Fundam. appl. Nematol., 1997, **20** (3), 213-228

Terrestrial nematodes from the Galápagos Archipelago. 7. Description of *Tylocephalus nimius* sp.n. and new data on the morphology, development and behaviour of *T. auriculatus* (Bütschli, 1873) Anderson, 1966 (Leptolaimina : Plectidae)

Paul De Ley and August Coomans

Instituut voor Dierkunde, Universiteit Gent, Ledeganckstraat 35, 9000 Gent, Belgium.

Accepted for publication 28 March 1996.

Summary – *Tylocephalus nimius* sp.n. is described from Isla Santa Cruz and Isla Floreana. It has cornua measuring 12-14 µm and cervical expansions extending well posterior to the anteriormost somatic setae. The known species *T. auriculatus* is described from soil and from a derived culture. On agar, *T. auriculatus* swings its lip region dorsally and ventrally during feeding, in a continuously oscillating sweeping motion independent of body locomotion. The hypothesis is proposed that this behaviour is functionally linked to the expanded and flattened lip region, and that *T. auriculatus* and other Wilsonematinae are specialized in sweeping up bacteria attached to flat microsurfaces in soils. Furthermore, morphological and developmental observations suggest that the species has only three juvenile stages, with marked metamorphosis of the lip region during the first and second moult.

Résumé – ***Nématodes terrestres de l'archipel des Galapagos. 7. Description de* Tylocephalus nimius *sp.n. et nouvelles données sur la morphologie, le développement et le comportement de* Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966 (Leptolaimida : Plectidae)*** – Description est donnée de *Tylocephalus nimius* sp.n., provenant des îles de Santa Cruz et Floreana, caractérisé par des " cornes " mesurant 12-14 µm et des expansions céphaliques dépassant considérablement les soies somatiques les plus antérieures. *T. auriculatus* est redécrit sur des spécimens provenant d'échantillons de sol et d'un élevage dérivé sur plaque d'agar. Pendant la prise de nourriture de *T. auriculatus*, la région labiale se balance dans le plan dorso-ventral dans un mouvement oscillant, continu et indépendant de la locomotion du corps. L'hypothèse est formulée que cette espèce et les autres Wilsonematinae se sont spécialisés à balayer les bactéries collées aux surfaces planes des éléments microscopiques du sol. De plus, les observations morphologiques et ontogéniques suggèrent que cette espèce ne passe que par trois stades juvéniles, avec une métamorphose marquée de la région labiale pendant les première et deuxième mues.

Key-words : behaviour, development, Galápagos, Nematoda, SEM, taxonomy, *Tylocephalus*.

This paper presents new data on the taxonomy, development and behaviour of the genus *Tylocephalus* Crossman, 1933, based on two species found on the Galápagos. One of these is a new species, characterized by longer cornua and cervical expansions than any known species. In addition, we also found the cosmopolitan species *Tylocephalus auriculatus* (Bütschli, 1873) Anderson, 1966, which has already been reported (but not described) by Gadéa (1977) from mosses in forests on Isla Isabela and Isla Santa Cruz. The species was previously cultured by Crossman (1933), but no details were provided on its behaviour or development. We established new cultures, in which individuals were observed to reach adulthood after only three moults instead of the usual four. Omission of a moult has hitherto only been reported in some *Longidorus* and *Xiphinema* species (Yagita, 1975; Shishida, 1983; Alkemade & Loof, 1989; Halbrendt & Brown, 1992). It could perhaps also occur in some Trichodoridae (see review in Decraemer, 1995) and in *Odontopharynx longicauda* de Man, 1912, as suggested by the work of Chitambar and Noffsinger (1989). The three juvenile stages of *T. auriculatus* are markedly different in seta distribution, bulbus morphology and especially labial structure, the first stage juvenile being similar in all respects to a first stage *Plectus* (*cf.* Maggenti, 1961) and the third stage largely resembling the adult, with the second stage being intermediate in morphology. Furthermore, juveniles and adults swing their lip region vigorously during feeding, a type of behaviour that must be linked to their anterior morphology, and which has not yet been reported for any nematode. The genus *Tylocephalus* was revised by Zell (1985), who included six species in it. A seventh species was recently described by Tahseen *et al.* (1995). Zell (1993) recently synonymized *Tylocephalus* with *Wilsonema* Cobb, 1915 and relegated the latter to subgeneric status within *Plectus* Bastian, 1865. We do not follow this classification, because in our view it is based on a highly inconsistent application of the monophyletic criterion of taxon validity, and because it fails to adequately represent morphological diversity.

Material and methods

We refer to De Ley *et al.* (1993) for details on the location, collection and fixation of the soil samples used in this study. Live specimens of *Tylocephalus auriculatus* were extracted with Baermann funnel on 13 March 1992 from one sample, D7, which was collected without fixation on 18 April 1991 in the littoral zone of Isla Española and stored at 4 °C for 11 months. Two suffocated females were fixed immediately upon extraction, while about fifteen active animals were transferred to agar gel for culture, along with other species from the sample. Cultures were kept at room temperature (16-24 °C) and originally consisted of pure 1 % agar agar, with a sprinkling of soil particles from sample D7 to inoculate the plates with naturally co-occurring bacteria. Specimens from one of these mixed cultures were heat-killed and fixed on 6 May 1992, and subsequently mounted in slides for taxonomic study and measurement. Further cultures were maintained by transferring single females to new plates. Progeny from one of these single-female cultures was sent to Dr. B. Sohlenius (Naturhistoriska Riksmuseet, Stockholm, Sweden) who transferred the animals to richer gels containing boiled soil extract, added salts (0.04 % $MgSO_4.7H_2O$; 0.14 % NaCl; 0.04 % K_2HHPO_4; 0.15 % KNO_3), peptone and mannit (each one knife-tip/300 ml). Upon our request, Dr. Sohlenius later sent back two plates of the Española strain of *T. auriculatus,* because our own cultures declined from November 1992 onwards and no longer inoculated well on new plates. Specimens and bacteria from Dr. Sohlenius' plates were transferred to mixed agar plates (1 % bacteriological agar + 0.05 % tryptone glucose yeast agar + 0.01 % beef-peptone nutrient agar) prepared with sterilized tap water instead of distilled water, enriched with cholesterol (about 0.025 %), and inoculated with *Escherichia coli* OP50. Cultures were thus maintained successfully until September 1994, when subculturing failed again.

Due to constraints of time and equipment, a round the clock investigation of the development of *T. auriculatus* under strictly controlled conditions was not possible, but a restricted study was made to determine the number of moults and to record the feeding behaviour of this species. Eggs were examined individually for evidence of a pre-hatching moult at immersion magnification (100 × 1.25 × 10 ×) under coverslip in a drop of water on a glass slide, or at lower magnification (40 × 1.25 × 10 ×) on a small piece of agar transferred from a culture plate to a glass slide and kept moist with water drops. The latter method allowed less detailed observation, but made it much easier to prevent desiccation and observe eggs over periods of up to 12 h.

Post-hatching moults were counted in developing juveniles under the following circumstances. Circular patches of mixed agar (prepared as above, but without *E. coli*) were made to keep individuals separate from each other and from the vertical rims of Petri dishes, where they could hide from view. Two methods were used to make these agar islands : either fifteen hot agar droplets were released from a pipette in the middle of 3 cm diameter Petri dishes (n = 5), or four-six agar droplets were dropped within each circular rim on the lid of a 6 × 4 well cluster, so that each island could be suspended upside down over a separate well of the cluster (n = 12). One ml of water was added to the wells of the cluster to keep air humidity high and slow down desiccation of the agar islands. The Petri dishes were easier to seal and slower to desiccate, while the inverted agar islands on the well cluster lid allowed easier tracking of specimens under a dissection microscope, as well as easier observation under light microscope with 25 × objective to assess their stage of development.

One egg with elongated juvenile was transferred with a fine needle to each of the seventeen agar islands thus created (preliminary attempts showed that eggs with earlier embryonic stages invariably ceased development after transfer). Sixteen eggs hatched, and in the subsequent days the emerging juveniles were scored as " active " or " moulting " at variable intervals (1-4 h during daylight, 2-17 h at night), depending on whether they were seen to feed and move, and (from the second moult onwards) whether moulting labial cuticle could be discerned or not. Fourteen juveniles were thus successfully monitored from hatching to maturity. Video recordings of developing eggs, active and moulting juveniles and active adults were made with a TK-1280E JVC camera mounted either on a Leitz Dialux 20 EB light microscope or a Wild M5 dissection microscope.

For the morphological descriptions, neck length was measured from the tips of the lip structures to the anterior end of the intestine (i.e., cornua included and cardia excluded). Stoma length was measured from the tips of the lateral lips to the point of origin of the radial tubuli (*cf.* De Ley & Coomans, 1994). Measurements are given as mean ± SD (range). Somatic setae are depicted as in De Ley and Coomans (1994). Upon request, we received additional *T. auriculatus* material studied by Crossman (1933) and Anderson (1966) from Dr. A. M. Golden, but these specimens were in too poor a condition to be useful. We did not receive any reply to repeated requests for type material of the *Tylocephalus* species described by Zell (1985) and deposited in the Landessammlungen für Naturkunde, Karlsruhe, Germany.

Four females of *T. nimius* sp.n. (one from samples 10 and 13, two from sample 4) and eight females of *T. auriculatus* (all from the mixed culture derived from sample D7) were submitted to critical point drying and sputter-coated for observation with a JEOL LSM-840 scanning electron microscope (SEM).

Tylocephalus nimius* **sp.n.**

(Figs 1-3)

MEASUREMENTS

See Tables 1, 3.

DESCRIPTION

Females : Body weakly to strongly ventrally or variously curved upon fixation. Cuticle up to 1 μm thick, finely divided into 0.6-1.0 μm wide annules. Lateral field consisting of two separate wings, 2.5-3 μm wide at mid-body, fading anteriorly at level of corpus and posteriorly on tail (ventral wing approaching tail tip while dorsal wing fades out on middle of tail : Fig. 1F). Neck region with sixteen to nineteen somatic setae (n = 6) distributed as follows (Fig. 3A) : one pair placed in the posterior half of each cervical expansion (holotype female with a single seta on the ventral expansion : Fig. 3B, I), one ventrosublateral pair near middle of corpus, a subventral pair at the excretory pore, four to seven subdorsal setae in an irregular row (one or two at corpus level), and two to four subventral setae in an irregular row (one or two at corpus level). Body with a total of 48-51 somatic setae (n = 6), including 9-14 setae between bulbus and vulva and 19-23 setae between vulva and anus. Most somatic setae are placed subdorsally or subventrally (Figs 1C, E; 3I). Deirids setiform, close to excretory pore (from one annule anterior to pore to two annules posterior), placed in lateral field. Amphids 2-3 μm wide, located 4-7 μm anterior to stoma base, their rims forming a rounded, irregular or flattened coil. Expanded sublabial region 17-25 μm long and 20-24 μm wide, each expansion bearing a series of 17-25 annules. Lip region dorsoventrally expanded, its precise appearance somewhat different under SEM *vs* under light microscope. Cornua 12-14 μm long, elongate-setiform with a basal cuticular flap visible only with SEM (Figs 1A; 2C) and usually with tips crossing those of the opposite cornua. Externally, the cornua carry a longitudinal incisure on their inner surface (Fig. 2A, B - small arrowheads) and have a rounded, shallow indentation at their base (Fig. 2A, B - large arrowheads; Fig. 1D). Internally, the base of each cornu contains a refringent " socket " under light microscope, which could be the fenestra of a nerve (Fig. 3A, B). Subdorsal and subventral lip pairs strongly modified, each pair fused into a curved median ridge projecting anteriad between two adjacent cornua, and bearing transverse lateral flanges that do not form acute projections over the mouth opening. A pair of outer labial sensilla sometimes visible near the top of each median ridge (distinct in Fig. 2A but not in 2B, despite being from same specimen), with short and faint innervation internally (Fig. 3C). Lateral lips modified and flattened, divided into two relatively small

Table 1. *Measurements in μm of adult females of* Tylocephalus nimius *sp.n.*

	Santa Cruz Island			Floreana Island
Sample	3	4	8	13
n	Holotype	5	1	1
L	443	452 ± 36 (394-488)	435	374
Body width	25	28 ± 3 (23-30)	26	25
Neck length	120	125 ± 3 (121-127)	113	116
Tail length	31	31-34	33	30
Anal body width	11	12-14	11	11
a	17	16 ± 2 (13-20)	17	15
b	3.7	3.6 ± 0.3 (3.1-4.0)	3.8	3.2
c	14	13-15	13	12.5
c'	3.0	2.4-2.7	3.0	2.7
Cornua length	14	12-14	13	14
Expansion length	25	22 ± 3 (17-26)	22	25
Expansion width	20	19-21	19	20
Stoma	14	13 ± 1 (11-15)	13	13
Corpus	43	42 ± 4 (35-46)	46	38
Isthmus	39	38 ± 3 (33-41)	36	36
Bulbus	17	17-19	19	18
Cardia	7	5-7	6	7
Rectum	12	12 ± 2 (11-15)	12	11
Nerve ring	60	68 ± 8 (55-77)	62	65
Excretory pore	70	71 ± 6 (61-77)	66	73
Deirid	69	72 ± 6 (61-77)	69	74
Nerve ring (% neck)	50	54 ± 6 (45-61)	54	56
Excretory pore (% neck)	58	57 ± 4 (50-61)	58	63
Deirid (% neck)	57	57 ± 4 (50-61)	61	64
V	48	49 ± 2 (46-51)	49	50
G_1 (%)	11	12 ± 2 (8-14)	14	9
G_2 (%)	10	12 ± 2 (9-14)	12	10
Vagina length	10	7-8	8	8

---

* The specific epithet is the Latin adjective for " exaggerated " and refers to the exceptional length of the cornua and cervical expansions of the new species.

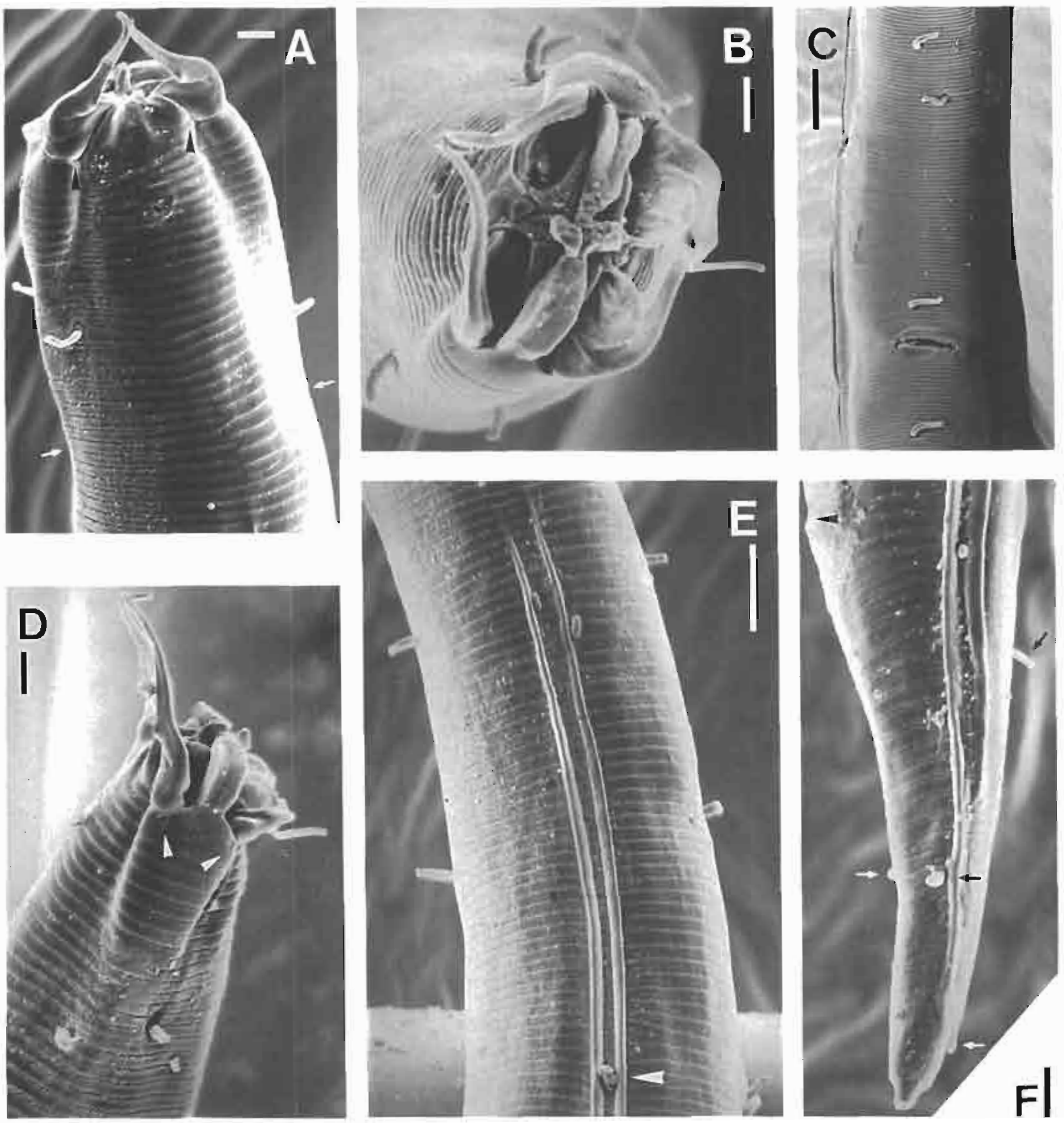

Fig. 1. Tylocephalus nimius *sp.n. (females only). A : Anterior end in lateral view (arrows point at posterior ends of cervical expansions, arrowheads at Y-shaped incisures); B : En face view; C : Vulva and subventral somatic setae; D : Anterior end in ventral view (arrowheads point at shallow submedian grooves); E : Lateral field and setae in neck region (arrowhead points at deirid); F : Tail (arrowhead points at anus, arrows at caudal setae). (Scale bars : 5 µm in C, E; 2 µm in all others).*

quadrants that do not cover the basal flaps of the cornua and do not overlap the cervical expansions (Fig. 2C). Grooves separate the base of each cornu from the adjacent lateral quadrant and cervical expansion, forming a Y-shaped incisure that is comparable to that of *T. auriculatus,* but less prominent and not overlapping the expansions (compare Figs 1A; 2C with Fig. 4E, F). Two very short and shallow additional submedian grooves extending posteriad from the anterior rim of each expansion (Fig. 1D; 2C). One protruding, contorted outer labial sensillum present on each lateral lip, placed very close to the mouth opening and the median ridges (Fig. 2A, B; 3C). Mouth opening closed off by the adaxial parts of the lips in all specimens seen. These parts appearing to contain the inner labial sensilla (Fig. 3C). Stoma 2.5-3.5 µm wide anteriorly, tapering posteriorly. Corpus cylindrical, plump, 1.1-1.3 times as long as isthmus, its walls with a faint transverse break at 9-10 µm from the stoma base. Isthmus plump, surrounded by nerve ring at its anterior end and by secretory-excretory gland cells at its posterior end. Bulb with simple valves. Cardia short, embedded in intestine. Ex-

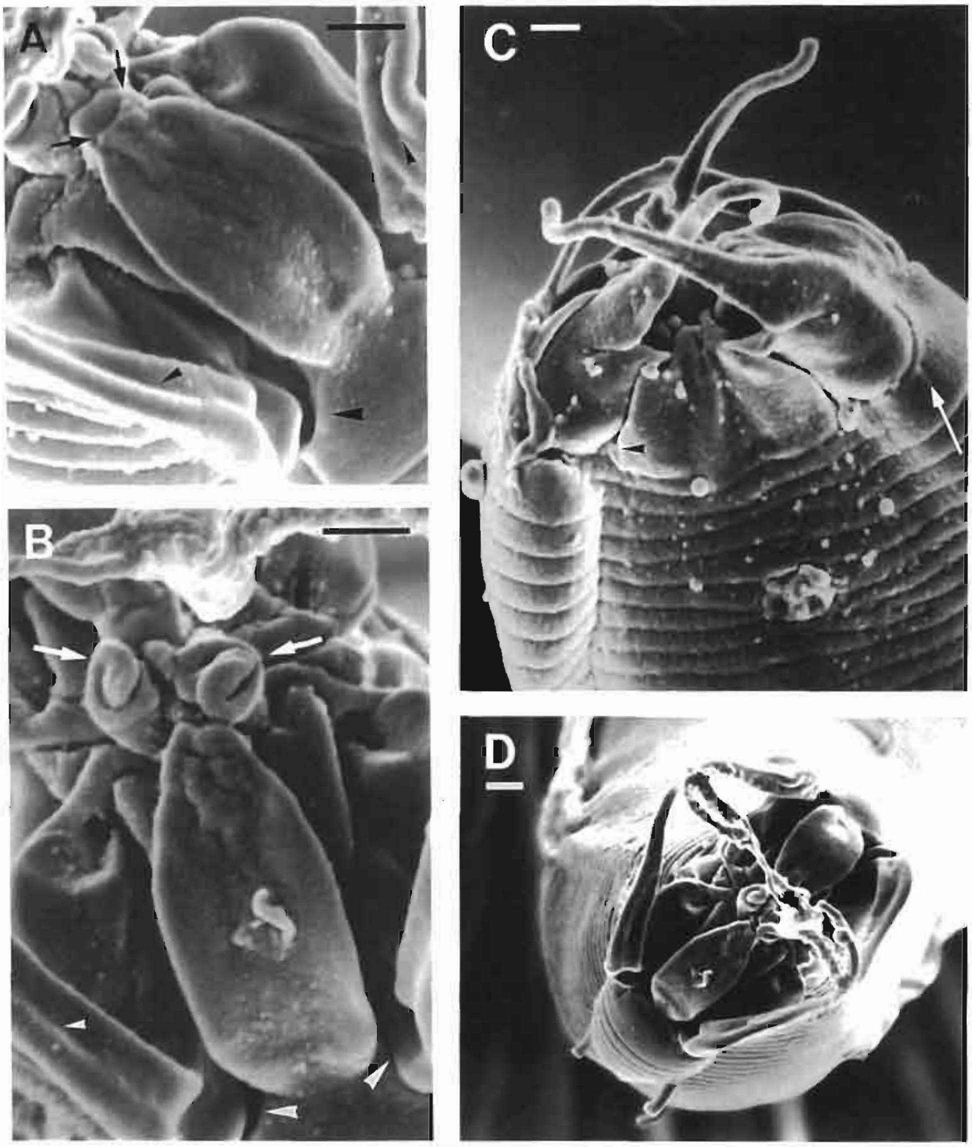

Fig. 2. Tylocephalus nimius *sp.n. (females only). A, B : Details of ventral and dorsal median ridge of one female respectively (arrows in A point at subventral outer labial sensilla, in B at lateral outer labial sensilla flanking the closed mouth, small arrowheads point at longitudinal incisures on cornua and large arrowheads at indented cornua bases); C : Lip region in lateral view (arrow points at shallow submedian groove, arrowhead at Y-shaped incisure); D : En face view of same female shown in A, B. (Scale bars : 1 µm).*

cretory pore slightly posterior to nerve ring, excretory canal fading rapidly proximally. Vulva inconspicuous, at or near mid-body, slit-shaped, with vulval lips about equally developed. Vagina always kinked in lateral view, without distal offshoot of the lumen. Reproductive system amphidelphic, with anterior branch on right side and posterior branch on left side of body in all females. Cytoplasm and nuclei of ovejector cells mostly located

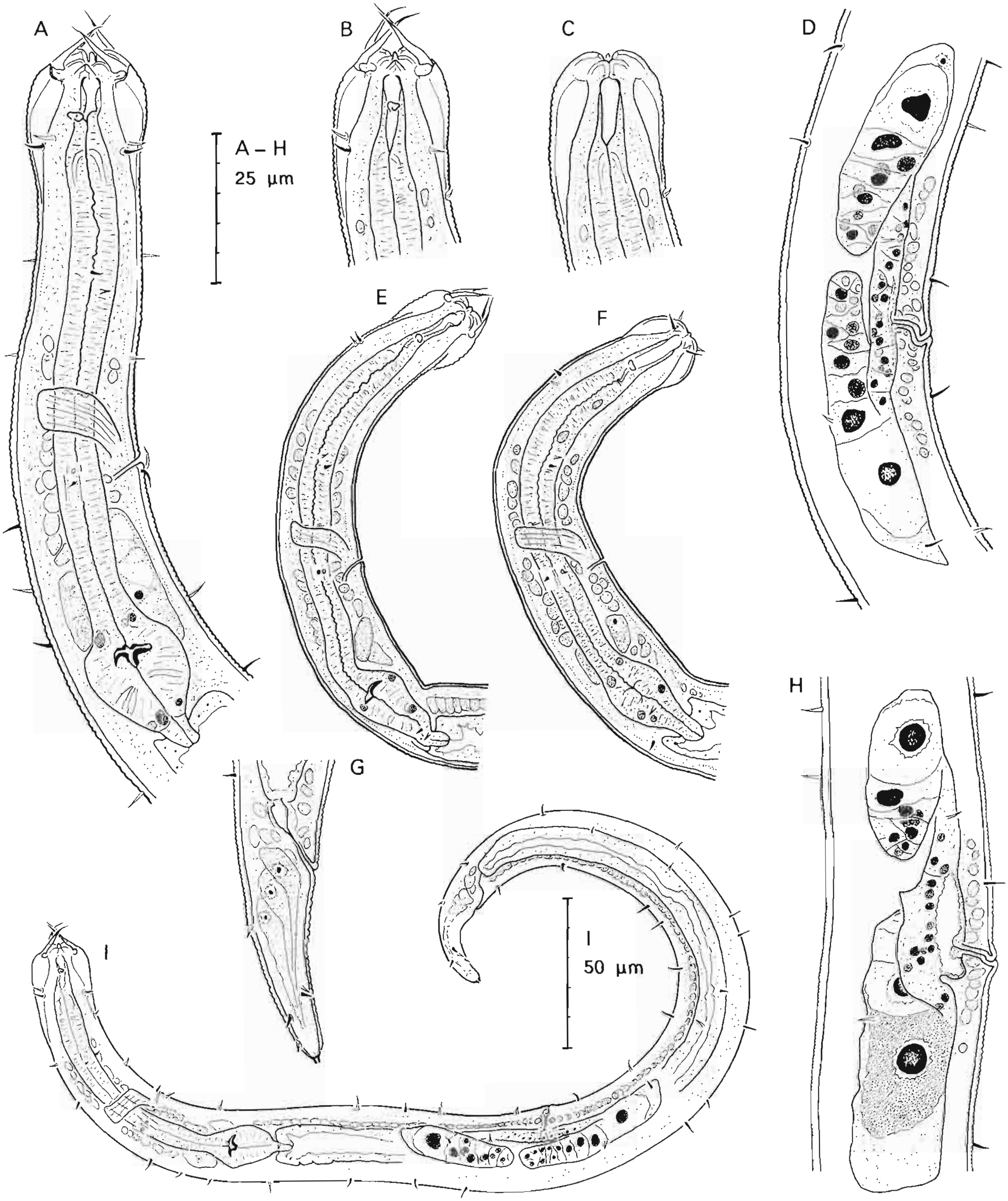

Fig. 3. Tylocephalus nimius *n.sp. (females) A : Neck region; B, C : Anterior end in submedian and median view; D, H : Female reproductive system; E, F : Neck region of third- and second-stage juvenile; G : Female tail; I : Entire female. (B, C, D, I from holotype).*

dorsally of ovejector lumen. Ovaries reflexed. One female with an oocyte or shell-less egg in ovejector measuring 48 by 19 µm. Rectum 0.8-1.1 times as long as ABW. Tail conical, bearing five caudal setae (the terminal one being located at 3-7 µm from the tail tip), containing three functional caudal glands, and ending in a small, tubular spinneret.

Male : Not found; no sperm observed in female genital tract.

First-stage juvenile : Not found.

Second-stage juvenile : Cuticle very finely annulated (annules 0.7 µm wide at mid-body), with distinct, 2.5 µm wide lateral field under light microscope. Neck region with one pair of subdorsal somatic setae near anterior end of corpus, one ventrosublateral pair slightly anterior to corpus-isthmus junction and one dorsosublateral pair at bulbus-cardia junction; tail with one subdorsal and two ventrosublateral caudal setae. Deirids setiform, level with excretory pore. Lip region and sublabial cuticle dorsoventrally expanded : expansions 8 µm long and bearing nine annules; corresponding body width 12 µm. Cephalic setae not modified into cornua, 2.5 µm long, reaching lip apices. Subdorsal and subventral lips not fused into median ridges. Specimen fixed with mouth closed. Bulbus clearly swollen, containing faint transverse valves. Gonad primordia with one germinal cell each.

Third-stage juvenile : Cuticle very finely annulated (annules 0.7 µm wide at mid-body), with distinct, 2.5 µm wide lateral field under light microscope. Neck region with one pair of subdorsal somatic setae near anterior end of corpus, one ventrosublateral pair slightly anterior to corpus-isthmus junction, and one dorsosublateral pair at bulbus-cardia junction; tail with three subdorsal and two ventrosublateral caudal setae. Deirids setiform, level with excretory pore. Lip region and sublabial cuticle dorsoventrally expanded : expansions 8-9 µm long and bearing eight to ten annules; corresponding body width 12-13 µm. Cephalic setae modified into cornua, 7-9 µm long, surpassing lip apices. Subdorsal and subventral lips modified into median ridges with blunt tips. No Y-shaped grooves. Both specimens fixed with mouth closed. Bulbus clearly swollen, containing " simple " transverse valves. Gonad primordia with one germinal cell each.

TYPE LOCALITY AND HABITAT

Sampling locality 4 (six females, one juvenile) : soil collected at 19.1 km from Itabaca at 630 m altitude on N slope of Los Gemelos, Isla Santa Cruz, Galápagos, Ecuador. Clearing in *Scalesia*-forest with grasses and sedges.

OTHER LOCALITIES

Isla Santa Cruz : Samples 3 (one female, one juvenile), 8 (one female) and 10 (one female); Isla Floreana : Samples 13 (two females, one juvenile) and 15 (one female).

TYPE SPECIMENS

Holotype and five paratype females kept on slides 3781, 3782, 3911, 3912, 3914 in the Nematode Collection of the Instituut voor Dierkunde, Universiteit Gent, Belgium; one paratype female deposited in the Collection Nationale de Nématodes, Muséum National d'Histoire Naturelle, Paris, France; one female paratype deposited in the USDA Nematode Collection, Beltsville, MD, USA.

DIFFERENTIAL DIAGNOSIS

The new species differs from all hitherto known *Tylocephalus* species in the 12-14 µm long cornua (*vs* up to 10 µm) and in the cervical expansions extending well beyond the anterior quartet of somatic setae and the stoma base (*vs* ending at or near setae and stoma base). It furthermore differs from all known species except *T. auriculatus* in having C-shaped " sockets " in the cornua bases and differs from all species except *T. auriculatus* and *T. palmatus* Tahseen, Ahmad & Jairajpuri, 1995 in having well developed, recurving median ridges. However, these two species differ from *T. nimius* sp.n. in numerous other details of the lip region : each median ridge bears two cuticular tips overhanging the mouth opening, the outer lateral labial sensilla are set further from mouth opening, the lateral lip quadrants are larger, and the Y-shaped incisures are more prominent and more dorsoventrally displaced. Finally, it also differs from *Tylocephalus* sp. on p. 32 in Sauer (1985), in particular in the absence of bifid tips on the median ridge and in the more centrally placed lateral sensilla.

Tylocephalus auriculatus (Bütschli, 1873) Anderson, 1966

(Figs 4-7)

MEASUREMENTS

See Tables 2, 3.

DESCRIPTION

Females : Body very weakly ventrally curved to tightly ventrally coiled upon fixation. Cuticle up to 1 µm thick, finely divided into 0.6-0.9 µm wide annules. Lateral field consisting of two separate wings, 4-5 µm wide at mid-body and fading anteriorly at the level of the corpus and posteriorly on the tail, with ventral wing nearly reaching tail tip and dorsal wing fading halfway down the tail (Fig. 5E, F). Neck region with twelve to seventeen somatic setae in eighteen cultured females and four females from soil, usually distributed as follows : a subdorsal pair at the base of the dorsal cervical expansion, a subventral pair just posterior to the ventral cervical expansion (Figs 4B, E, F; 6C, D), three pairs at corpus level (anterior one subdorsal, middle one ventrosublateral and posterior one subventral), and two pairs near isthmus-bulbus junction (anterior one subdorsal, post-

Table 2. *Measurements in µm of adult females of* Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966 from soil samples and an agar culture.*

	Santa Cruz Island	Fernandina Island	Española Island			
Sample	1	17	21	D7	Culture (from D7)	CV*
n	2	4	1	2	25	
L	411-474	293-439	495	363-370	458 ± 27 (409-515)	5.8
Body width	24-27	24-34	30	20-21	27 ± 3 (21-33)	11.7
Neck length	119-124	103-109	128	119	114 ± 4 (105-126)	3.6
Tail length	34-37	29-40	37	33-34	40 ± 3 (32-46)	8.5
Anal body width	11-13	11-14	14	10-11	13 ± 1 (10-15)	7.7
a	15-19	12-16	16.5	18	17 ± 1 (14-20)	8.0
b	3.5-3.8	2.8-4.2	3.9	3.1	4.0 ± 0.1 (3.7-4.4)	3.7
c	12-13	10-12	13	11	11.5 ± 0.6 (9.8-13)	5.4
c′	2.8-3.0	2.5-2.9	2.6	3.1	3.2 ± 0.2 (2.7-3.8)	7.6
Cornua length	9	8-9	10	8	8-10	6.9
Expansion length	14-15	10-13	17	16-17	17 ± 1 (15-19)	6.0
Expansion width	22-23	20-25	23	20-21	21 ± 1 (18.5-23)	5.1
Stoma	11-13	11-13	14	15-16	19 ± 1 (17-22)	5.7
Corpus	42-46	37-40	46	39-40	38 ± 1 (35-40)	3.5
Isthmus	38	32-36	37	36-37	35 ± 2 (32-38)	5.2
Bulbus	17-20	16-18	18	16	19 ± 2 (15-22)	9.5
Cardia	7-8	6-7	6	6	3-6	20.1
Rectum	12-15	11-12	14	12-13	12 ± 1 (8-14)	10.6
Nerve ring	70-72	57-61	76	63-64	64 ± 2 (60-68)	3.6
Excretory pore	75-79	51-68	84	70	73 ± 4 (67-87)	5.1
Deirid	80-84	54-72	85	72-70	74 ± 3 (69-83)	4.2
Nerve ring (% neck)	58-59	55-56	59	53-54	56 ± 2 (52-60)	3.4
Excretory pore (% neck)	63-64	50-63	66	59	63 ± 4 (59-77)	5.6
Deirid (% neck)	64-68	53-67	66	59-61	65 ± 2 (61-70)	3.4
V	49-52	47-52	50	51-52	48 ± 1 (47-50)	1.6
G_1 (%)	11-20	9-20	15	8	7-11	11
G_2 (%)	8-18	3-26	20	7-10	9 ± 2 (6-13)	18
Vagina length	7-10	8-10	10	8-11	8 ± 1 (6-10)	13

* CV : coefficient of variation.

Table 3. *Measurements in µm of juveniles of* Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966 from agar culture and juveniles of* T. nimius *sp.n. from soil.*

	T. auriculatus				*T. nimius* sp. n.	
	J1	J2	J3	J3	J2	J3
Nr of germinal cells	2	2	2	2 x 2	2	2
n	10	10	10	2	1	2
L	190 ± 8 (180-209)	236 ± 12 (222-257)	306 ± 9 (283-316)	326-337	234	269-276
Body width	12-14	13-16	15-18	17-18	16	14-19
Neck length	70 ± 3 (66-74)	79 ± 3 (74-84)	93 ± 3 (86-97)	97	86	94-97
Tail length	22-25	26 ± 1 (23-29)	31 ± 1 (29-34)	31-34	–	23
Anal body width	7-9	7-9	8-11	10-11	11	8-9
a	13-15	15-18	17-19	19	15	14-19
b	2.7 ± 0.1 (2.6-2.9)	3.0 ± 0.1 (2.8-3.3)	3.2-3.5	3.4-3.5	2.7	2.8-2.9
c	8.1 ± 0.3 (7.7-8.6)	9.1 ± 0.8 (8.5-11.3)	9.8 ± 0.3 (9.3-10.4)	9.9-10.5	–	12
c′	3.0 ± 0.2 (2.8-3.4)	3.2 ± 0.3 (2.7-3.5)	3.3 ± 0.2 (2.8-3.6)	3.0-3.1	–	2.6-2.7
Stoma	11-14	14-17	16-19	17-18	14	12-13
Corpus	23 ± 1 (21-25)	26 ± 1 (23-28)	28-31	30-32	30	32-34
Isthmus	20 ± 2 (17-23)	25 ± 1 (23-28)	29 ± 2 (26-32)	30-31	27	30-31
Bulbus	11-13	10-12	12-15	13-15	13	12-15
Cardia	2-4	2-4	3-5	4	5	6
Corpus : isthmus	1.0-1.3	0.9-1.1	0.9-1.1	1.0	1.1	1.0-1.1
Rectum	7-9	7-10	9-11	9	–	8-9
Nerve ring	38 ± 2 (36-42)	45 ± 2 (40-49)	54 ± 1 (51-56)	54-55	49	55-56
Excretory pore	43 ± 3 (39-47)	50 ± 2 (47-53)	60 ± 2 (58-62)	62-63	55	60-61
Deirid	43 ± 3 (39-48)	51 ± 2 (47-54)	61 ± 2 (58-63)	62-63	55	61
Nerve ring (% neck)	55 ± 1 (53-57)	57 ± 2 (54-61)	58 ± 2 (56-62)	56-57	57	57-60
Excretory pore (% neck)	61 ± 2 (58-65)	64 ± 1 (62-67)	64 ± 2 (61-67)	64-65	64	62-65
Deirid (% neck)	61 ± 2 (58-67)	65 ± 1 (63-68)	65 ± 2 (61-69)	64-65	64	63-65

erior one subventral). Less common conditions (*e.g.*, Fig. 6A) include : an extra subventral pair at the base of the ventral cervical expansion (n = 1), only one subdorsal seta at expansion plus only one subventral seta near expansion (n = 2), only four or five setae at corpus level (resp. n = 1 and n = 3), one to three additional setae at isthmus level (n = 5), and five (n = 1) or three (n = 5) setae near isthmus-bulbus junction instead of four. Body with a total of 40-49 somatic setae in cultured females (n = 10), including 8-12 setae between bul-

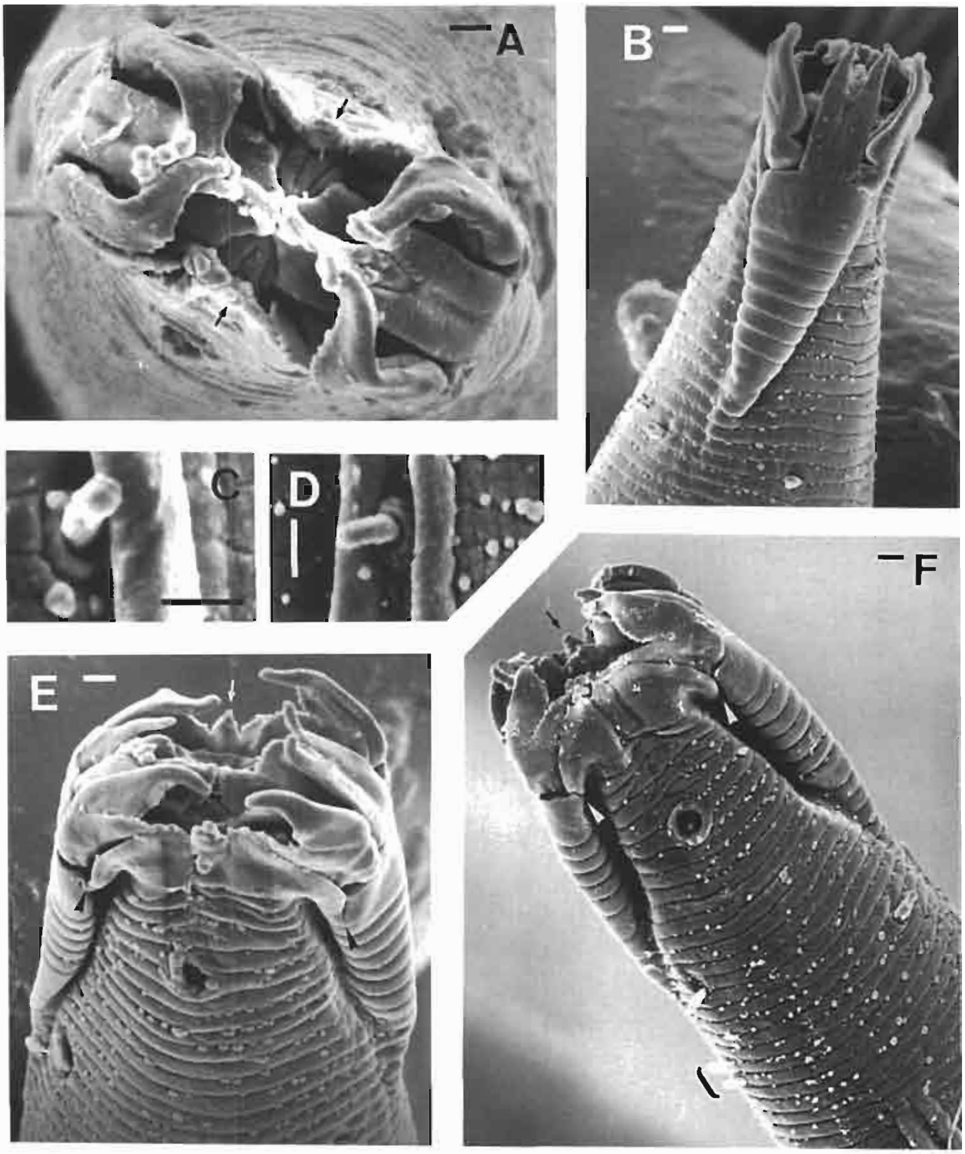

Fig. 4. Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966 (females only). A : En face view; B : Anterior end in ventral view; C : Dorsosublateral somatic seta posterior to cardia; D : Deirid in lateral field; E, F : Lip region in resp. anteriolateral and lateral view (arrow points at lateral outer labial sensilla, arrowheads point at Y-shaped incisures, bracket in F lies opposite bacteria, not setae). (Scale bars : 1 μm).*

bus and vulva and 18-24 setae between vulva and anus, but with 34-50 somatic setae in specimens from soil (n = 4), of which 5-14 between bulbus and vulva and 17-22 between vulva and anus. Deirids setiform, 0-6 annules posterior to excretory pore, placed in lateral field. Amphids 1.5-2.5 μm wide, located 6-10 μm anterior to stoma base, their rims usually forming a rounded coil, but sometimes irregular or flattened. Expanded sublabial region 10-19 μm long and 18-25 μm wide, each expansion bearing a series of 6-13 annules that is often interrupted by a band of smooth cuticle (Figs 4F; 6B). The expansions may carry one or more conspicuous transverse " creases " (Fig. 6C, D). Lip region dorsoventrally expanded, its precise appearance being quite different under light microscope (Fig. 6A-D) and SEM (Figs 4A, B, E, F; 5A, B) due to the transparency of some structures. Cornua 8-10 μm long, appearing setiform under light microscope but actually more leaf-shaped through the presence of an anterior flap, and also carrying an additional basal cuticular flap, both of which are only visible with SEM (Fig. 4F). Externally, each cornu has a longitudinal incisure on its inner surface

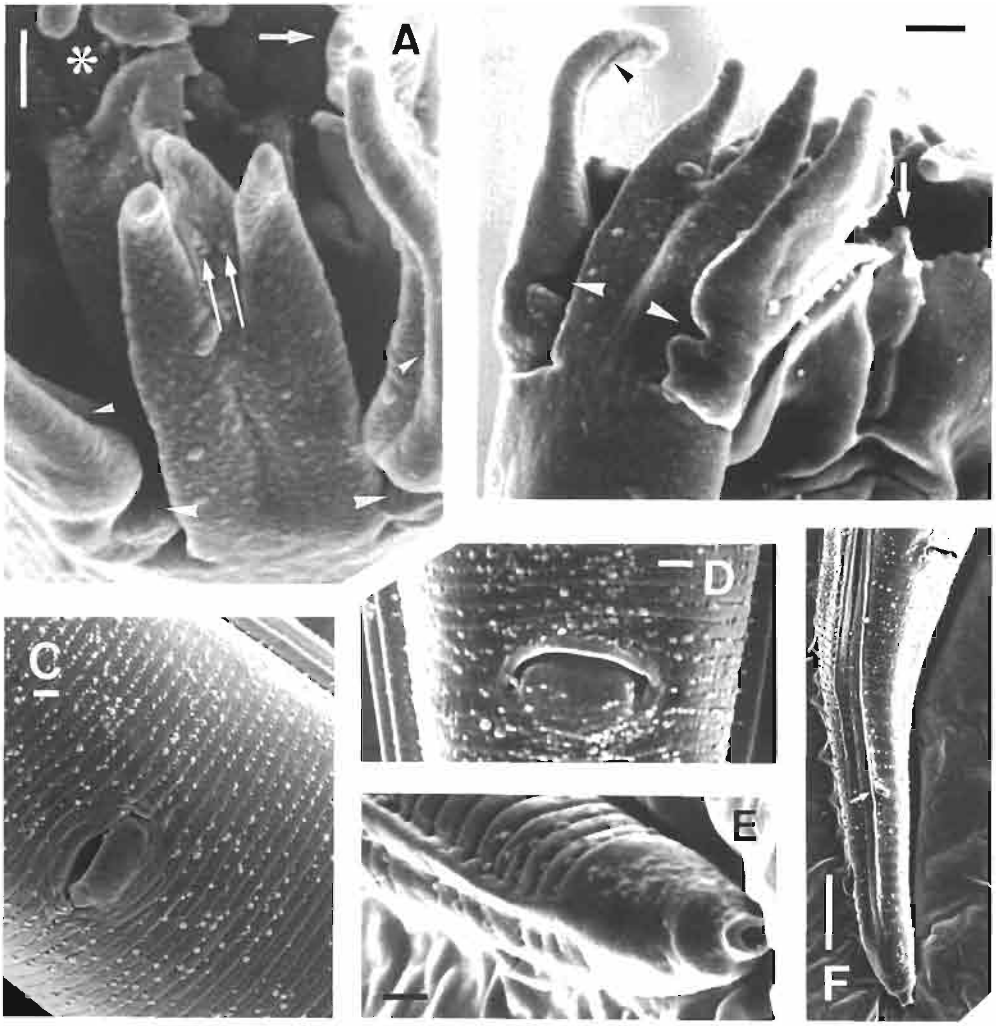

Fig. 5. Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966 (females only). A : Detail of dorsal median ridge (closed mouth opening lies right of asterisk, long arrows point at subdorsal outer labial sensilla, short arrow at lateral outer labial sensilla, small arrowheads at longitudinal incisures on cornua, large arrowheads at indented cornua bases); B : Same median ridge and cornua in subdorsal view (arrow points at lateral outer labial sensilla, arrowheads as in A); C : Vulva (lower right is anteriad); D : Anus; E : Spinneret and terminating ventrolateral wing of lateral field; F : Tail (arrow points at caudal seta). (Scale bars : 5 μm in F, 1 μm in rest).*

(Fig. 5A, B - small arrowheads) and a fairly deep indentation at the base (Fig. 5A, B - large arrowheads). Internally, the base of each cornu is visible under the light microscope as a C-shaped refringent " socket ", which could be the fenestra of a nerve. Subdorsal and subventral lip pairs modified into two median ridges, each median ridge projecting anteriad between two adjacent cornua, and bearing lateral transverse flanges that each end in an acute, flattened slip of cuticle (Figs 4B; 5A, B; 6B, C). Thus, each median ridge ends in a pair of setiform tines, in between which lies the blunt top of the ridge itself, carrying a pair of closely adjacent outer labial sensilla (Fig. 5A). Faint nerve endings are usually visible under light microscope within the ridges, in positions corresponding with these sensilla (Fig. 6B). Lateral lips also modified, bearing two sublateral cuticular plates shaped like large quadrants that partly cover the basal flaps of the cornua (Figs 4E, F; 5B). Rims of these quadrants meeting grooves at the bases of the cornua and continuing posteriorly over a variable distance, forming distinct Y-shaped incisures overlaping the cervical expansions and separating the cornua from all adjacent external structures (Figs 4E, F; 6A, C, D). Lateral lips midlaterally extending in between the quad-

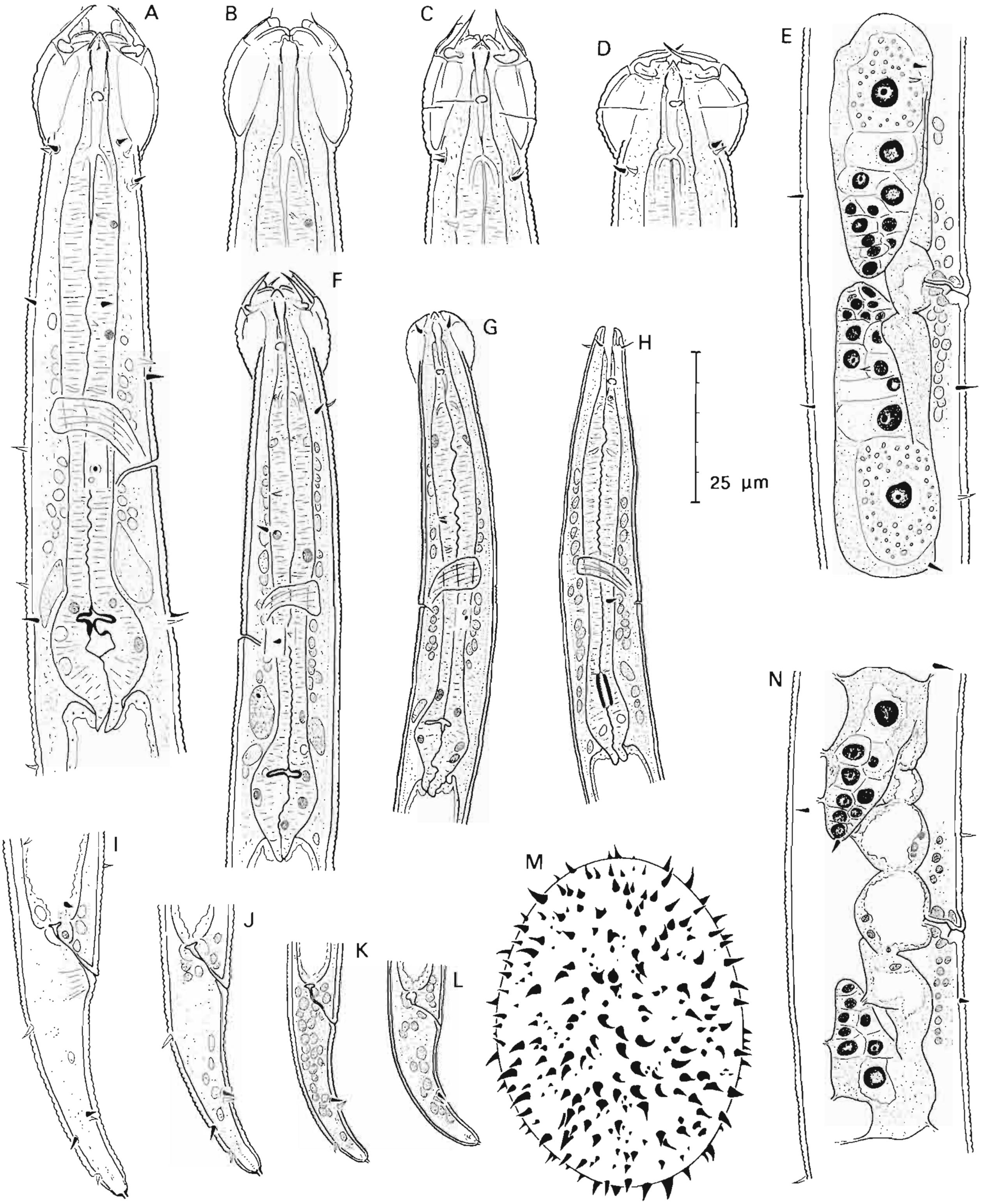

Fig. 6. Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966. A : Neck region of female from culture; B : Anterior end in median view (same female as A); C, D : Anterior ends of females from soil; E, N : Reproductive system of females from culture; F-H : Neck region of third-, second- and first-stage juvenile; I-L : Tail of adult female, third-, second- and first-stage juvenile; M : Surface ornamentation of egg.*

rants, each forming a tapering tip appearing to contain a nerve ending under the light microscope, and being therefore thought to be a lateral outer labial sensillum (Figs 4E, F; 5A, B; 6A, B, C). Lateral lip tips and Y-shaped incisures visible with light microscope, but lateral lip quadrants only visible with SEM. Mouth opening closed off by the adaxial parts of the lips in all specimens seen; these parts appearing to contain the inner labial sensilla (Fig. 6B). Stoma 2.5-3.5 µm wide anteriorly, tapering posteriorly. Corpus cylindrical, plump, 1.0-1.2 times as long as isthmus, with a faint transverse break in its walls at 8-11.5 µm from the stoma base. Isthmus plump, surrounded by nerve ring at its anterior end and by secretory-excretory gland cells at its posterior end. Bulb with simple valves. Cardia short, embedded in intestine. Excretory pore slightly posterior to nerve ring, excretory canal fading rapidly proximally. Vulva inconspicuous, at or near mid-body, crescentic with swollen anterior vulval lip. Vagina always with two kinks in lateral view, and in cultured specimens usually with the posterior wall folded back on itself, resulting in a characteristic short " offshoot " of the lumen at the distal kink (Fig. 6E, N; less clear in specimens from soil). Reproductive system amphidelphic, with anterior branch on right side and posterior branch on left side of body in all females. Distal parts of uteri fixed in markedly distended condition in many cultured females, with an offset, similarly swollen ovejector at the junction of the uteri (Fig. 6E, N). Ovaries reflexed; in one female clearly attached to the body wall by support fibres (Fig. 6N). No fixed gravid females. Seven eggs with elongated juveniles in water measuring 53 ± 3 (50-57) µm long by 38 ± 2 (35-39) µm wide (possibly slightly flattened by coverslip) and having a spiny surface ornamentation (Fig. 6M). Rectum 0.6-1.2 times as long as ABW. Tail conical, bearing five caudal setae (of which the terminal one is placed at 5-7 µm from its tip), containing three caudal glands, and ending in a small, tubular spinneret (Fig. 6I).

Male : Not found. No sperm observed in female genital tract.

First-stage juvenile (Fig. 6H, L) : Cuticle annulated very finely, lateral field not visible under light microscope. Neck region without somatic setae, tail with two subventral caudal setae. Deirids setiform, at level of excretory pore. Lip region *Plectus*-like, 5.5-6.5 µm wide, not expanded. Cephalic setae 2-2.5 µm long, not reaching lip apices. All specimens fixed with mouth open. Bulbus hardly swollen, containing very finely serrated, 4-5 µm long bar-shaped longitudinal valves. Gonad primordia with only one germinal cell each.

Second-stage juvenile (Fig. 6G, K) : Cuticle finely annulated (annules 0.6-0.8 µm wide at mid-body), with distinct, 2-2.5 µm wide lateral field under light microscope. Neck region with one pair of ventrosublateral somatic setae slightly anterior to corpus-isthmus junction; tail with one dorsal and two ventrosublateral caudal setae. Deirids setiform, at level of excretory pore. Lip region and sublabial cuticle dorsoventrally expanded : expansions 7-10 µm long and bearing 8-14 annules; corresponding body width 10.5-13 µm. Cephalic setae not modified into cornua, 2-2.5 µm long, almost reaching lip apices. Subdorsal and subventral lips not fused into median ridges. All specimens fixed with mouth closed. Bulbus clearly swollen, containing " simple " transverse valves. Gonad primordia with only one germinal cell each.

Third-stage juvenile (Fig. 6F, J) : Cuticle finely annulated (annules 0.8-1.0 µm wide at mid-body), with 2.5-3 µm wide lateral field visible under light microscope. Neck region with one pair of subdorsal somatic setae slightly posterior to cervical expansions, one pair of ventrosublateral setae slightly anterior to corpus-isthmus junction, and one pair of dorsosublateral setae at or slightly posterior to bulbus-cardia junction; tail with three subdorsal and two ventrosublateral caudal setae. Lip region and sublabial cuticle dorsoventrally expanded : expansions 10-12 µm long and bearing 8-11 annules; corresponding body width 16.5-18 µm. Cephalic setae modified into cornua, 7-8 µm long, surpassing lip apices. Subdorsal and subventral lips modified into curved ridges with paired acute tips. Lateral lips demarcated by Y-shaped grooves. All specimens fixed with mouth closed. Bulbus clearly swollen, containing " simple " transverse valves. Gonad primordia with one germinal cell each in smaller (presumably younger) juveniles, but with two germinal cells in larger (presumably older) juveniles.

LOCALITIES

Isla Fernandina : Sample 17 (four females, four juveniles); Isla Santa Cruz : Sample 1 (two females); Isla Floreana : Samples 13 one female) and 14 (one female, two juveniles); Isla Española : Samples 21 (one female, one juvenile); 22 (24 females, 69 juveniles) and D7 (from soil : two females, one juvenile; from culture : 25 females, 32 juveniles).

REFERENCE SPECIMENS EXAMINED

– Material of Crossman (1933) : one female and one late third-stage juvenile in a slide labelled " *Tylocephalus bacillivorus* stained in carmin - F128 - 2♀'s [corrected to 1♀] "; nine juveniles (one first, seven second and one third stage) in a slide labelled " G2 - Slide # 15 - *Tylocephalus bacillivorus* - F128 - 9-18-33 - various 3 & 4th stage larva - # 5/5 - soil imported roots... [illegible] Brazil ". These slides are kept as Nrs. T-3113p and T-3114p as paratypes of *T. bacillivorus* Crossman, 1933 in the USDA Nematode Collection, Beltsville, MD, USA.

– Material of Anderson (1966) : five females and three juveniles (all third stage) in a slide labelled " Canadian National Collection Nematodes - Host : Alfalfa -

Loc : Goshim Ont. - Date : June 3/58 - Coll : J. M. Schinzel - Mnt Lacto - Sig Mol - C.No 1325 - Plectidae - *Tylocephalus auriculatus* 5♀3Y - By R. V. Anderson ". This slide is kept as Nr. 1325 in the CNC, Ottawa, Ontario, Canada.

COMMENTS ON IDENTIFICATION

The currently accepted diagnosis of *T. auriculatus* (*cf.* Andrássy, 1984; Zell, 1985) is based on the detailed description of Anderson (1966), and its most important features include the presence of " sockets " at the cornua bases, distinct Y-shaped incisures and recurved median ridges with acute projections (*cf.* Fig. 4A-D *in* Anderson, 1966; " Kopflamelle " *in* Zell, 1985). Our species is identified as *T. auriculatus,* since it agrees with the description and specimens of Anderson (1966) in these and other respects. We accept Anderson's synonymization of *T. bacillivorus* Crossman, 1933 with *T. auriculatus.* At present, *Tylocephalus* contains eight more recognized species : *T. andinus, T. annulatus, T. becki, T. cornutus, T. laticollis* (all described by Zell, 1985), *T. palmatus* Tahseen, Ahmad & Jairajpuri (1995) and the poorly known *T. cephalatus* (Cobb, 1893) Anderson, 1966, which we do not include in *Wilsonema,* contrary to Zell (1985). *T. cephalatus* and the five species of Zell (1985) apparently differ from *T. auriculatus* in lacking the aforementioned features, but it must be noted that they have not been studied with SEM. Apart from several differences mentioned by Tahseen *et al.* (1995), *T. palmatus* differs from *T. auriculatus* in the absence of cornua " sockets ", and in the straight and symmetrical vulva and vagina. Sauer and Annells (1982) and Sauer (1985) examined two species with SEM and identified these as *Tylocephalus* sp. and *T. auriculatus* (no biometric or light-microscopical data given). *Tylocephalus* sp. has bifid median ridges but lacks Y-shaped incisures, and may therefore be an as yet undescribed species. Our specimens of *T. auriculatus* conform with those of Sauer and Annells (1982) and Sauer (1985) in all specified aspects, except for the presence of larger anterior flaps on the cornua.

DEVELOPMENT, MOULTING AND FEEDING BEHAVIOUR

T. auriculatus eggs are apparently laid at the one-celled stage, and take 2 to 4 days to hatch at 19-27 °C. The earliest stage in which muscular activity was observed was a " tadpole larva " exhibiting general pulsations. Elongated juveniles move with greater coordination, periodically wriggling back and forth inside the egg shell. After differentiation of the intestine, pharynx, stoma and lips, this wriggling is accompanied by occasional corpus and/or bulbus pumping motions, and sideways twitching or swinging motions of the anterior end. Caudal spinneret and cephalic setae are distinguishable in all juveniles with differentiated pharynx, but the cuticle apparently takes longer to become functional than the digestive tract. Eggs under coverslip often burst open and expelled juveniles, upon which all except the most developed juveniles died and plasmolysed in less than a minute. No duplication of the lip region or spinneret indicative of a moult was ever observed in over 40 juveniles examined prior to hatching or upon premature eclosion, and no shed cuticle was ever seen inside or near empty egg shells.

Seven eggs containing elongated juveniles with differentiated pharynx were monitored continuously for 10 h (magnification 40 × 1.25 × 10 ×). During this period, two eggs hatched (one after 8 h and another after 10 h). In all these eggs and over the entire observation period, short bursts of activity were separated by periods of up to 15 min of rest. No prolonged period of inactivity corresponding to a possible pre-hatching moult was ever observed. Of the two eggs followed till hatching, the shell was seen to thin, fade and soften in the last hour of development, suggesting enzymatic breakdown of its lipid layer (*cf.* review *in* Bird and Bird, 1991). Simultaneously, the lip and body cuticle of the juvenile became more and more distinct, the intestine cleared due to yolk consumption, and resting periods became shorter and shorter. Increased wriggling, pumping and lip region swinging of the juvenile suggested that shell-digesting enzymes were secreted from the pharynx and smeared out over the egg shell by the juvenile. Due to the various changes, eggs close to hatching were easily recognized in culture plates (even under stereomicroscope), but nearly impossible to manipulate, as the slightest disturbance resulted in eclosion.

In addition to the two eclosions observed with 40 ×-objective, two other eclosions were seen with immersion 100 ×-objective. All four juveniles emerged with their lip region first and began to feed within a few minutes, pumping their pharynx and swinging their lip region dorsoventrally. On the separate agar islands and in group cultures, all stages kept up this swinging movement nearly continuously during pharyngeal pumping, irrespective of their body being stationary or in motion. Brief interruptions occurred only rarely and were triggered by external influences, *e.g.,* when an animal encountered a very dense bacterial patch or was disturbed with a needle. Contact with other nematodes did not cause cessation of motion. The point of flexure of the oscillatory motion was always located at the level of the anterior third of the corpus. While all animals oscillated at approximately the same speed, motion amplitude increased markedly with body size as the nematodes matured. On average, a single complete oscillation of the neck region lasted 1.2 s in feeding specimens of all stages, videotaped at room temperature in group cultures (Fig. 7).

Most juveniles stayed on the exposed surface of their agar island during feeding, but crept between the agar and its plastic carrier to moult : of a total of 46 moulting periods observed (including three juveniles that died

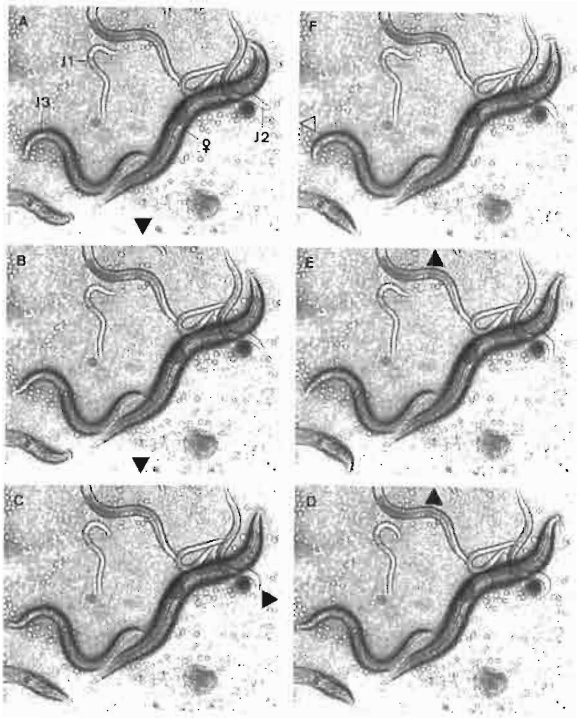

***Fig.** 7. Rhythmic dorsoventral swinging of feeding* Tylocephalus auriculatus *(Bütschli, 1873) Anderson, 1966. A-F: Videoprints taken at 0.16 s intervals, showing one motion cycle of the lip region in feeding animals of all stages. Sequential order indicated by black triangles. The situation 0.16 s after F roughly equalled the situation in A (represented by open triangle in F).*

before adulthood), all but two occurred between the agar and plastic surfaces. Juveniles in group cultures frequently formed moulting clusters, mostly on the bottom and sides of the plate rather than on the open surface. The first moult of isolated animals occurred 1 or 2 days after hatching and lasted about 1 day (exceptionally 2), during which the juveniles assumed a C-shaped body posture, interrupted occasionally by stationary flexing movements. This pattern was maintained in subsequent days : moults and active periods each lasted between 1 and 2 days, exceptionally slightly less than 1 day. At room temperature (19-27 °C), maturity was reached 7 to 9 days after hatching of the separated juveniles. In eleven animals, only three moulting periods were observed, while in three others four periods were scored. In the latter three cases a single discrepancy (one " moulting " score between two " active " scores or vice versa) led to this aberrant result, and these discrepancies were mutually contradictory.

It is therefore assumed that only three moults occur during the development of *T. auriculatus,* as supported by : the scores for the eleven other individuals; the long durations of other moulting and intermoult periods; the observation that juveniles sometimes moved during moulting or rested during feeding; and the distinct morphological differentiation into three stages of the fixed juveniles. It cannot be excluded with absolute certainty that the first of four moults was overlooked and actually occurs a few hours after hatching (*cf., e.g.,* Morton & Perry, 1968), but this is quite unlikely in view of the fact that this would require the first- and second-stage juveniles to be completely similar in qualitative morphology. After the third moult, adults started ovipositing within 1 day, laying one egg at a time at intervals of 1 to 3 h. Thus, the generation time of *T. auriculatus* in the 19-27 °C temperature interval is roughly estimated at 10 to 14 days. Individual fertility was not determined.

Discussion

HOMOLOGY OF LABIAL STRUCTURES

Our observations on the transformation of the lip region of *T. auriculatus* during development appear to be ontogenetic corrollaries of Zell's (1985) description of two species (*T. cornutus* and *T. becki*) with cornua strongly resembling enlarged setae. Both these facts confirm the suggestion of Sauer and Annells (1982) that the cornua are in fact modified cephalic setae. The less developed lateral lip quadrants of *T. nimius* sp.n. also confirm the extent to which the lateral lips have become dorsoventrally extended in *T. auriculatus,* and our observation of nerves in the appropriate positions inside the mediolateral lip tips and the median ridges support the assumptions of Sauer and Annells (1982) that these contain the outer labial sensilla, and that the median ridges are fused pairs of submedian lips. As in *Plectus,* we believe that the inner labial sensilla of *Tylocephalus* have migrated into the rim of the mouth opening, so that these are (always?) hidden from view under SEM.

FEEDING BEHAVIOUR

The observed movements of the lip region during feeding of *T. auriculatus* are constant and regular, suggesting that these are its natural behaviour and not an artefact of the culturing conditions. In particular, the lateral flattening, dorsoventral expansion and complex anterior morphology of this and other Wilsonematinae become easier to interpret functionally : they are probably specialized adaptations allowing these animals to sweep up bacteria attached to more or less flat surfaces in the soil, such as leaf or shell fragments and other particles of organic origin. Sauer and Annells (1982) observed variations in the inclination of the cornua and interpreted these as signs of lateral motility and scooping actions, coupled with the opening and closing of the mouth. We have not seen evidence for or against autonomous motility, but assume that the sweeping behaviour allows the cornua to function as bacterial scrapers and scoops regardless of such motility.

It must be noted that many (if not all) nematodes move their lip region away from the body axis during feeding and/or exploration. However, these movements are much more regular and pronounced in *T. auriculatus* than usual for bacterial feeders, and in combination with the complicated labial processes this must allow them to gather bacteria from a wider search path and thus feed with greater efficiency. We assume that this functional combination of behaviour and morphology is most advantageous for nematodes crawling along a flat surface, and therefore hypothesize that Wilsonematinae are specialized in feeding on microscopic flat surfaces in soils.

Acknowledgements

We wish to thank Drs A. M. Golden and R. V. Anderson for their help in finding the consulted reference material. We are also grateful to Dr. H. Zell for sharing his results with us prior to the publication of his monograph on *Plectus*. The first author is postdoctoral researcher with the Belgian National Fund for Scientific Research.

References

ALKEMADE, J. R. M. & LOOF, P. A. A. (1989). Observations on the ontogeny of some *Xiphinema* species (Nematoda : Dorylaimida). *Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 54 : 1177-1186.

ANDERSON, R. V. (1966). An emendation of the diagnosis of both the subfamily and two genera of Wilsonematinae and a new genus, *Ereptonema* n. g. *Can. J. Zool.*, 44 : 923-935.

ANDRÁSSY, I. (1984). *Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida)*. Stuttgart, Germany, Gustav Fischer Verlag. 509 p.

BIRD, A. F. & BIRD, J. (1991). *The structure of nematodes. 2nd edition.* San Diego, USA, Academic Press, 316 p.

CHITAMBAR, J. J. & NOFFSINGER, E. M. (1989). Taxonomy and postembryonic stages of the nematode predator *Odontopharynx longicauda* de Man, 1912 (Diplogasterida). *J. Nematol.*, 9 : 189-201.

CROSSMAN, L. (1933). Preliminary observations on the life history and morphology of *Tylocephalus bacillivorus* n. g.; n. sp.; a nematode related to the genus *Wilsonema*. *J. Parasit.*, 20 : 106-107.

DE LEY, P. & COOMANS, A. (1994). Terrestrial nematodes from the Galápagos Archipelago IV : The genus *Plectus* Bastian, 1865, with description of three new species (Leptolaimina : Plectidae). *Bull. Inst. Roy. Belge Sci. nat., Biol.*, 64 : 43-70.

DE LEY, P., LOOF, P. A. A. & COOMANS, A. (1993). Terrestrial nematodes of the Galápagos Archipelago II : Redescription of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968, with review of similar species and a nomenclature for the vagina in Dorylaimida (Nematoda). *Bull. Inst. Roy. Belge Sci. nat., Biol.*, 63 : 13-34.

DECRAEMER, W. (1995). *The family Trichodoridae : stubby root and virus vector nematodes.* Dordrecht, The Netherlands, Kluwer Academic Publishers, 360 p.

GADÉA, E. (1977). Nematofauna muscícola de las islas Galápagos. *Misc. Zool.*, 4 : 17-22.

HALBRENDT, J. F. M. & BROWN, D. F. J. (1992). Morphometric evidence for three juvenile stages in some species of *Xiphinema americanum sensu lato*. *J. Helminth.*, 24 : 305-309.

MAGGENTI, A. R. (1961). Morphology and biology of the genus *Plectus* (Nematoda : Plectidae). *Proc. helminth. Soc. Wash.*, 28 : 118-130.

MORTON, H. V. & PERRY, V. G. (1968). Life cycle and reproductive potential of *Trichodorus christiei*. *Nematologica*, 14 : 11 [Abstr.].

SAUER, M. R. (1985). *A scanning electron microscope study of plant and soil nematodes.* Australia, CSIRO, 64 p.

SAUER, M. R. & ANNELLS, C. M. (1982). An SEM study of *Tylocephalus auriculatus* (Nematoda : Plectidae). *Nematologica*, 28 : 123-126.

SHISHIDA, Y. (1983). Studies on nematodes parasitic on woody plants. 2. Genus *Xiphinema* Cobb, 1913. *Jap. J. Nematol.*, 12 : 1-14.

TAHSEEN, Q., AHMAD, I. & JAIRAJPURI, M. S. (1995). Two new species of Plectidae from India (Nematoda : Araeolaimida). *Fundam. appl. Nematol.*, 18 : 471-477.

YAGITA, H. (1975). The life-history and biology of the needle nematode, *Longidorus martini* Merny. I. Morphological measurements and developmental stages. *Jap. J. Nematol.*, 5 : 10-15.

ZELL, H. (1985). Nematoden eines Buchenwaldbodens. 5. Die Wilsonematinae (Nematoda, Araeolaimida). *Carolinea*, 43 : 77-92.

ZELL, H. (1993). Die Gattung *Plectus* Bastian, 1865 *sensu lato* (Nematoda, Plectidae) - Ein Beitrag zur Ökologie, Biogeographie, Phylogenie und Taxonomie der Plectidae. *Andrias*, 11 : 3-171.