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Scanning electron microscopy of the cephalic structures of Pneumospiruridae

(Thelazioidea - Nematoda)

Revision of the family

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Summary.

In a study with the scanning electron microscope (SEM) five different types of cephalic structure were found in five species of Pneumospiruridae recorded in Israel. The description, in the literature, of a sixth type, based on a study with the light microscope, was also considered in evaluating the results. By comparing descriptions and drawings of the known Pneumospiruridae with the SEM micrographs the species in the family were grouped into two genera : 1. *Vogeloides* Dougherty, 1952 in which the form of the cephalic structures could be correlated with the structure of the oesophagus ; 2. *Metathelazia* Skinker, 1931 in which the cephalic structures could not be correlated with any other morphological or anatomical character. The species included in the genus *Metathelazia* were therefore grouped on the basis of the form of cephalic structures in 5 types : *booliati, multipapillata, acomysi, capsulata* and *rodentium.*

The genus *Pneumospirura* Wu and Hu, 1938 is considered a subjective synonym of *Metathelazia.*

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Résumé.

Etude des structures céphaliques des *Pneumospiruridae* (*Thelazioidea* - *Nematoda*) à l'aide du microscope à balayage. Révision de la famille.

Les cinq espèces de Pneumospiruridae récoltés en Israël ont chacune une structure céphalique particulière. Un 6e type de structure est connu par ailleurs.

Les espèces sont scindées en deux genres : 1. *Vogeloides* Dougherty, 1952, où il y a corrélation entre la structure céphalique et l'anatomie de l'œsophage ; 2. *Metathelazia* Skinker, 1931 (= *Pneumospirura* Wu et Hu, 1938), où l'on ne trouve pas de corrélations entre les structures des différents organes. La création de 5 genres particuliers compliquerait la systématique. Les 13 espèces décrites sont donc simplement réparties en 5 groupes.

Introduction

The nematode family Pneumospiruridae contains species parasitic in the respiratory system (lungs and/or bronchi) of mammals. The main hosts appear to be the Carnivora in which 17 species were described : 4 species in Canidae (Gerichter, 1948 ; Price, 1928 ; Romanov, 1961 ; Sadychov, 1953 ; Schaldybin, 1950), 6 — in Mustelidae (Biocca and Chabaud, 1952 ; Chabaud, 1949 ; Gerichter, 1948 ; Singh and Pande, 1966 ; Wu and Hu, 1938), 2 — in Viverridae (Biocca and Chabaud, 1963 ; Vuylsteke, 1956) and 5 — in Felidae (Alwar, Lalitha and Seneviratna, 1958 ; Chabaud and Biocca, 1950 ; Davtian, 1933 ; Skinker, 1931 ; Vogel, 1928). From other hosts 8 species were described : 4 — from monkeys (von Linstow, 1879 ; Travassos, 1921 ; Vogel, 1928), 1 - from Erinacidae (Gerichter, 1948), 1 from Tupaiidae (Quentin and Krishnasamy, 1977) and 2 — from Muridae (Greenberg, 1969 ; Wertheim and Giladi, 1977).

The group has an almost worldwide though sporadic distribution. Only 5 species were recorded from the American continent (Price, 1928 ; Skinker, 1931 ; Travassos, 1921 ; Vogel, 1928). Most of the species were found in Africa (Biocca and Chabaud, 1952 ; Biocca and Chabaud, 1963 ; Chabaud, 1949 ; Chabaud and Biocca, 1950 ; Vuylsteke, 1956) and in a wide belt beginning in south-east Europe [Moldavia (Schaldybin, 1950), southwest Bulgaria (Kanchev, 1961), Armenia (Davtian, 1933), Uzbekistan (Miminov, 1968), Azerbijan (Sadychov, 1953)] and continuing through south and southeast Asia [India (Alwar, Lalitha and Seneviratna, 1958 ; Singh and Pande, 1966), Hainan (Wu and Hu, 1938), Malaysia (Quentin and Krishnasamy, 1977)] to Indonesia [Celebes (Vogel. 1928)]. Five species have been recorded from Israel.

Gerichter (1948) described *Metathelazia multipapillata* from the lung parenchyma of *Erinaceus europeus* and *E. auritus ; M. capsulata* from the bronchi of *Meles meles, Vulpes nilotica* and *Vormela peregusna* and *M. œsophagea* from the bronchi of *Herpestes ichneumon. M. acomysi* was found in *Acomys cahirinus* (Greenberg, 1969) and

Pneumospirura rodentium in *Gerbillus dasyurus* and *Meriones crassus*, both in the lung parenchyma (Wertheim and Giladi, 1977).

All the Pneumospiruridae show similar morphological and anatomical characters, however, they differ strikingly in the structure of the cephalic region. Gerichter (1948) was aware of this variation in the three species studied but considered the genus uniform in other characters and claimed that the differences in cephalic structure did not justify establishing a separate genus for each species, as suggested by Dougherty (1952).

Since the cephalic structures are considered of primary importance in the taxonomy of nematodes (Chitwood and Wehr, 1934), a study of the perioral region of the five pneumospirurids in our collection with scanning electronmicroscope was made.

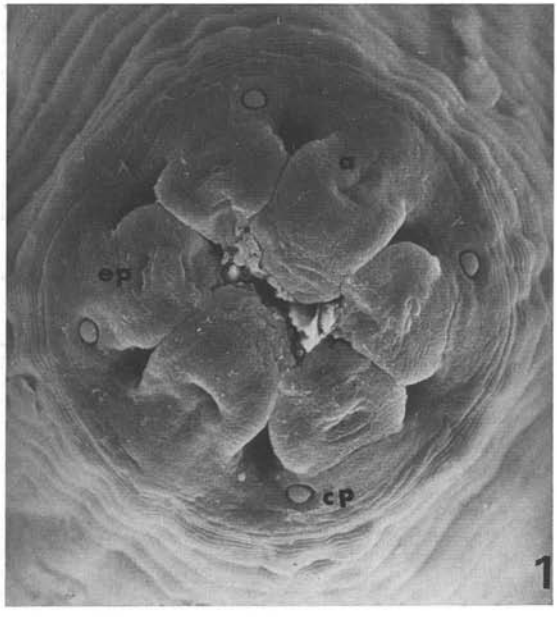
Materials and methods

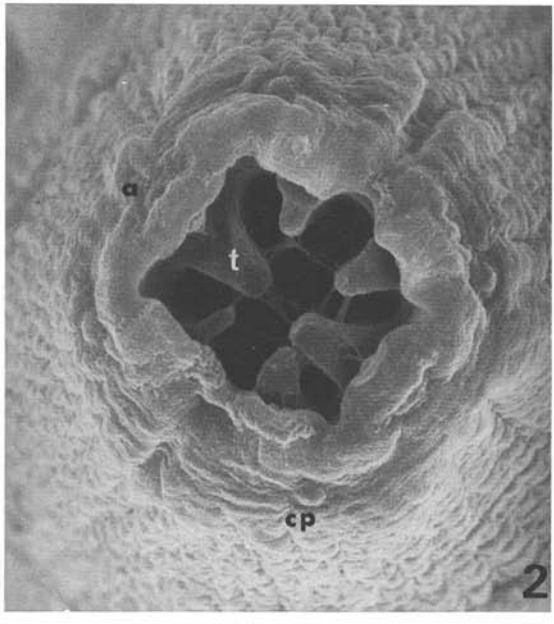
Two groups of samples were studied : Nematodes recovered from rodents, preserved for from 6 to 10 years, originally fixed in hot AFA and stored in this solution. Samples of worms described by Gerichter (1948), fixed and stored in 70 % ethanol for over thirty years. For light-microscopy studies the specimens were cleared in lactophenol. For scanning electron microscopy specimens were dehydrated in an ascending series of ethanol solutions 70-80-96 %. The anterior 4-5 mm of each worm were cut off and placed in small cellulose extraction thimbles immersed in 100 % ethanol overnight (Madden and Tromba, 1976). The thimbles were transfered to the boat of the critical point dryer containing absolute ethanol. The dried fragments were mounted « head on view » on metal stubs with double sided sellotape, gold-coated and examined with a Cambridge S4-10 scanning electromicroscope. Cleaning the worms of adhering host tissue debris by sonication was not made to avoid damage.

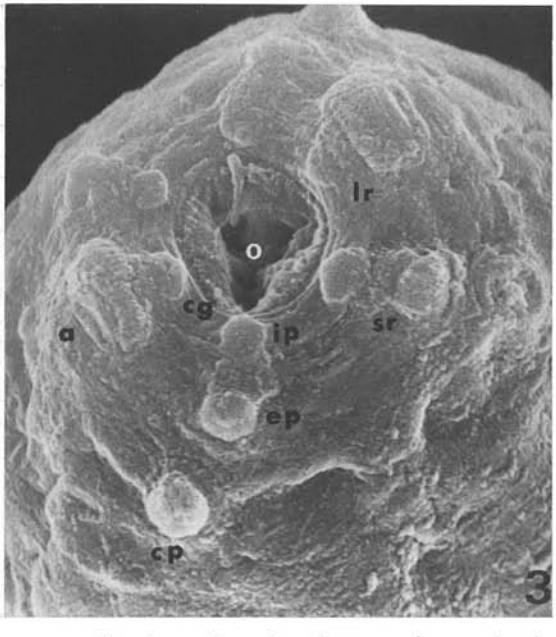
Results and discussion

Processing for SEM

Because of the small number of specimens available and their brittleness after prolonged storage (some for over 30 years), preliminary tests of the preparative techniques for scanning electron microscopy were made with two species of nematodes available in large numbers : *Mastophorus muris* from experimental infections of laboratory rats fixed in AFA immediately after recovery and *Streptopharagus kutassi* from field rodents preserved in AFA for 4 to 5 years (*fig. 1, 2*). Passage of worms in an ascending series of ethanol followed by critical point drying (CPD) gave sharper and clearer pictures than the « glycerine » technique (Allison, Ubelaker, Webster and Riddle, 1972) and CPD in alcohol proved superior to CPD in amylacetate (Madden and Tromba, 1976). Even with the method most suitable for the fresh material the labial papillae on the micrographs of the pneumospirurids were not always clearly visible and the cuticle appeared folded and shrunken. However, information from the SEM micro-







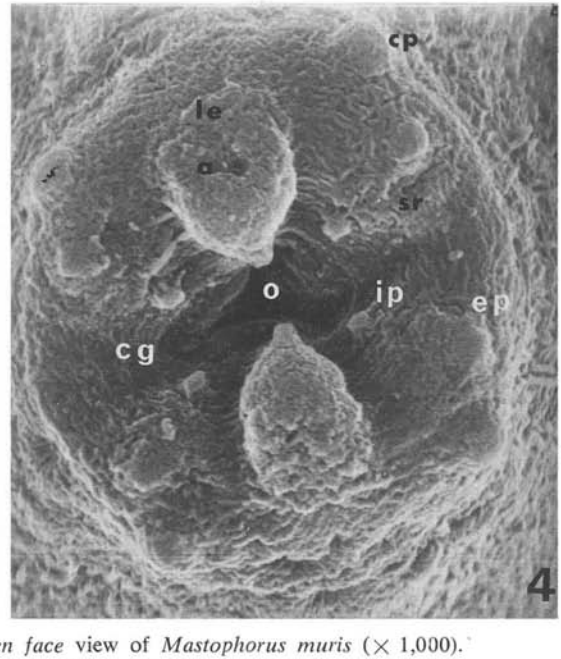


FIG. 1. — Scanning electron micrograph of *en face* view of *Mastophorus muris* (× 1,000).

FIG. 2. — Scanning electron micrograph of *en face* view of *Streptopharagus kutassi* (× 7,200).

FIG. 3. — Scanning electron micrograph of *en face* view of *Metathelazia multipapillata* (× 13,000).

FIG. 4. — Scanning electron micrograph of *en face* view of *Metathelazia acomysi* (× 13,500).

graphs combined with observations made with the light microscope (LM) provided a clear picture of the cephalic structures of each species.

The SEM micrographs of the Pneumospiruridae confirmed observations made with the light microscope on the variability of the cephalic region in this group (Dougherty, 1952 ; Gerichter, 1948). Each of the 5 species studied showed a different type of oral and perioral structure.

In *Metathelazia multipapillata* the triradiate mouth is formed by a plate-like structure arising from a demarcated circular groove at the apex. The circumoral elevation is thickened forming six (4 submedian and 2 lateral) ridges. The 6 papillae of the internal circle are situated on the proximal ends of the ridges. The distal ends of the submedian ridges bear each a papilla of the external circle. The lateral papillae of the external cycle are lacking. The large, knoblike distal ends of the lateral ridges bear the amphibial glands. The cephalic papillae are situated posterior to the submedian ridges (*fig. 3*).

The mouth of *M. acomysi* is triradiate and formed by a plate-like structure resembling that of *M. multipapillata*. The submedian ridges on the circumoral elevation are small. The lateral ridges are prominent lip-like formations, overhanging the mouth, broad at the base and tapering at the proximal end. The 6 papillae of the internal circle are situated at the proximal end of the ridges. The 4 papillae of the external circle are situated on the submedian ridges. The lateral ridges bear the amphibial glands. The cephalic papillae are situated posteriorly between the submedian and lateral ridges (*fig. 4*). In some micrographs the submedian ridges have the form of triangular, tooth-like structures (*fig. 5*). Figures *6 a* and *b* illustrates the appearance of this type of cephalic structure in LM.

The oval mouth of *M. capsulata* is partly covered by two lateral, broadly oval lobe-like — and 4 submedian, small, tooth-like structures resulting from extension and thickening of the circumoral elevation. Four prominent papillae of the external circle are situated on the submedian teeth. The amphids form elongated slits on the posterior margin of the lateral lobes (*fig. 7*). The papillae of the internal circle and the cervical papillae are not visible on this micrograph. Both types of papillae were illustrated by Gerichter (1948) in specimens examined with LM.

The mouth of *Pneumospirura rodentium* is surrounded by 6 tooth-like, triangular lobes, the lateral lobes slightly larger than the submedian (*fig. 8*). Because of the shranken cuticle papillae are not seen on this micrograph. When examined with LM four external papillae and 4 cephalic papillae were seen (Wertheim and Giladi, 1977). The internal papillae were not observed with LM and are not seen in micrograph, however, it is possible that the round structure observed on the margin of the mouth close to the left subventral lobe, represent an internal papilla (*fig. 8*).

The oval, dorso-ventral mouth of *M. oesophagea* is flanked on each side by 3 almost rectangular prominent elevations formed by thickening of the circumoral margin. The submedian elevations bear each a papilla of the external circle and a cephalic papilla. Small pits on the outer surface of the lateral elevations indicate the location of the amphids (*fig. 9*). The papillae of the inner circle are lacking in

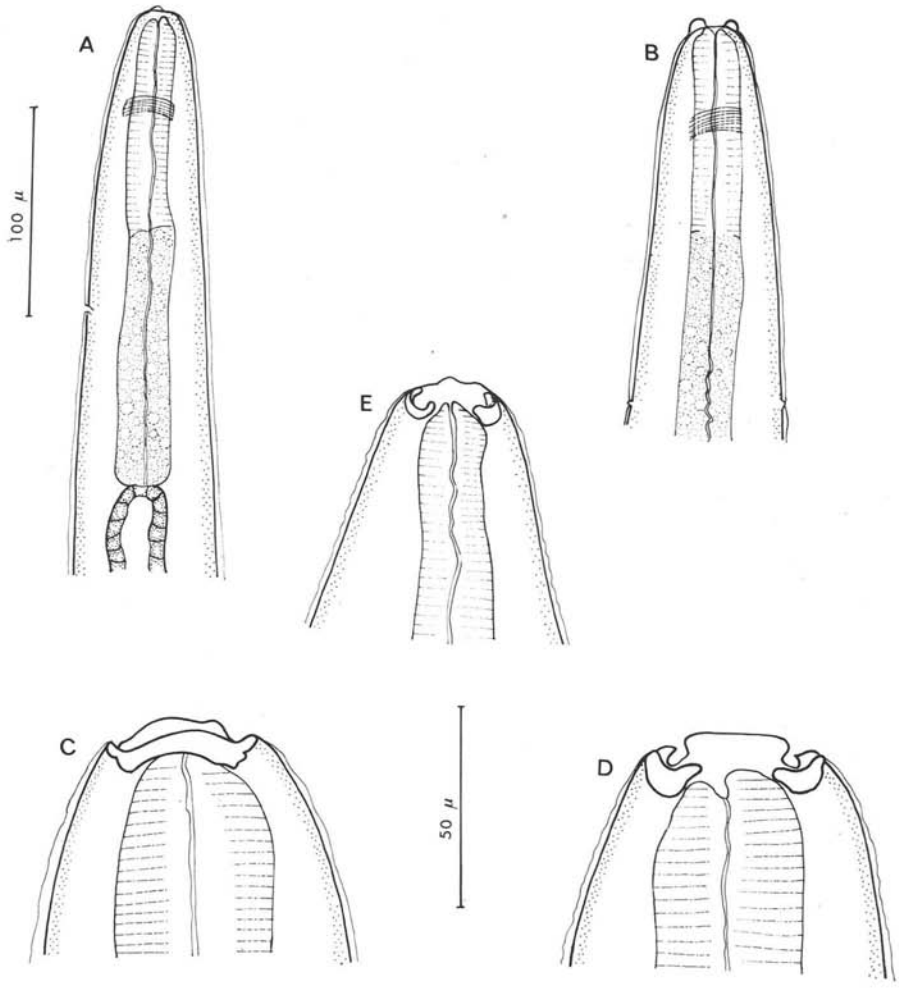
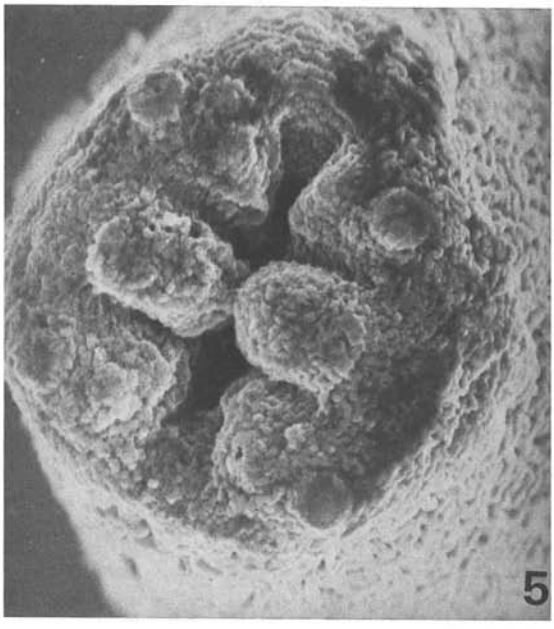
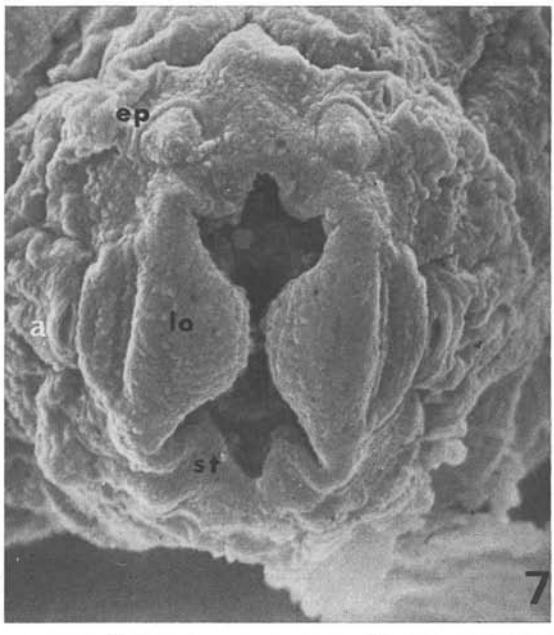


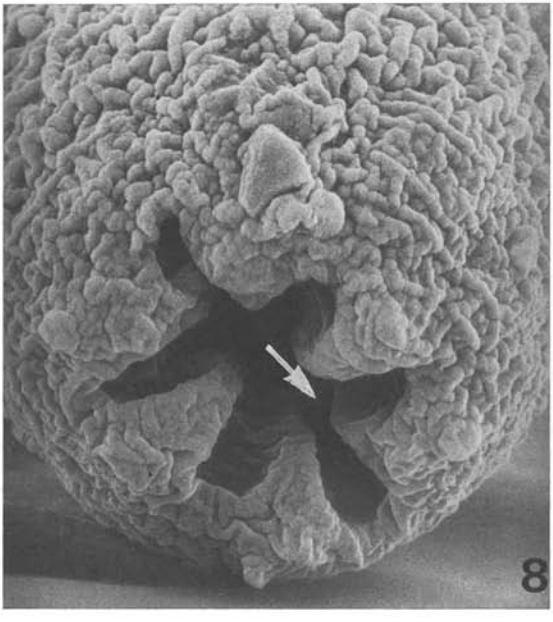
FIG. 6. — *Metathelazia acomysi* female : A) lateral view of anterior end ; B) ventral view of anterior end. *M. capsulata* female ; C) lateral view of anterior end ; D) lateral view of anterior end, deeper optical section ; E) ventral view of anterior end. A, B, E : 100 µ ; D, C : 50 µ.

this micrograph, they were, however, illustrated in specimens examined with LM (Gerichter, 1948).

The five pneumospirurids studied represent five different types of cephalic structure characterized by thickenings, in various forms and sizes, of the cuticle (?) surrounding the actual oral opening.







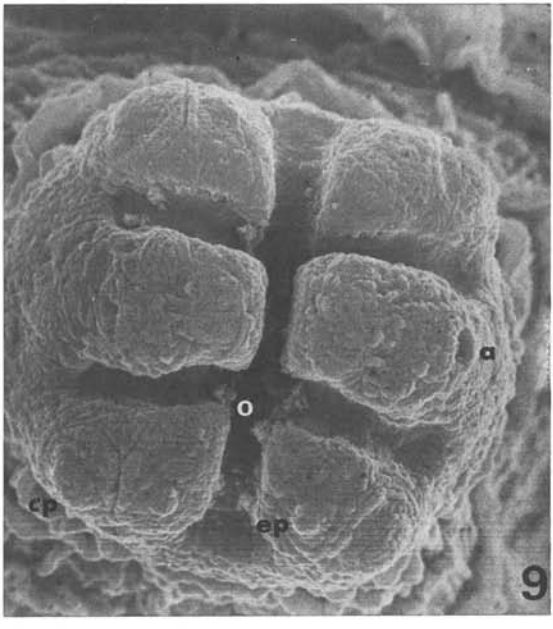


FIG 5. — Scanning electron micrograph of *en face* view of *Metathelazia acomysi* (× 14,000).

FIG. 7. — Scanning electron micrograph of *en face* view of *Metathelazia capsulata* (× 7,200).

FIG 8. — Scanning electron micrograph of *en face* view of *Pneumospirura rodentium* (× 7,200).

FIG. 9. — Scanning electron micrograph of *en face* view of *Metathelazia œsophagea* (× 9,000).

A sixth type of cephalic structure was recently described by Quentin and Krishnasamy (1977) for *M. booliati*. The mouth is triangular, flanked by six flat ridges similar to those seen in *M. multipapillata*. The papillae of the internal circle have the form of small denticles. There are 6 papillae of the external circle as compared to 4 in *M. multipapillata ;* the 2 additional papillae are very small and are situated on the lateral ridges close to the amphids.

The close relationship between *M. booliati* and the family Seuratidae enables a better understanding of the evolutionary trend in the cephalic structures of the Pneumospiruridae. The evolution of the structures flanking the mouth seems to have progressed along a line of hypertrophy of 6 perioral radially arranged ridges, beginning with the lateral ones.

In *M. booliati,* the most primitive type, the triradiate mouth is flanked by 6 flat ridges ; there are 6 papillae of the internal cycle and 6 of the external cycle. A similar structure is found in *M. papillata* in which, however, the lateral papillae of the external cycle are lacking. In *M. acomysi* and *M. capsulata* the mouth remains triradiate or becomes oval ; the lateral ridges become thickened. In *M. rodentium* all 6 circumoral ridges become thickened, forming tooth-like projections ; the papillae of the internal circle disappear or move deeper to surround the actual mouth opening. The six ridges attain maximum development on *M. oesophagea* in which the papillae of the internal circle are no longer visible.

By comparing descriptions and drawings of the Pneumospirurids in the literature with the forms studied the species in the family can be arranged in 6 groups corresponding to the above 6 types of cephalic structure. Each of these groups could perhaps be considered a separate genus or subgenus. However, in most cases the type of the cephalic structure could not be correlated with any other character (e.g., buccal capsule, rugosities or coeca in the intestinal wall, the number of caudal papillae in the male, etc.) neither with the type of host or with geographical distribution. The family appears to represent a collection of archaic forms without obvious phylogenetic relationships among the species.

Only in the group of species including *M. oesophagea* could the form of the cephalic structures be correlated with another anatomical character namely the oesophagus. Whereas in all other groups the oesophagus is a somewhat club-shaped structure in which the differentiation between the muscular and glandular is not clearly demarcated (similar to the oesophagus of Seuratidae), in the *oesophagea* group the oesophagus becomes divided into an anterior, narrow muscular part and a wide, glandular posterior part.

The characters used to separate the 3 existing genera in the family, vise *Vogeloides* (Orlov, Davtian and Lubimov in Skriabin, 1933) Dougherty, 1952, *Metathelazia* Skinker, 1931 and *Protospirura,* Wu and Hu, 1938, were the presence or absence of a well developed, buccal capsule and the size of the lips (Dougherty, 1952 ; Chabaud, 1975). Actually, a weakly developed buccal capsule is present only in *M. servalis* Chabaud and Biocca (1950), in all other species this structure was not demonstrated. The thick buccal capsule of *M. capsulata,* illustrated by Gerichter (1948), is actually

a drawing of the submedian teeth and the lateral lobes in optical section (*fig. 6 C, D, E*).

It is therefore proposed to leave in the family two genera *Vogeloides* and *Metathelazia.*

In the genus *Vogeloides,* characterized by cephalic structure of the *oesophagea* type, an oesophagus divided into a narrow anterior and wide posterior part and the absence of buccal capsule, the following species are included :

V. ascaroides (V. Linstow, 1879), Dougherty 1952, possibly a synonym of *V. massinoi* (Davtian, 1933), Dougherty, 1932.
V. cynopitheci (Vogel, 1928), Dougherty, 1952.
V. oesophageus (Gerichter, 1948), Dougherty, 1952).
V. lerouxi (Biocca and Chabaud, 1952), Dougherty, 1952.
V. congolense (Vuylsteke, 1956), n. comb.
V. chabaudi (Singh and Pande, 1956), n. comb.
V. ramanujacharii Alwar, Lalitha and Sarevirathna, 1958.

All other species are considered as belonging in the genus *Metathelazia* (= *Pneumospirura*) in which 5 types of cephalic structures can be distinguished :

1. *Type booliati* with *M. booliati* Quentin and Krishnasamy, 1977.

2. *Type multipapillata* with :

M. californica Skinker, 1931.
M. felis (Vogel, 1928) n. comb.
M. multipapillata Gerichter, 1948.
M. petrovi Schaldybin, 1950.
M. skriabini Sadychov, 1953.
M. vulpis Romanov, 1961.

3. *Type acomysi* with :

M. exilis (Biocca and Chabaud, 1952) n. comb.
M. acomysi Greenberg, 1969.

4. *Type capsulata* with *M. capsulata* Gerichter, 1948.

5. *Type rodentium* with :

M. hainanensis (Wu and Hu, 1938) n. comb.
M. servalis Chabaud and Biocca, 1950.
M. rodentium (Wertheim and Giladi, 1977) n. comb.

The genus *Pneumospirura* Wu and Hu 1938 thus becomes a subjective synonym of *Metathelazia* Skinker, 1931. The family name Pneumospiruridae is retained according to Article 40 of International Code of Zoological Nomenclature, London, 1961.

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Abbreviations : a, amphids ; cg, circumoral groove ; cp, cephalic papillae ; ep, papillae of the external circle ; ip, papillae of the internal circle ; le, lateral elevations ; lo, lateral lobes ; lr, lateral ridges ; o, oral opening ; sr, submedian ridges ; st, submedian teeth, t, teeth-like projections.

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