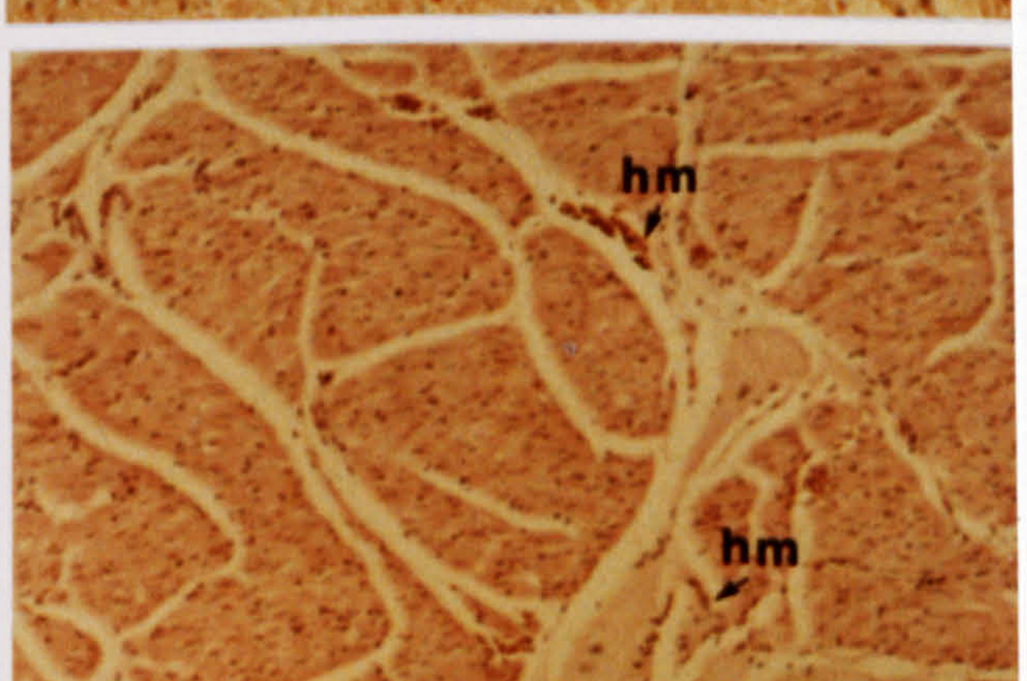
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DISCUSSION

The heartworm of dogs, *Dirofilaria immitis*, belongs to the same family of nematodes (Onchocercidae) as *S. eurycerca* , the heartworm of swans. As a potentially fatal disease, the dog heartworm has been extensively researched and is used as a model for the study of diseases caused by filarial nematodes (Otto, 1970; Melby and Altman, 1974; Knight, 1977, Jubb et al, 1985). The pathological findings from this research are compared with the findings from the present study on the pathology of *S. eurycerca*.

In dogs, adult heartworms of *D. immitis* are usually located in the right side of the heart and in pulmonary arteries. Adult male heartworms measure from 12 - 20 x 10^3µm in length and females from 25 - 31 x 10^3µm in length (Jubb et al, 1985). Like *S. eurycerca* , adult female dog heartworms are ovoviviparous and release microfilariae directly into the blood stream.

Clinical diagnosis of dirofilariasis, as for *S. eurycerca* , depends on the detection of microfilariae in the blood. Clinical signs of infection include weakness in infected dogs, anorexia and progressive respiratory difficulties. Some dogs develop a cough associated with the respiratory infection which can progress to congestive heart failure. There is a rough correlation between the number of adult worms in the heart of a dog and the severity of clinical signs (Jackson et al, 1966). Dogs with no clinical signs of heartworm can harbour up to approximately 30 nematodes and those exhibiting signs usually have between 50 - 60 nematodes (Jubb et al, 1985). Few clinical signs have been described in swans infected with *S. eurycerca*. Holden and Sladen (1968) suggested that infected Whistling Swans might delay or abandon migratory flights due to the presence of the nematode. Most swans do not have more than 25 - 30 nematodes per heart (Table 30) and it is possible that these represent low infections which are not detrimental to the birds.

Heartworm infestation of dogs has long been known to cause pulmonary hypertension (Porter, 1951; Hennigar and Ferguson, 1957; Wallace and Hamilton, 1962). Lesions are produced by the parasites in the heart and lungs and are characterised by inflammation of the endothelial tissues of the pulmonary arteries. The effect of widespread endothelial damage is a reduction in the cross-sectional area of the pulmonary vascular bed which in turn, impedes blood-flow and gradually elevates the pressure in the

vascular bed leading to pulmonary hypertension. When pulmonary flow is increased, by exercise for example, the right ventricle of the heart has to generate an increasingly greater pressure and this produces hypertrophy of the right ventricle. Radiographic techniques which identify enlargement of the right ventricle are often used to diagnose infected dogs in the absence of microfilariae (Knight, 1977).

It is unlikely that *S. eurycerca* produces the same pathological manifestations in swans as described for *D. immitis*. It has been shown in the present study that *S. eurycerca* adults are located all over the swan heart and are not usually concentrated in the pulmonary arteries. In addition, the results suggest that gross morphological changes do not occur in hearts infected with *S. eurycerca*, although researchers have shown otherwise (Boughton, 1965; Irwin, 1975). It was difficult to make judgements from only 8 infected hearts, 6 of which had been taken from lead poisoned and emaciated swans.

The histological pathology of *D. immitis* is not similar to that of *S. eurycerca* . Heartworm disease of dogs is primarily a pulmonary vascular disease and much of the histological reactions are seen in the pulmonary arteries and lungs. For *S. eurycerca* , most of the histopathology is visible in the heart tissue associated with dead or dying nematodes.

Clearly, larger samples of infected swan hearts are required for more extensive investigations into the effects of *S. eurycerca*. Nevertheless, results from this study suggest that *S. eurycerca* is not as pathogenic as *D. immitis* in dogs.

3. HAEMATOLOGY AND CLINICAL CHEMISTRY OF SWAN BLOOD

The histopathological effects of *S. eurycerca* in swan hearts have been shown to be attributable to mechanical damage to the tissue by the nematode and the reaction of the host to the presence of the nematode (p.154). Similar gross effects are seen in dog hearts infected with *D. immitis* where chronic inflammation and lesions are commonplace.

As part of the intensive research on the dog heartworm, *D. immitis*, researchers have compared the haematology and blood chemistry of infected and non-infected dogs. Even though some workers have recorded blood values to differ significantly between normal dogs and those with heartworm, the values may still remain within a normal range (Snyder *et al*, 1967; Tulloch *et al*, 1970). There is sometimes a need for an alternative means of diagnosing filariasis when microfilariae are absent and an examination of blood components could provide such a diagnostic tool. Certain trends have been noted in infected dogs: they commonly have a higher eosinophilia than normal dogs which accompanies microfilaremia (Wong *et al*, 1973) but the mean total white blood cell count remains unchanged (Weiner and Bradley, 1973). The more advanced the heartworm infection, the more important it is to establish baselines for complete blood counts and creatinine levels (Knight, 1977).

There is little baseline data on the haematology and clinical chemistry of swan blood. The only published information is contained in the I.S.I.S. book of biological data (1983) and within these records, only 11 haematological parameters have been measured for 2-7 swans. The present investigation was undertaken first, to compare the levels of certain blood constituents and haematological values of swans with *S. eurycerca* microfilariae and for non-infected swans and second, to provide baseline data on swan blood haematology and clinical chemistry.

(Source: I.S.I.S. BOOK OF BIOLOGICAL DATA)

TABLE 35 : Haematological data summary for non-infected Whooper and Mute Swans

VALUE	Whooper Swans			Mute Swans		
	N	Mean	SD	N	Mean	SD
Haematocrit (gldl)	7	40.3	6.0	5	43.1	11.6
White blood Count (ml)	6	22.6	19.5	6	7.1	3.2
Red blood Count (10:12/6)	6	3.0	0.0	6	3.0	0.0
Mean cell volume (fl)	6	144.0	26.0	4	177.0	5.0
Lymphocyte (%)	2	28.0	12.0	6	13.0	3.0
Monocytes (%)	2	12.4	17.5	6	6.3	2.6
Eosinophils (%)	2	0.0	0.0	6	0.0	0.0
Basophils (%)	2	3.1	4.4	6	1.9	1.7

3.1 MATERIALS AND METHODS

3.1.1 Haematology and Clinical Chemistry of swan blood

Blood samples were taken from Whooper Swans in Caerlaverock (Dumfries) (N = 56) and from Mute Swans in Montrose (Tayside) (N = 103). In addition, blood from three captive swans was examined: one swan (Z53705) had exhibited microfilaremia after 5 months of capture; a second swan (SIDNEY) had *S. eurycerca* microfilariae when first captured but subsequently lost the infection; a third swan (Z48891) remained free from infection for the course of the captive period (18 months).

Fresh blood was collected initially in non-heparinised syringes (10.0 cm^3) and separated into vessels for three tests:

1) 2.0 cm^3 was placed in K^+ - coated tubes for haematological analysis;

2) 1.0 cm^3 was transferred into lithium-heparin coated tubes for centrifugation, from which the plasma was removed into non-coated tubes for clinical chemistry analysis;

3) 2.0 cm^3 was removed for sedimentation to determine the presence of microfilariae of *S. eurycerca*.

3.1.2 Haematological analysis

Haemoglobin was determined by the oxyhaemoglobin method. Blood (2.0 cm^3) was washed with 0.4 cm^3 ammonia (0.4 cm^3 per dm^3) in a tube with a slightly fitted stopper. The solution was mixed by inverting the tube several times. The solution was measured in a colourimeter using a yellow-green filter (Ilford 625). A standard of known haemoglobin concentration was measured at regular intervals (every ten samples).

Packed cell volumes (haematocrit) were determined using the micro-haematocrit method. Capillary tubes (7.5 cm, 0.1 cm diameter) were filled with blood and sealed at one end using a plasticine filler. The tube was placed in a microhaematocrit centrifuge and spun for 5 minutes and at 12,000 g. The haematocrit was then measured using a reading device.

Differential counts of white blood cells were performed on blood films prepared on microscope slides. The film was stained by a Hema Tek staining machine using Romanowsky stains. The stained blood film was examined under the microscope and a count of 100 consecutive white cells was performed, using a multiple manual register to record the types of white cells counted. This produced a percentage differential count.

3.1.3 Clinical Chemistry analysis

Potassium was measured on an IL 943 Flame Photometer using a Cesium internal standard (IL - Instrumentation Laboratories). *Creatinine* was determined by the Alkaline picrate method (Jaffé, 1886), alanine aminotransferase (*ALT*) and aspartate aminotransferase (*AST*) were measured using an Optimised Method according to the Scandinavian Committee on Enzymes (SCE, 1974). Creatinine, ALT and AST were all measured on a 'Centrifichem 400' centrifugal fast analyser at 37°C.

Creatine phosphate kinase was estimated using an optimised standard method according to Deutsche Gesselschaft fÜr Klinische Chemie (DGKC) (Anon, 1977; Szasz, 1976; Gruber, 1978).

3.1.4 Sedimentation analysis

The sedimentation technique (p. 18) was used to determine the presence of microfilariae of *S. eurycerca* in swan blood.

Mean values were determined for all measured parameters. A t-test was used to evaluate the statistical significance of differences between infected and non-infected swans.

For each parameter the data was grouped in the following way:

i) combined values for Whooper and Mute Swans;
ii) separated values for Whooper Swans;
iii) separated values for Mute Swans;
iv) mean values for Mute Swans compared to values of three captive Mute Swans.

3.2 RESULTS

3.2.1 Haematological analysis

i) Mute and Whooper Swans (combined group)

As a combined group, infected swans showed a significantly higher ($p < 0.01$) lymphocyte level and a significantly lower ($p < 0.01$) eosinophil level when compared to non-infected swans (Table 36). There were no significant differences between the monocyte and basophil values for infected and non-infected swans.

ii) Whooper Swans

Significant differences were found in the haematocrit percentage and red blood corpuscle counts of infected and non-infected Whooper Swans. In swans with *S. eurycerca* , the red blood cell count ($p < 0.05$) and the haematocrit ($p < 0.05$) were lower than in Whooper Swans with no microfilariae (Table 36).

All other measured parameters were not significantly different in the infected and non-infected groups.

iii) Mute Swans

The proportion of lymphocytes, monocytes, eosinophils and basophils showed no significant differences in infected and non-infected Mute Swans.

iv) Captive Swans

Single values were obtained for each of three captive swans and are compared to the equivalent mean values from the group of Mute Swans.

The lymphocyte value (83%) of the infected captive swan (Z53705) was within one standard deviation of the mean lymphocyte percentage (74.75%) of the infected Mute Swan group. The non-infected captive swans had higher lymphocyte counts (91% and 98%) than the mean value for non-infected Mute Swans (62.88%).

Monocyte counts for all captive Mute Swans were within one standard deviation of the mean values calculated for infected and non-infected Mute Swans (Table 37).

For the infected captive Mute Swan (Z53705), the eosinophil count was greater (14%) than for the swan which had been infected (8%) which in turn was greater than for the non-infected swan (2%) (Table 37). Both values for non-infected swans were lower than the mean value for the group of non-infected Mute Swans (36.82%). The infected captive swan had an eosinophil value within one standard deviation of the mean count (24.5%) for the infected group.

TABLE 36 : Haematological analysis of infected and non-infected swans

VALUE	INFECTED SWANS(*)			NON-INFECTED SWANS			
	N	Mean	SD	N	Mean	SD	P
MUTE AND WHOOPER SWANS (Combined Group)							
Lymphocytes (%)	9	75.60	7.60	125	66.10	14.14	p< 0.01
Monocytes (%)	9	0.33	0.71	125	0.22	0.57	NS
Eosinophils (%)	9	24.00	8.03	125	33.40	14.44	p< 0.01
Basophils (%)	9	0.11	0.33	125	0.23	0.55	NS
WHOOPER SWANS							
Haemoglobin (g/dl)	5	15.88	1.46	37	17.05	1.46	NS
Red blood corpuscles (10:12/l)	5	2.52	0.08	37	2.65	0.22	p< 0.05
Haematocrit (%)	5	46.80	1.30	37	49.30	4.17	p< 0.05
Mean cell Hb conc. (g/dl)	5	33.92	2.58	37	34.63	1.49	NS
Mean cell Hb (pg)	5	62.92	5.52	37	64.05	3.99	NS
Mean cell volume (fl)	5	185.6	5.73	37	184.89	9.61	NS
Lymphocytes (%)	5	76.20	2.68	32	73.58	8.92	NS
Monocytes (%)	8	3.50	4.57	60	3.78	4.31	NS
Eosinophils (%)	5	23.60	2.70	36	25.47	8.76	NS
Basophils (%)	8	3.38	4.66	60	3.98	4.18	NS
MUTE SWANS							
Lymphocytes (%)	4	74.75	11.95	103	62.88	15.38	NS
Monocytes (%)	4	0.50	1.00	103	0.18	0.57	NS
Eosinophils (%)	4	24.50	12.71	103	36.82	15.67	NS
Basophils (%)	4	0.25	0.50	103	0.06	0.23	NS

(Note: values for Haemoglobin, red blood corpuscles, Haematocrit, mean cell Hb, conc., Mean cell Hb and mean cell volume not available for Mute Swans and combined group.)

(* Infected swans diagnosed by detection of *S. eurycerca* microfilariae using the sedimentation technique.)

TABLE 37 : Clinical chemistry and Haematological values of captive Mute Swans

VALUE	SWAN (Z53705) *	SWAN (SIDNEY) **	SWAN (Z48891) ***
CLINICAL CHEMISTRY			
Potassium (mmol/1)	3.04	3.05	3.67
Creatinine (µmol/1)	45.0	39.0	42.0
AST (IU/1)	31.0	19.0	24.0
ALT (IU/1)	13.0	8.0	12.0
Creatine phosphate kinase (IU/1)	123.0	99.0	105.0
HAEMATOLOGY			
Haemoglobin (g/dl)	16.4	16.3	18.4
Red blood corpuscle count (10:12/1)	2.44	2.49	2.79
Haematocrit (%)	45.0	42.0	56.0
Mean cell Hb conc. (g/dl)	36.4	38.8	32.9
Mean cell Hb (pg)	67.2	65.4	65.9
Mean cell volume (fl)	184.0	169.0	201.0
Lymphocytes (%)	83.0	91.0	98.0
Monocytes (%)	2.0	1.0	0.0
Eosinophils (%)	14.0	8.0	2.0
Basophils (%)	1.0	0.0	0.0

(* Swan (Z53705 : with microfilaremia;
** Swan (SIDNEY) : previously infected but no detectable microfilariae;
*** Swan (Z48891) : no microfilariae.)

Basophil counts for captive swans were all within one standard deviation of the mean values derived for infected and non-infected Mute Swans (Table 37).

In summary, significant differences were recorded in the lymphocyte, eosinophil and red blood corpuscle counts and the haematocrit percentage in groups of infected and non-infected swans (Table 39).

3.2.2 Clinical Chemistry analysis

i) Mute and Whooper Swans (combined group)

There were no significant differences in any of the blood chemistry values for infected and non-infected Mute and Whooper Swans (Table 38).

ii) Whooper Swans

No significant differences were found in the blood chemistry of infected and non-infected Whooper Swans (Table 38).

iii) Mute Swans

No significant differences were found in the blood chemistry of infected and non-infected Mute Swans (Table 38).

iv) Captive Swans

The potassium levels of captive swans (Table 37) were slightly lower than for the infected and non-infected group of Mute Swans (Table 38), but all values were within one standard deviation of the mean. Similarly for creatinine, the values for captive swans were within one standard deviation of those recorded for both infected and non-infected Mute Swans (Table 37).

The AST value of 31.0 for the infected captive swan (Z53705) was close to the mean value (31.5) for the infected group of swans. The AST value for SIDNEY of 19.0 and for Z48891 of 24.0 were within two standard deviations of the mean non-infected value (35.19)(Tables 37A & 38).

ALT values for captive swans were all within one and two standard deviations of the mean values for the group of swans (Table 37).

The mean creatine phosphate kinase value for infected swans 157.3 and the infected captive swan (Z53705) had a creatine phosphate kinase value of 123.0, within one standard deviation of this mean. For non-infected Mute Swans, the mean value for creatine phosphate kinase was 169.4. Creatine phosphate kinase values for SIDNEY of 99.0 and for Z48891 of 105.0 were within one standard deviation of the mean.

TABLE 38 : Clinical chemistry analysis of infected and non-infected swans

VALUE	INFECTED SWANS(*)			NON-INFECTED SWANS			
	N	Mean	SD	N	Mean	SD	P
MUTE AND WHOOPER SWANS (Combined Group							
Potassium (mmol/1)	10	4.63	2.38	143	5.13	1.88	NS
Creatinine (µmol/1)	11	48.00	12.62	147	46.19	18.42	NS
AST (IU/1)	11	36.64	10.18	145	37.42	14.16	NS
ALT (IU/1)	11	16.55	3.83	146	19.60	7.36	NS
Creatine phosphate kinase (IU/1)	11	302.70	215.50	148	258.20	317.30	NS
WHOOPER SWANS							
Potassium (mmol/1)	-	-	-	54	4.34	1.54	-
Creatinine (µmol/1)	7	53.14	10.46	59	61.34	19.21	NS
AST (IU/1)	7	39.57	11.69	57	41.40	18.42	NS
ALT (IU/1)	7	16.14	3.80	58	17.88	7.71	NS
Creatine phosphate kinase (IU/1)	7	385.9	234.0	59	403.50	432.00	NS
MUTE SWANS							
Potassium (mmol.1)	4	5.82	3.48	103	5.49	1.85	NS
Creatinine (µmol/1)	4	39.00	11.92	102	36.03	7.79	NS
AST (IU/1)	4	31.50	4.12	102	35.19	10.19	NS
ALT (IU/1)	4	17.25	4.35	102	20.51	6.81	NS
Creatine phosphate kinase (IU/1)	4	157.30	30.2	103	169.40	163.60	NS

(* Infected swans diagnosed by detection of *S. eurycerca* microfilariae using the sedimentation technique.)

TABLE 39 : Summary of statistically significant differences between the clinical chemistry and haematology of infected and non-infected swans

VALUE HAEMATOLOGY	INFECTED SWANS Mean	NON-INFECTED SWANS Mean	P
Red blood corpuscle count (10:12/1) (Whooper Swans)	2.52	2.65	$p< 0.05$
Haematocrit (%) (Whooper Swans)	46.80	49.30	$p< 0.05$
Lymphocytes (%) (Mute & Whooper)	75.60	66.10	$p< 0.01$
Eosinophils (%) (Mute & Whooper)	24.00	33.40	$p< 0.01$

DISCUSSION

The primary aim of this section was to compare the level of blood constituents and haematological values in infected and non-infected swans. 'Infected' swans were diagnosed using a sedimentation technique which detects microfilariae of *S. eurycerca* . In an earlier discussion of the sensitivity of this test (p.43), certain conditions were outlined where swans infected with *S. eurycerca* would not be detected. To reiterate, those swans with young adult heartworms, with heartworms of one sex or with very low microfilaremias, would go undetected by the sedimentation method and would therefore be categorized as non-infected. Such 'indetectable' heartworms are still likely to cause mechanical damage to cardiac tissue and subsequent physiological changes in blood components (Archer, 1963). It is important therefore when considering the results of the present section to bear in mind that any differences that do occur are between swans with blood-dwelling microfilariae of *S. eurycerca* and swans with no detectable microfilariae.

Statistically significant differences between infected and non-infected swans were demonstrated in red blood corpuscle counts, haematocrit (percentage of red blood corpuscles), lymphocyte and eosinophil percentages.

Red blood cell counts were significantly lower in infected swans. This does not appear to be a common phenomenon in other filarial infections.

Low haematocrit percentages were recorded in infected Whooper Swans which suggests possible anaemia. Further indications of anaemic conditions were found in infected Whooper Swans where low values were recorded in haemoglobin levels, mean cell haemoglobin concentration and mean cell haemoglobin.

A significant rise in lymphocytes was detected in microfilaremic swans. Such physiological responses have been previously associated with chronic infections (Wakelin, 1984; Jubb *et al* , 1985).

Eosinophilia of circulating blood is probably more commonly associated with parasitism than any other clinical entity (Archer, 1963). The mechanism of eosinophilia is probably due to histamine release consequent upon tissue damage caused by the parasite. In the present study, lower eosinophil levels were found in infected Mute and Whooper Swans. These findings are somewhat contrary to what might have been expected of a tissue-dwelling heartworm. Indeed, dogs with *D. immitis* have been shown

to have eosinophilia which decreases around the third to fourth month when young adult worms reach the heart (Weiner and Bradley, 1972).

Creatine phosphate kinase is an enzyme widely associated with heart tissue damage following cardiac infarction in man (Pentecost, 1987). It was thought that similar tissue damage could be caused by *S. eurycerca* adults in swan hearts and therefore elicit a similar enzymic response. Although high levels of creatine phosphate kinase were recorded for some swans, overall the values were not significantly different between infected and non-infected swans. It may be, that once established in heart tissue and producing microfilariae, female nematodes do not cause as much damage as when they initially enter the heart and migrate through the tissue as immature worms.

Even though the total sample size of swans tested in this section was large, the proportion of infected birds was relatively small and only tentative conclusions have been drawn. However, the data as a whole provide a baseline of haematology and clinical chemistry values of swan blood.

CHAPTER DISCUSSION

S. eurycerca is regarded by some researchers as a pathogenic nematode significantly contributing to the deaths of many swans (Seegar, 1977; Irwin, 1973). Even though Irwin (1973) records widespread tissue damage in hearts of infected swans, he states that there was little or no inflammatory response from the host to the parasite. It is thought that tissue damage caused by *Sarconema eurycerca* could have impaired cardiac function in seven Whistling Swans which failed to undertake spring migrations (Holden and Sladen, 1968). MacNeill (1986, pers.comm.) has attributed death due to *S. eurycerca* in numerous swans but in most cases the nematode was found in conjunction with tapeworm and gizzard worm infections.

The extent to which *S. eurycerca* contributes to overall mortality in wild Whistling Swan populations still remains unassessed (Seegar, 1977). From investigations into the histology and blood chemistry of infected British swans, it appears that *S. eurycerca* is not as pathogenic as suggested by Seegar's results. It is difficult to assess whether *S. eurycerca* has contributed to swan deaths in this country. It is felt, however, for swans already afflicted with other diseases an additional infection with *S. eurycerca* might well accentuate their decline.

CHAPTER V

GENERAL DISCUSSION

The role of an insect as an intermediate host of a filarial parasite has two main functions; first, the insect acts as a vehicle of transmission of the parasite from one definitive host to another, thus perpetuating the filarial species; and second, the intermediate host provides an environment in which larval stages of the filariae can develop. *Trinoton anserinum* is the only insect which has been implicated as an intermediate host of *Sarconema eurycerca* in swans. Seegar (1977) first presented evidence for this relationship in American Whistling Swans. The present study has extended his research by examining the insect and filarial parasite in British Swans.

When an intermediate host is included in the life-cycle of a filarial nematode, dispersal of the nematode in order to locate the next host is entirely dependent on the relationship between the intermediate and definitive host. In other words, once inside *T. anserinum* , the final destination of *S. eurycerca* larvae is determined by the louse and its activities on swans.

This study has demonstrated that *T. anserinum* has a close spatial and temporal relationship with the swan and is found only on closely related bird species (swans and geese), all of which are also susceptible to infection by *S. eurycerca* (Seguy, 1944; p.51). When considering mosquito species however, few if any feed solely on one vertebrate species susceptible to a single filarial infection (Smith, 1982). Indeed, it is believed that one of the major factors contributing to the spread of filarial infections by mosquitoes is the probability that an individual mosquito will bite a susceptible vertebrate species at least twice; once, to ingest microfilariae and a second time to return infective larvae to the host (Smith, 1982; Balashov, 1984). Unlike mosquitoes, *T. anserinum* is a wingless insect. It has a continuous association with its host and therefore has more opportunity of taking frequent blood meals from the same host species thus becoming a more effective disseminator of *S.eurycerca* than winged insects are of other filariae.

If the dispersal of *S. eurycerca* is entirely determined by the louse, factors affecting the incidence of lice will also affect the incidence of *S. eurycerca*. Indeed, similarities were found in the incidence of both parasites. Both the louse and nematode were recorded in more juvenile than adult swans, and both parasites were equally distributed between

male and female birds. In addition, geographical distribution of both parasites largely coincided, although fewer swans were screened for *T. anserinum* (N = 387) than for *S. eurycerca* (N = 1128). The overall incidence of *S. eurycerca* recorded in this study was 15.0%, as determined by a new, sensitive technique which has been shown to be more accurate than the methods adopted by Seegar (1977). The incidence of *T. anserinum* (8.3%) is thought to be an underestimate due mainly to the inefficiencies of the sampling technique and the contagious nature of *T. anserinum's* distribution on swans. With contagious populations, the likelihood and degree of underestimation increases with increasing contagiousness and decreasing sample size (Marshall, 1981). It is difficult to speculate what the true incidence of *T. anserinum* might be. If it was estimated at about 10 - 15%, the question then arises as to whether such an incidence of *T. anserinum* could produce the recorded incidence of *S. eurycerca*.

As part of numerous campaigns against insect vectors of debilitating human diseases, researchers have estimated the capacity of insects to transmit disease in an attempt to predict the long-term epidemiological consequences of vector control (Dye and Baker, 1986). The Vectorial Capacity, for example, is the average number of inoculations which arise from one case of disease per unit time. Some components of the Vectorial Capacity however are difficult to measure, such as the proportion of bites of infective flies which lead to the establishment of infection. Other indices require information on the biting rate, the latent period of parasites in flies and incidences of infection. Clearly, in order to predict whether *T. anserinum* could produce the reported incidence of nematode infection in swans, other variables need to be identified and measured.

The incidence of *S. eurycerca* in Whistling Swans was recorded by Seegar (1977) to be slightly higher (20.0%) than in the present study (15.0%). The small disparity of 5.0% could be accounted for in several ways. First, Seegar (1977) used a different method to detect microfilariae in swan blood. The greater efficiency of the sedimentation technique over Seegar's capillary tube method has already been discussed (p.43). If Seegar had adopted the sedimentation technique, he could probably have detected more Whistling Swans with *S. eurycerca* and therefore widened the disparity between the incidences in USA and Britain.

A second explanation for the difference in incidences could relate to host density. Rates of transmission of disease are determined primarily by host density (Wakelin, 1984). An increased efficiency in transmission of *S. eurycerca* by *T. anserinum* could arise from differences between the behavioural and population density factors of Whistling Swans produced by a greater host density compared to swans in Britain. Seegar (1977) reports approximately 100,000 Whistling Swans leave the breeding grounds of Alaska and Canada destined for wintering grounds on the east and west coasts of America. It may be that larger aggregations of Whooper Swans compared to relatively small flocks of a few hundred Mute and Whistling Swans (Minton, 1971; Perrins and Ogilvie, 1971; Bacon, 1980; Brazil, 1980; Hardman and Cooper, 1980; Black and Rees, 1984) provide more opportunities for lice to transfer and therefore distribute *S. eurycerca* to a larger proportion of Whistling Swans.

Further differences between Seegar's results and those in the present study have been identified. Higher infection levels, that is, the number of microfilariae per volume of blood, were found in Whistling Swans than in Mute and Whooper Swans. Several factors could be put forward to explain this. First, the physiology of Whistling Swans might be sufficiently different from Mute and Whooper Swans to make the species more susceptible to the nematode. Whistling Swans, for example, may have more adult nematodes developing in the heart, thus producing more microfilariae than in the other swan species. From the small amount of information available between 2 - 36 adult nematodes per Whistling Swan heart were recorded by Holden and Sladen (1968), 4 - 26 per heart (McDonald, 1974) and 52 in a single Whistling Swan (MacNeill, 1975) compared to between 1 - 13 recorded in Mute Swans in the present study.

A second explanation for the higher infection levels in Whistling Swans might be associated with variations in the ability of *T. anserinum* to develop *S. eurycerca* larvae, one of the major roles of an intermediate host in the life-cycle of the nematode. In other words, *T. anserinum* might be a more effective dispersal agent of *S. eurycerca* in Whistling Swans than on swans in Britain. If more third stage larvae are able to develop in *T. anserinum* and are successfully inoculated into Whistling Swans, then the potential for developing more adult nematodes and thus, more microfilariae is greater. Indeed, Seegar (1977) found significantly more larvae developing in *T. anserinum* than were recorded in the lice in the present study.

What factors could cause *T. anserinum* to develop filariae more effectively on Whistling Swans than Mute and Whooper Swans? The ability to develop filariae has been studied in several species of arthropod, most notably in mosquitoes (Ludham and Jachowski, 1970). Research has shown that larvae of *D. immitis* , the dog heartworm, develop better in one strain of *A. aegypti* in mainland USA than in another strain of the same mosquito species in Hawaii (Gubler, 1966). Similarly, an Indochinese strain of *A. albopictus* which was very susceptible to *D. immitis* from Tonkin, showed complete resistance to development of the same parasite from the Eastern Pyrenees (Galliard, 1937).

Different strains of nematode species can also produce variations in the ability of insects to develop filariae. Nelson (1962) demonstrated one strain of *B. patei* in man is not infective to *A. pembaenis* on the African mainland, but a few miles off shore on Pate Island a different filarial strain is successfully transmitted by the same mosquito species. Furthermore, a strain of *W. bancrofti* is infective and dispersed by *C. fatigans* in Singapore but in neighbouring Malaya, another strain of *W. bancrofti* is much less infective to *C. fatigans* (Wharton, 1966). Furthermore, it has been suggested that differences in susceptibility of mosquitoes to *D. immitis* could be due to different biological strains of *D. immitis* (Kartman, 1954).

Numerous authors have explained the differences in susceptibility of insects to filariae on a genetic basis (Huff, 1941; Trager, 1942; Kartman, 1953; McDonald, 1967). A single gene was found to control the development of *W. bancrofti*, *B. malayi* and *B. pahanji* in the mosquito, *A. aegypti* (McDonald, 1967). One might suppose then if one gene also controls the development of filariae in other insects, it is possible that geographically-separated insect populations could diverge into different strains with slightly different susceptibilities to filariae. The examples given above of such variations have all occurred in island populations or in areas separated by long distances. It is not impossible that similar variations have occurred in populations of *T.anserinum* on American Whistling Swans separated from their British counterparts by the Atlantic Ocean and that different strains of lice exist with variable susceptibilities to *S. eurycerca*. .

Geographical separation may not only produce strains of filariae with varying infectivities, but can also produce filariae with different periodic rhythms. The infective strain of *B. patei* on Pate Island is

non-periodic but the non-infective strain in mainland Kenya exhibits nocturnal periodicity (Wharton, 1960). It is thought that the same mechanism could have produced a nocturnal sub-periodic rhythm of *S. eurycerca* in Whistling Swans (Seegar, 1977) whilst *S. eurycerca* in Mute Swans was shown to exhibit diurnal sub-periodicity in the present study.

It is generally accepted that the periodic cycle of microfilariae is linked to the feeding habits of the intermediate insect host (Hawking, 1962). *D. immitis* exhibits nocturnal periodicity which coincides with the feeding habits of the intermediate mosquito hosts (Kartman, 1953; Otto, 1970). From the limited investigations of the activities of *T. anserinum* , it appears that lice have no specific feeding times and have continuous opportunities to ingest *S. eurycerca*. It might seem unnecessary therefore, for *S. eurycerca* to exhibit any periodicity at all. Indeed, the same question was raised by Gubler (1966) who observed two peaks in the periodic cycle of *D. reconditum* (in dogs), one diurnal and one nocturnal. These did not seem to coincide with the habits of the intermediate host, a flea, which is not known to have a specific feeding time. The sub-periodic rhythm of *S. eurycerca*, with a maximum microfilaremia between 11.00 and 19.00 hours, may represent a compromise between providing maximum opportunity for filarial transmission through an intermediate host with variable feeding times and obtaining some of the benefits of a more favourable physiological environment in the lungs. Further investigations into the feeding habits of *T. anserinum* might throw further light on these relationships.

An insect's role as an intermediate host is undoubtedly an essential part of the life-cycle of any filarial nematode. It is believed that *T. anserinum* performs the two main functions required of an intermediate host namely, it acts as a vehicle of transmission between one swan and another and it provides an environment in which larval stages of filariae can develop. Another essential part of the life-cycle of *S. eurycerca* is its relationship with the swan. Investigations and understanding of 'heartworm' disease in wildfowl have only recently broadened in scope. Two factors could well have delayed current research interest; the first relates to the lack of evidence supporting the nematode as being a major cause of mortality in birds. Secondly, the disease has no observable signs and is therefore more easily overlooked.

The pathogenicity of *S.eurycerca* relates to many interdependent factors: the characteristics of the host (swan), its susceptibility and infectiousness; characteristics of the nematode, its infectivity, virulence and stability.

Susceptibility of swans to *S..eurycerca* has been considered in early discussions (p. 45). The differing susceptibility to *S. eurycerca* of Whistling Swans and Mute Swans was proposed as a possible explanation of disparities between Seegar's work and results of the present study. Susceptibility is a complex issue relating to a variety of acquired and environmental influences (Smith, 1982) and it is sufficient for the purposes of this study simply to mention a selection of these influences (p.178),

Infectiousness of a definitive host defines both the duration of the period when the host is infective and the relative amount of infection it is capable of transmitting. The long-term nature of *S. eurycerca* infections has been demonstrated and some swans have remained infectious for more than 18 months. During this time the microfilariae varied in peripheral blood but there was a continuing opportunity for transmission of the nematode by *T. anserinum*.

The pathogenicity of *S.eurycerca* also depends on several attributes of the nematode itself. The infectivity or dose of the infective larvae required to establish an infection, has been a difficult factor to determine. Difficulties in maintaining laboratory populations of *T. anserinum* prevented the study of third stage larvae and their transmission to the swan. Virulence is another parameter which should be considered in relation to pathogenicity of an organism. Virulence describes the capacity of a pathogen to cause disease in terms of both frequency and severity (Smith, 1982). Virulence is subject to natural selection and it was suggested earlier that a more virulent strain of *S. eurycerca* might infect American Whistling Swans causing a higher incidence and level of infection when compared to British Swans. The stability of *S. eurycerca* outside its definitive host is yet another important epidemiological characteristic. Filarial nematodes like *S. eurycerca* cannot survive outside the definitive host and require an intermediate host for their survival. As long as *T.anserinum* is part of the life-cycle, *S. eurycerca* can remain infective within the insect and its stability is assured.

Investigations into the pathology of *S. eurycerca* have shown a limited immunologic response from the swan to the worm. Slight increases in inflammatory cells were recognized in infected heart tissues and some eosinophilic deposits were noted around degenerating nematodes. Raised blood eosinophil levels, normally associated with chronic parasitic infections (Archer, 1963) were not recorded in infected swans in this study. On the whole, both the effects of *S. eurycerca* on the swan and the response of the swan to *S.eurycerca* were minimal. Theories have been postulated that during evolution there is always a tendency towards a mutual adjustment between two species, the parasite reducing its pathogenicity and immunogenicity so as to elicit weaker host responses. The result is a reduced antigenic disparity so that parasite control is effected without any pathological change (Wakelin, 1984).

From the evidence gathered here it could be that *S.eurycerca* might have undergone such a process and become a "successful" parasite. In such an 'adapted' host-parasite relationship, any immunological response from the host is directed towards control of the parasite burden rather than to cause complete elimination of the infection (Dineen, 1963).

To conclude, the present thesis had three main aims:

1) to determine the incidence and infection levels of *Sarconema eurycerca* in British swans;

2) to examine the relationships between *Trinoton anserinum, Sarconema eurycerca* and the swan; and

3) to investigate the pathological effects of *S. eurycerca* on swans.

The substance of the investigation has shown that:

1) the overall incidence of *S. eurycerca* in British swans was 15.0% and infection levels ranged from 1 - 46 microfilariae per 0.25 cm^3 blood;

2) *T. anserinum* has been shown to have all four attributes of an intermediate host of a filarial nematode and fulfills the role of intermediate host in the life-cycle of *S. eurycerca* in swans;

3) *S. eurycerca* elicits a limited immunological response and causes only slight mechanical tissue damage in infected swans. There is little evidence to suggest the nematode has a significant effect on swan mortality.

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APPENDICES

Appendix 1 : May-Grünwald and Giemsa staining procedure

Dried blood smears were stained in vertical staining jars. Smears were stained first with undiluted May-Grünwald solution for 3 minutes then with May-Grünwald diluted with buffered distilled water (1:1) pH 6-8 for 2 minutes. Smears were rinsed briefly in buffered distilled water and stained for 10 minutes in Giemsa, diluted with buffered distilled water (1:6 parts water). The slides were rinsed briefly under tap water and allowed to air dry. Once dried, the preparations were cleared in xylene for 5 minutes and mounted with a covership in Hystomount.

APPENDIX 2 : Z scores for statistical comparison of weights of infected and non-infected swans

SPECIES, AGE, SEX OF SWANS	INFECTED vs NON-INFECTED	
Mute, adult ♂	1.76	n/s
Mute, adult ♀	1.32	n/s
Mute, juvenile ♂	0.156	n/s
Mute, juvenile ♀	0.703	n/s
Whooper, adult ♂	0.881	n/s
Whooper, adult ♀	3.425	$p < 0.01$
Whooper, juvenile ♂	1.03	n/s
Whooper, juvenile ♀	1.74	n/s

APPENDIX 3 : REFERENCES TO STUDIES CONCERNED WITH FEEDING OF MALLOPHAGA

DATE	AUTHOR	AMBLYCERA (blood & feathers)	ISCHNOCERA (feathers)	GENERA
1921	Barber, B.A.		+	*Nirmus vulgatus* (K)
				Docophorus communis (N)
1926	Waterston, J.		+	*Gonoides bicuspidatus* (P)
			+	*Esthiopterum monile* (G)
1933	Wilson, F.H.	+		*Menopon stramineus* (N)
1934	Martin, M.		+	*Columbicola columbae* (L)
1934	Wilson, F.H.		+	*Lipeurus heterographus* (N)
1943	Crutchfield & Hixson	+		*Menopon stramineus* (N)
		+		*Menacanthus stramineus*(N)
		+		*Menopon gallinae* (L)
			+	*Gonoides gigas* (Ta)
			+	*Goniocotes hologaster* (N)
			+	*Lipeurus caponis* (L)
		+		*Menacanthus sp.* (N) (fowl body louse)
1953	Waterhouse, D.	+		*Eomenacanthus stramineus* (N)
			+	*C.Columbae* (L)
			+	*Lipeurus caponis* (L)
1957	Clay, T.	+		*Ricinus sp.* (De)
1971	Nelson, B. & Murray, M.	+		*Hohorstiella lata* (P)
		+		*Colocephalum turbinatum* (D)
			+	*Campanulotes bidentatus copar* (B)
			+	*C. columbae* (L)
1976	Eveleigh, E. & Threlfall, W.		+	*Saemundssonia fraterulae* (O)
			+	*Cummingsiella helgovauki* (G)
		+		*Austromenopon nigropleurum* (D)
		+		*Austromenopon uriae* (T)
1977	Seegar, W.S.	+		*Trinoton anserinum* (F)
1988	Cohen, S.	+		*Trinoton anserinum* (F)

N = Nitzsch. K = Kellogg P = Piaget Ta = Taschenberg
L = Linnaeus T = Timmerman D = Denny De = Deeger
G = Geibel O = Overgaard F = Fabricius

Appendix 4 : Chromagen test for iron in blood

(Method provided by M. Stotherd, Fisons plc, Pharmaceutical Division, Bakewell Road, Loughborough, Leics.)

Materials

1) Chromagen - 2,2' - azino-di- (3-ethyl benzthiazoline suphonic acid) or ABTS (Cat. No. A1838, Sigma Chemical Co. Ltd., Fancy Road, Poole, Dorset.):

2) Glacial acetic acid SLR;

3) L-ascorbic acid SLR 1 g per 100 cm^3;

4) Hydrogen peroxide, 20 volumes.

Preparation of reagent

ABTS (20 mg) was dissolved in 50 cm^3 of distilled water. Glacial acetic acid (50 cm^3) was added and mixed. Ascorbic acid solution was added, dropwise, to remove the green colouration. The chromagen reagent was stored in a dark bottle at room temperature.

Method

The test sample was placed in a plastic bottle and one drop of hydrogen peroxide was added followed by one drop of chromagen reagent. A blue-green colour developed if blood was present.

Appendix 5 : Z scores for statistical comparison of crop contents of age-classes of *T. anserinum*

Size class comparison

Crop contents	Ad♂:Ad♀	Ad:N_3	Ad:N_2	Ad:N_1	N_3:N_2	N_3:N_1	N_2:N_1
Feathers	0.2675 N/S	0.084 N/S	0.2287 N/S	5.620 p<0.01	0.1085 N/S	6.160 p<0.001	6.475 p<0.001
Blood	0.5745 N/S	0.7448 N/S	1.1780 N/S	6.370 p<0.001	0.2932 N/S	2.68 p<0.01	3.080 p<0.01
Feathers and blood	0.5324 N/S	0.180 N/S	0.757 N/S	2.596 p<0.01	0.75 N/S	2.2 p<0.05	1.50 N/S

Appendix 6 : Rapid Fixation Technique for Preparation of adult nematodes for Scanning Electron Microscopy

Nematodes were stored in formalin solution (2.0%) and transferred into sodium phosphate buffer (0.05M pH 6.8) for 24 hours. Specimens were then fixed in gluteraldehyde (3.0%) for 1½ hours at room temperature. The nematodes were washed in five complete changes of buffer for 15 minutes each to remove the fixing agent. Specimens were post-fixed in osmium tetroxide (2.0%) for 2 hours at room temperature. Excess osmium was washed from the tissues with six complete changes of buffer for 10 minutes each.

Dehydration of specimens was performed using a fluid that is miscible with the critical point drying solution. A six-graded series of acetone in water was used to dehydrate the nematodes each for 15 minutes. Specimens were placed in fresh solutions of 100% acetone three times.

Following dehydration the nematodes were critically point dried to ensure removal of all liquid from the specimen.

The nematodes were coated with a thin layer of gold and examined using a Scanning Electron Microscope.

Appendix 7 : Procedure for preparation of heart tissue section

1. Processing of tissue:

   a. Tissue was fixed in 10% buffered formalin.
   b. Industrial methylated spirits (IMS) - 70% for 90 minutes
   c. " " " - 70% for 90 minutes
   d. " " " - 90% for 90 minutes
   e. " " " - 95% for 90 minutes
   f. Absolute ethanol - 100% for 90 minutes
   g. " " - 100% for 90 minutes
   h. Chloroform and ethanol (50:50 ratio)- for 90 minutes
   i. " " " (75:25 ratio)- for 90 minutes
   j. Absolute chloroform - for 90 minutes
   k. " " - for 90 minutes
   l. Wax heated to 60° and vacuumed - for 90 minutes
   m. " " " " - for 60 minutes

2. Tissue embedding:

   Wax was moulten on leaving stages 'l' amd 'm' in the fixing process and was transferred into a metal container, (2.5 cm x 2.5 cm). The container was placed on a warm plate whilst the tissue was orientated in the wax for sectioning. A plastic top was put on the metal container which later formed the embedding block from which the tissue was sectioned. The plastic top was filled with moulten wax and placed on a cold plate to set for 15 minutes.

3. Microtome sectioning:

   The plastic container was placed in ice for 10 minutes to produce the best conditions for section cutting. It was secured in a microtome and the wax was trimmed until the area containing the tissue was reached. Sections of tissue (approximately 4 μm wide) were cut and floated in a water bath to flatten out. Cleaned, labelled slides were immersed in the water and brought under each wax section to lift them out. Slides with sections were drained and dried on a heater for a few minutes, then heated further to remove the wax.

4. Staining tissue sections:

   a) Haematoxylin and Eosin

   Reagents: Gill's haematoxylin
   Saturated sodium tetraborate
   1% Eosin in distilled water.

Method: Take sections to water - place in xylene for 5-10 minutes, wash in fresh xylene, agitate for 1 minute in ethanol, agitate in IMS (two changes) for 1 minute, wash well in tap water. Stain nuclei in Gill's for 4-5 minutes. Wash in tap water. Wash in 1% acid alcohol to remove excess background stain. Blue in saturated sodium tetraborate for about 30 seconds. Wash in tap water. Counterstain in 1% Eosin for about 30 seconds.

Results: Muscle fibres, keratin, cytoplasm, connective tissue and red blood cells stain varying shades of red and pink.

b) PAS and Alcian Blue method

Method: Take sections to water (see (a)). Stain sections in Alcian blue for 25 minutes. (This step can be omitted if PAS only is required.) Wash in running water for 5 minutes. Ox idise in 1% periodic acid for 5 minutes. Wash in running water for 5 minutes. Rinse in distilled water. Stain in 50% Schi ff's reagent for 10-20 minutes. Wash in tap water for 10 minutes. Stain nuclei in haematoxylin for 2 minutes. Wash in tap water. Wash in 1% acid alcohol and rinse again in water. Blue in borax for 0.5 minutes then wash in water. Dehydrate in alcohol then stain in tartrazine/cellosolve mixture. Dehydrate - that is, the reverse process of taking to water.

Results:		
	Acid and most well sulphated mucopolysaccharides	Blue
	PAS positive material	Red
	Nuclei	Blue
	Background	Yellow

c) Perl's Prussian Blue for Haemosiderin

Method: Take sections to water. Rinse in distilled water. Make up stain fresh as follows: equal parts of 2% potassium ferrocyanide and 2% hydrochloric acid for 30 minutes. Wash in distilled water. Counterstain in 1% netural red. Wash in distilled water to remove excess stain. Dehydrate - that is reverse process of taking to water.

Results:		
	Haemosiderin and ferric salts	Deep Blue
	Nuclei	Red.

APPENDIX 8 :

Occurrence of the mite, *Myialges trinotoni* on *T. anserinum*

During the collection and investigation of *Trinoton anserinum* mites were observed attached to some of the specimens. The mites were invariably secured to the integument of the louse's thorax in a lateral position between the legs. Each mite was surrounded by approximately 20-40 oval-shaped eggs which remained firmly secured to the insect.

Out of 326 *T. anserinum* specimens, twelve (3.7%) had mites attached. Eleven lice with mites were adults (4♀ , 7♂) and one was a third instar nymph.

The mites were identified as *Myialges trinotoni* (courtesy Professor Alex Fain) and it is the first time the mite has been recorded on *T. anserinum* from the Mute Swan (*Cygnus olor*). *M.trinotoni* was previously recorded on *Trinoton* sp. from *Plectropterus gambensis* .*

*(Spur-winged Grouse) Thompson (1936)