OCULAR OXYSPIRUROSIS OF PRIMATES IN ZOOS: INTERMEDIATE HOST, WORM MORPHOLOGY, AND PROBABLE ORIGIN OF THE INFECTION IN THE MOSCOW ZOO

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Summary:

Over the last century, only two cases of ocular oxyspirurosis were recorded in primates, both in zoos, and two species were described: in Berlin, Germany, *Oxyspirura (O.) conjunctivalis* from the lemurid *Microcebus murinus*, later also found in the lorisid *Loris gracilis*; in Jacksonville, Florida, *O. (O.) youngi* from the cercopithecid monkey *Erythrocebus patas*. In the present case from the Moscow zoo, oxyspirurosis was recorded in several species of Old World lemuriforms and lorisiforms, and some South American monkeys. *i*) The intermediate host was discovered to be a cockroach, as for *O. (O.) mansoni*, a parasite of poultry. The complete sequence identity between ITS-1 rDNA from adult nematodes of the primate and that of the larval worms from the vector, *Nauphoete cinerea*, confirmed their conspecificity. *ii*) Parasites from Moscow zoo recovered from *Nycticebus c. coucang* were compared morphologically to those from other zoos. The length and shape of the gubernaculum, used previously as a distinct character, were found to be variable. However, the vulvar bosses arrangement, the distal extremity of left spicule and the position of papillae of the first postcloacal pair showed that the worms in the different samples were not exactly identical and that each set seemed characteristic of a particular zoo. *iii*) The presence of longitudinal cuticular crests in the infective stage as well as in adult worms was recorded. Together with several other morphological and biological characters (long tail and oesophagus, cockroach vector), this confirmed that *Oxyspirura* is not closely related to *Thelazia*, another ocular parasite genus. *iv*) The disease in the Moscow zoo is thought to have started with *Nycticebus pygmaeus* imported fromVietnam, thus the suggestion was that Asiatic lorisids were at the origin of the Moscow set of cases. The natural host(s) for the Berlin and Jacksonville cases remain unknown but they are unlikely to be the species found infected in zoos. Consequently the notion of type hosts is artificial and the three agents of oxyspirurosis are provisionally placed in the taxon *O. (O.) conjunctivalis*.

KEY WORDS : *Oxyspirura (O.) conjunctivalis, O. (O.) youngi*, Thelazioidea, Nematodes, Primates, Lorisids, *Nycticebus* spp., life cycle, cockroache, ITS-1 rDNA.

Résumé : OXYSPIRUROSE OCULAIRE DE PRIMATES DE ZOOS: HÔTES INTERMÉDIAIRES, MORPHOLOGIE DU VER, ET ORIGINE PROBABLE DE L'INFECTION DANS LE ZOO DE MOSCOU

Deux cas seulement d'oxyspirurose ont été signalés chez les primates au cours du siècle dernier, tous deux dans des zoos, et deux espèces ont été décrites : à Berlin, Allemagne, Oxyspirura (O.) conjunctivalis *chez le lémuridé* Microcebus murinus, *retrouvé aussi chez le lorisidé* Loris gracilis*; à Jacksonville, Floride,* O. (O.) youngi *chez le cercopithecidé* Erythrocebus patas. *L'oxyspirurose a été observée chez plusieurs espèces de lémuriformes et lorisiformes de l'Ancien Monde, et chez des singes d'Amérique du Sud dans le cas étudié ici, au zoo de Moscou.* i*) L'hôte intermédiaire a été identifié, c'est une blatte, comme pour* O. (O.) mansoni, *parasite des poules. La séquence d'ITS-1 ADNr des nématodes adultes récoltés chez le primate est identique à celle des larves récoltées chez le vecteur,* Nauphoete cinerea, *ce qui confirme leur conspécificité.* ii*) L'analyse morphologique comparée des parasites du zoo de Moscou récoltés chez* Nycticebus c. coucang *et de ceux des autres zoos a été effectuée. Le gubernaculum, utilisé précédemment comme caractère distinctif, apparaît variable en taille et forme. Cependant, l'arrangement des bosses cuticulaires de la région vulvaire, l'extrémité distale du spicule gauche et la position des papilles de la première paire postcloacale montrent que la morphologie des spécimens de diverses provenances n'est pas identique et semble caractéristique de chaque zoo.* iii*) Des crêtes cuticulaires longitudinales chez le stade infectant ainsi que chez l'adulte sont mises en évidence. Ce trait, joint à d'autres caractères morphologiques et biologiques (queue et oesophage longs; blatte comme hôte intermédiaire), confirment qu'*Oxyspirura *est éloigné de* Thelazia, *également parasite oculaire.* iv*) Au zoo de Moscou, il semble que la maladie ait débuté avec* Nycticebus pygmaeus *importé du Vietnam, et les lorisidés asisatiques seraient ici à l'origine des cas observés. L'hôte (ou les hôtes) naturel(s) des cas de Berlin et de Jacksonville restent inconnus, mais il est peu probable que ce soient les espèces trouvées infectées dans ces zoos. Il en résulte que la notion d'hôte-type est ici artificielle et les trois agents d'oxyspirurose sont placés provisoirement dans le taxon* O. (O.) conjunctivalis.

MOTS CLÉS : Oxyspirura (O.) conjunctivalis, O. (O.) youngi, *Thelazioidea, Nématodes, Primates, Lorisidés,* Nycticebus *spp., cycle, blatte, ITS-1 ADNr.*

INTRODUCTION

An ocular disease of several species of lower and higher primates in the Moscow zoo was registered since 1995. It was caused by nematodes a few millimetres long, living in the eye orbit. Sick primates were treated regularly against nematodes but

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infection restarted every several months. As far as we know no attempts to identify the nematode species had been made until 2003 when it was incorrectly identified as *Thelazia callipaeda* Railliet & Henry, 1910, an ocular parasite of dogs transmitted by muscid flies. Later, examination of the worms showed the long conical tail and posterior vulva characteristic for another thelazioid genus, *Oxyspirura* Drasche *in* Stossich, 1897 (Alshinetski *et al.*, 2005). Five of the six subgenera ascribed to the genus are parasitic in birds and the sixth one in primates and birds (Barùs, 1963; Chabaud, 1975). The Moscow specimens with their divided buccal cavity belonged to the latter subgenus, *Oxyspirura*.

Ocular oxyspirurosis among primates was reported only twice (Linstow, 1907; Addison *et al.*, 1986). Interestingly, like in Moscow, the cases were observed in zoos (Berlin, Germany; Jacksonville, Florida). Two causative agents were described. *O.* (*O.*) *conjunctivalis* (Linstow, 1907) was recovered from the lemuriform *Microcebus murinus* J. Miller, 1777. The brief description by Linstow was later improved by Baer (1935) who studied the types and specimens from the lorisiform *Loris gracilis* (É. Geoffroy, 1812), also from the Berlin zoo. *Oxyspirura (O.) youngi* Addison *et al.*, 1986 was recovered from the African cercopithecid monkey *Erythrocebus patas* Schreber, 1775 and was distinguished by the longer gubernaculum only.

The great similarity of the two *Oxyspirura* species despite the unrelated zoological and geographical position of their hosts suggested that the ocular disease observed in zoos might be the result of an intense transmission in captivity, leading to extension of infection to non-natural hosts. We suspected cockroaches to be the intermediate hosts, as in the case of *O. (O.) mansoni* (Cobbold, 1879) from poultry, the only life-cycle known in the genus (Anderson, 2000). The vectors were sought among cockroaches present in the Moscow zoo, the morphology of the ocular worms from Moscow, Berlin and Jacksonville zoos was compared and the history of infection in the Moscow zoo was traced.

MATERIALS AND METHODS

An experimental infection of nematode-free cockroaches from laboratory cultures was performed, and naturally infected cockroaches trapped in and around the monkey cages were collected. For experimental studies, 30 *Nauphoete cinerea* (Olivier) were fed with nematode eggs, recovered from a gravid female from an eye orbit of *N. coucang coucang*, offered on fruits to nymphs and in a small amount of water through a pipette to adult cockroaches. These experimental infections did not succeed (15 nymphs and 15 adults were dissected three days, seven days and a month after start of experiment).

Four species of cockroaches were trapped in glass jars containing small amount of beer, *Periplaneta americana* L., 1758, *Gromphadorrhina portentosa* Schaum, 1853, *B. germanica* L., 1767 and *N. cinerea* (Olivier). Cockroaches were dissected in one-quarter Ringer's solution. Also several specimens of *Blaberus cranifer* L., 1758 from laboratory cultures bred for feeding to primates were examined.

To prove conspecificity of adult nematodes from eye orbits and nematode juveniles from cockroaches' haemocoel, highly species-specific ITS rDNA sequences were obtained and compared. Material for DNA extraction was collected from cockroaches (*N. cinerea* and *B. germanica*) and eye orbit of *N. c. coucang* Boddaert, 1785 and preserved in 70 % ethanol. Ethanol-fixed nematodes were rinsed in autoclaved HPLC-quality water overnight. Anterior body part of nematode was removed by blade and transferred into the tube with 8 µl of worm lysis buffer (100 mM KCl, 20 mM Tri-HCl pH 8.3, 3 mM $MgCl_2$, 2 mM DTT and 0.9 % Tween 20), 10 µl of autoclaved water and 2 µl of autoclaved (600 µg ml^{-1}) solution of proteinase K. Specimens were incubated in such a mix for one hour under 65° C plus 10 min treatment at 95° C for enzyme inactivation. Aliquots (1-6 µl) of this homogenate were used as template in PCR of 20 µl total volume (Master-mix and conditions of PCR were according to Phan *et al.*, 2006). Two sets of primers for ribosomal DNA were tested (for ITS rDNA: 18S and 26S by Vrain *et al.*, 1992, TW81 and AB28 by Curran & Driver, 1994). None of these pairs produced visible amplification. For further study primers were constructed on the basis of the ITS-1 rDNA sequences for taxonomically close nematodes of the genus *Thelazia* (Otranto *et al.*, 2001; Otranto & Traversa, 2004). According to selected conservative regions on the 3' and 5' ends of *Thelazia* ITS-1, two primers were designed: SPIR18 (5' - TGA ACC TGC GGA AGG ATC ATT-3') SPIR58 (GCA GCT RGC TGC GTK CTT CAT). Obtained PCR-products (about 720 bp for nematodes from *Nycticebus* eye orbit and *Nauphoete* cockroach), and about 1,400 bp for spirurid juveniles from *B. germanica*) were run in 0.8 % agarose gel for 1.5 hours, then band with product was excised. DNA was extracted from gel blocks and used for ligation with PGemT vector. *Escherichia coli* competent cells (JM109 from Promega™) were transformed with ligation product according to Promega protocols. Plasmids from white colonies were extracted with standard Qiaquick Qiagene kits. Cleaned DNA was precipitated in ethanol and used for sequencing in ABI Prism Big-Dye Termination Mix with plasmid primers SP6 and T7. Sequences obtained in "ab1" format were checked in Chromas 1.45., exported as FASTA files and aligned in Clustal × 1.81. Obtained *Oxyspirura* sequence was

deposited in NCBI GenBank under accession number EF 417873. Sequences for *Thelazia* (*T. callipaeda* Railliet & Henry, 1910 – AY207464; *T. gulosa* Railliet & Henry, 1910 – AF 337897; *T. lacrymalis* (Gurlt, 1831) – AY 208137; *T. rhodesi* (Desmarest, 1827) – AF 337895 and *T. skrjabini* Erschov, 1929 – AF 337896) nematodes were used to construct alignments for obtained spirurid sequences. Live nematodes for morphological study were recovered from *N. c. coucang*. They were removed by rinsing the eye orbits with Ringer's solution and collected from eye surface by forceps during the veterinary treatment of the sick animal, then transported in Ringer's solution to the laboratory for examination. Nematodes for morphological study were fixed in 5 % hot formaldehyde. For SEM, they were dehydrated in ethanol and acetone series, dried at critical point dryer, coated with gold and studied in Jeol and Camscan microscopes. For light microscopy, they were processed into glycerol (Seinhorst, 1959) and mounted on slides, or they were cleared in lactophenol, the slide and coverslide not sealed in order to orient the worm in different positions. Apical views of the head was made on adult worms and infective larvae. Study of gubernaculum and ovejector was performed on dissected specimens. Drawings and measurements were made using Jenaval or Wild light microscopes equipped with a drawing tube. DIC photographs were taken using AxioImager A1 microscope. Measurements are given in µm. Moscow samples were compared morphologically to the three lots from primates conserved in national collections: the syntypes of *O. (O.) conjunctivalis*, the second sample of this species from Berlin (Museum, Berlin), and four paratypes of *O.(O.) youngi* (US parasite collection, Beltsville). In addition, females of *O (O.) mansoni* were observed to assess the morphology of the body cuticle (specimens from *Gallus gallus*, Vietnam, number 223 JW, Muséum National d'Histoire Naturelle, Paris, recovered by Mathis & Léger, date not stated).

RESULTS

IDENTIFICATION OF THE INTERMEDIATE HOST

Among the four cockroach species trapped, the German cockroach *B. germanica*, known as a highly pestilent natural resident in all zoo lodgings, was present in high density. *N. cinerea* and *P. americana* were also frequently observed in primates' lodgings indicating that wild populations of these laboratory cultured cockroach species were already established there. *G. portentosa* were found only occasionally outside culturing containers.

Spirurid nematodes were found in *N. cinerea* inhabiting monkeys'cages. On thirty three specimens captured and dissected (seven males and 26 females), 20 cockroaches were infected (seven males and 13 females). A total of 223 capsules 650-900 µm long and 450-750 µm wide were found in the fat body. Number of capsules in cockroaches ranged from 2 to 99. Each capsule contained one larva 2,378-3,983 µm long, except one with two larvae. In three cockroaches, some capsules (26/96) were melanized and empty. Larvae in capsules were motionless but activated in 20-30 sec time, then penetrated the capsule wall into Ringer's solution. They stayed actively moving for two days then died. PCR-product of similar size (720 bp) was obtained for these larvae from *N. cinerea* and adults of *Oxyspirura* nematodes from orbital cavity of *N. c. coucang*. Alignment of ITS1 rDNA sequences demonstrated the complete identity of the sequences of larvae and adults.

Spirurid larvae morphologically different from those from *N. cinerea* were recovered from *B. germanica*. Five cockroaches of 43 trapped and dissected were infected by 1-8 shorter (around 800 long) spirurid nematodes, each in a separate capsule. PCR-product for these larvae was much larger (1,400 bp) than obtained for larvae from *N. cinerea*. These larvae were excluded from the further analysis.

No spirurid nematodes were found in the three other species examined: *P. americana, G. portentosa* and *Blaberus cranifer* (21, 10 and 5 specimens, respectively).

MORPHOLOGICAL STUDY OF WORMS FROM PRIMATES' EYE ORBITS AND INFECTIVE LARVAE FROM THE COCKROACH *N. CINEREA*

• Specimens from the lorisiform *Nycticebus c. coucang*, Moscow

Two samples: nine males, 14 females and nine larvae, recovered from one host in January 2004; 18 males, 24 females and 15 larvae recovered from another animal in February 2005. Deposited in the Museum of helminhological collections of the Centre for Parasitology of Severtsov Institute of Ecology and Evolution RAS; five slides (four males, four females, two juveniles), collection numbers 14185 to 14189. Some of these specimens were deposited in the Museum National d'Histoire Naturelle, Paris: two males, collection number 43 JW and 44 JW; one female 46 JW; males and females 48 JW, from the first loris; one male and one female 201 JW from the second loris; molting juveniles 47 JW and 219 JW, recovered in 2004.

Adult worms

The general morphology is as described by Baer (1935). Several previously undescribed characters were detailed. Cuticle (Figs 1J & K, 2 K & L, 3B & C, 4A): ornate in both sexes with longitudinal discontinuous crests, transverse annulations and transverse thin striae (6-9

per annulations); this ornamentation begins anterior to nerve ring and ends at mid-tail. Head (Figs 1B & C, 2I, 3A): mouth subround; six rudimentary lips, each with one salient internal labial papilla; four atrophied external labial papillae not reaching the cuticle surface and identified by their deep sensory nerve (not seen at SEM); posterior circle of four prominent button-like cephalic papillae and amphids. Buccal capsule (Figs 1B, C & 2I): base of its anterior third delineated by a thickened transverse ring which is uniform or forms tooth-like irregularly placed thickenings, 3-5 in number; in some specimens, tooth posterior to ring present. Buccal cavity subhexagonal in the anterior third, posteriorly flattened in the lateral plan. Apex of oesophagus sclerotized at its junction with the buccal capsule. Deirids like tiny pin, situated between nerve ring and excretory pore. Excretory pore and cell conspicuous; two sinuous excretory ducts can be traced to tail in the lateral chords, which are 22-28 µm wide. Phasmids at posterior third of tail, a small boss at their apex.

Female

Vulva: longitudinal slit opening in an area delimited by circular ridge 30-35 µm in diameter. On mid-line near vulva, numerous large cuticular bosses (Fig. 3C); often extend more posteriorly than anteriorly to vulva (180 µm and 30 µm from vulva, respectively, Fig. 1J). Vagina 200 µm long, lumen twisted near vulva. Ovejector: 2,100 µm long in the dissected specimen (Fig. 1L), composed of pouch 350 µm long with thin wall, short sphincter with thick epithelium, a tube 1,200 µm long with thick eptihelium and thin muscular layer; in the proximal portion of the tube, epithelium composed of high and narrow cells. The pouch often contained embryonated eggs within a mass of spermatozoa, 6-7 µm in diameter (Figs 1L & 4B). Mature oocytes elongated, bullet-shaped, thin-walled. Oval embryonated eggs with smooth shell.

Female measurements (n = 10): Body length 10,898 ± 1,237 (6,200-12,795); width at mid-body 266 ± 30 (206-308), at anus 73 ± 14 (52-101); nerve ring 191 ± 11 (175-210) from apex; excretory pore 281 ± 34 (231-320) from apex; deirids 212 ± 15 (190-250) from apex; buccal capsule length 36 ± 4 (30-42); oesophagus length 767 ± 54 (686-863); tail length 254 ± 33 (210-304); phasmids 50-70 from tail extremity; vulva 774 ± 853 (660-904) from tail tip; egg 46 ± 2.3 (43-50) long and 26 ± 2 (24-30) wide. First stage larva (n = 4): length 165 (the single larva not broken), maximum width 8-9, excretory pore 65-75 from apex.

First stage larva extracted from egg (Fig. 2N, O, P, Q): body wider in the anterior third, then tapering; lateral alae and transverse striations conspicuous; head narrow in median view; a small left cephalic hook and no other cephalic spines; excretory pore conspicuous and sclerotized; conical tail with constricted extremity.

Male

Left spicule thin with short handle and very long lamina; round membranous distal extremity (Fig. 1E). Gubernaculum: composed of solid thick posterior portion and thinner lateral walls of variable shape, more or less elongated anteriorly, fused with sclerotized left or right spicular sheeth (Fig. 1D, F); the anterior end is difficult to clearly define, preventing accurate measurements. Caudal papillae (Figs 1I, 3D): an unpaired precloacal papilla; three precloacal subventral pairs; one paracloacal sublateral pair; two postcloacal pairs, not joined on median line, situated asymmetrically; phasmids at posterior third of tail. Tail nearly straight, slightly bent in ventral direction. Tail tip conical; in several specimens, distinct subterminal transverse stria (Fig. 1H).

Male measurements (n = 12): Body length 7,234 ± 880 (5,801-8,213); width at mid-body 220 ± 29 (180-270), at anus 105 ± 9 (94-120); nerve ring 188 ± 29 (150-270) from apex; excretory pore 244 ± 47 (170-312) from apex; deirids 204 ± 32 (160-288) from apex; buccal capsule length 34 ± 6.2 (27-45); oesophagus length 664 ± 30 (589-698); tail length 271 ± 35 (214-323); phasmids 50-70 from tail extremity; left spicule length 1,282 ± 106 (1,078-1,433); right spicule length 182 ± 39 (125-251); gubernaculum length 40-120.

Fourth stage larvae

Head structure as in adult worms (Fig. 1R). Deirids 25-35 µm posterior to nerve ring. Buccal cavity less sclerotized, shorter and narrower than in adult nematodes; division in two parts less distinctive. Tooth-like thickenings in buccal cavity less prominent than in adult worms (Fig. 1R). Tail of both sexes ends with short onion-shaped pointed mucron (Fig. 1U, V). Female larvae: vulvar and ovarial primordia appear separately, do not connect in earlier stage; vulva posterior (Fig. 1T). Male larvae: right spicule appears earlier than left one and gubernaculum (Fig. 1S).

Measurements (n = 10): Body length 3,922 ± 466 (3,195-4,845); width at mid-body 117 ± 23 (78-150), at anus 59 ± 10 (51-81); nerve ring 162 ± 14 (130-180) from apex; excretory pore 238 ± 21 (210-275) from apex; oesophagus length 552 ± 32 (492-588); tail length 198 ± 35 (165-270).

• Specimens from the lemuriform *Microcebus murinus*, Berlin

Syntypes of *O. (O.) conjunctivalis* (Linstow, 1907), collection number 4463, Museum für Naturkunde der Humboldt-Universität zu Berlin; re-examined by Baer (1935). Three males and five females examined in this study. Worms were well fixed but brownish. To not damage the fragile specimens, no ventral view of male was observed

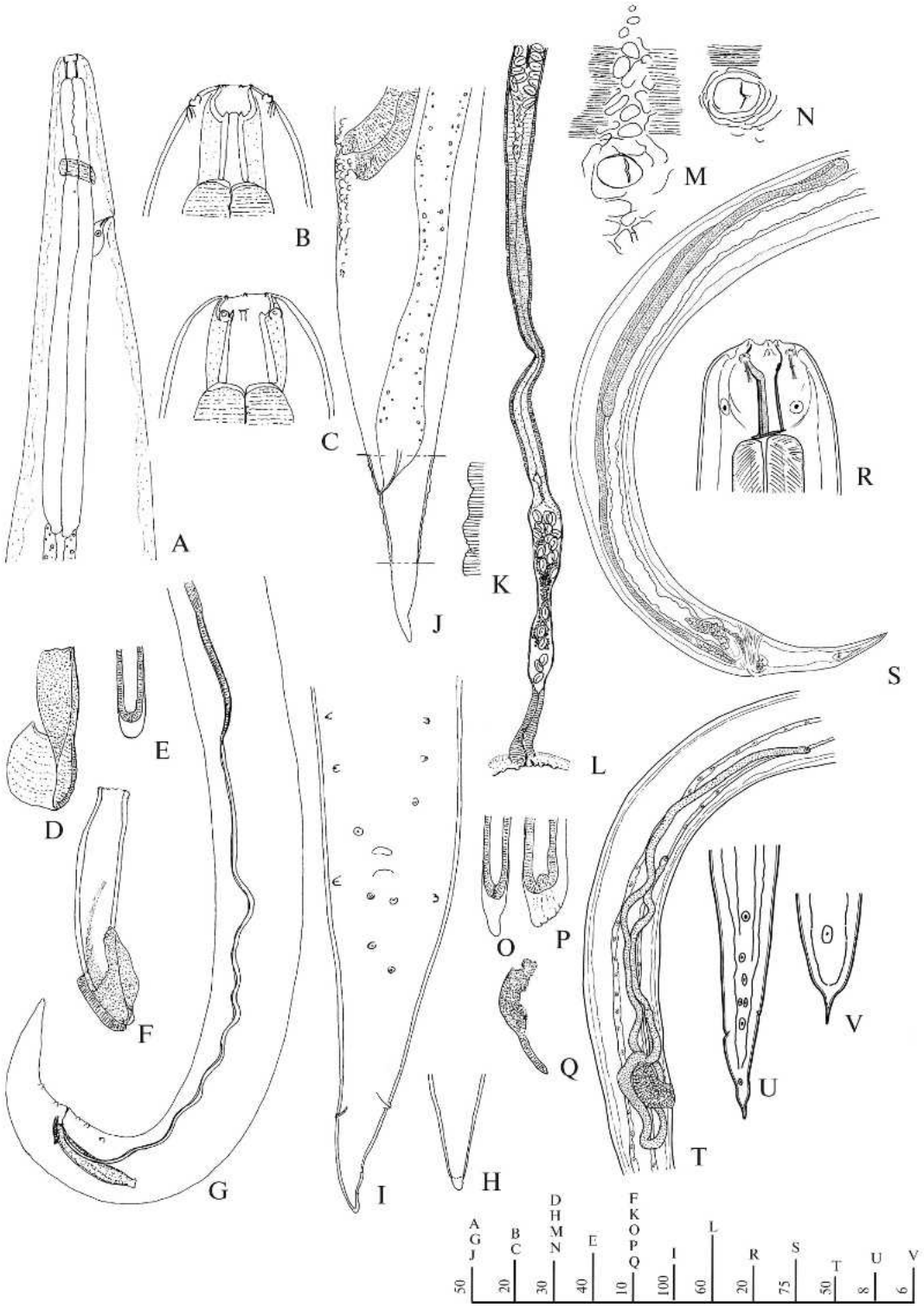

Fig. 1. – *Oxyspirura (O.) conjunctivalis*. A-Q. Adult specimens. A-L. From *Nycticebus c. coucang*. A-G. Male. A. Anterior region, left lateral view. B. Head, dorsal view. C. Head, lateral view. D. Gubernaculum, left lateral view, with a long anterior part. E. Posterior extremity of left spicule, ventral view. F. Right spicule and gubernaculum of another specimen, right lateral view. G. Posterior region of body, left lateral view. H. Tail extremity, ventral view. I. Tail, caudal papillae and phasmids, ventral view. J-L. Female. J. Posterior region and vulvar bosses (more extended posterior to vulva), left lateral view. K. Cuticular striations and annulations, at level of oesophagus, lateral view. L. Ovejector dissected out from a female; pouch with embryonated eggs and spermatozoa. M & N. Cuticular bosses near vulva, ventral view, from *Erythrocebus patas* (more developed anteriorly) and *Loris gracilis* (6101), respectively. O & P. Posterior extremity of left spicule of two specimens, lateral and median view, from *L. gracilis* (6101). Q. Gubernaculum, left lateral view, from *Microcebus murinus* (4463; syntype). R-V. Larvae from *N. c. coucang*. R. Head, lateral view. S. Genital primordium, male larva, right lateral view. T. Genital primordium, female larva. U & V. Tail extremity, lateral and ventral view, respectively. Scale bars in µm.

The majority of characters (cuticle ornamentation, deirids, left spicule with handle and lamina, excretory cell and ducts, deirids, phasmids) similar to Moscow specimens. However vulvar bosses are absent or form one or two close circles of few narrow bosses or ridges; the membranous extremity of the left spicule, seen in one male, longer than wide. Gubernaculum difficult to analyse (Fig. 1Q), with or without lateral walls extending anteriorly, these not strongly sclerotized. Caudal papillae salient and conical, as figured by Baer (1935); phasmids inconspicuous.

Measurements
Female (n = 5). Body length 9,942 ± 1,038 (8,650-11,000); width at mid-body 298 ± 14 (280-315); nerve ring 195 ± 25 (160-270) and excretory pore 284 ± 33 (245-320) from apex; deirids 247 from apex (identified in one specimen); buccal capsule length 28 ± 3 (25-32); oesophagus length 635 ± 71 (560-730); tail length 245 ± 37 (200-280) ; vulva 660 ± 201 (450-1200) from tail tip.
Male (n = 3). Body length 6,017 ± 126 (5,900-6,750); width at mid-body 183 ± 23 (170-210); nerve ring and excretory pore 191 ± 13 (180-205) and 270 ± 36 (230-300) from apex, respectively; deirids 228 ± 32 (210-270) from apex; buccal capsule length 26 ± 4 (23-30); oesophagus length 568 ± 41 (540-615); tail length 258 ± 61 (190-308); left spicule length 1,192 ± 250 (1,000-1,415); right spicule length 192 ± 50 (162-250); gubernaculum length 53 ± 8 (42-85).

• Specimens from the lorisiform *Loris gracilis (= Stenops gracilis)*, Berlin

Collection number 6101 (= Q 1940), Museum für Naturkunde der Humboldt-Universität zu Berlin; examined by Baer (1935). Four males (one without tail) and nine females examined in this study, in excellent condition of preservation. Two male tails were observed in ventral view.
General morphology as described earlier. Vulvar cuticular bosses poorly developed, one to three circles of narrow bosses or ridges (Fig. 1N). Membranous extremity of left spicule longer than wide (Fig. 1O & P). Salient conical caudal papillae in male; first pair of postcloacal papillae not joined on median line. Gubernaculum without/with lateral walls extending anteriorly, the latter not strongly sclerotized if present. Female and male tail extremity with/without subterminal transverse stria.

Measurements
Female (n = 9). Body length 8,309 ± 1,767 (5,000-10,700); width at mid-body 278 ± 22 (250-310); nerve ring 206 ± 11 (200-230) and excretory pore 311 ± 21 (280-340 from apex); deirids 264 ± 37 (205-280) from apex; buccal capsule length 33 ± 2 (29-35); oesophagus length 705 ± 58 (630-810); tail length 257 ± 19 (240-280); phasmids 50-78 from tail tip; vulva 664 ± 81 (600-800) from posterior extremity.
Male (n = 4). Body length 6,013 ± 541 (5,750-6,750); width at mid-body 195 ± 13 (180-210); nerve ring 184 ± 23 (155-205) and excretory pore 272 ± 40 (230-310) from apex; deirids 241 ± 35 (205-280) from apex; buccal capsule length 30 ± 1 (29.5-30); oesophagus length 585 ± 9 (575-595); tail length 222 ± 30 (190-250); left spicule length 1,093 ± 90 (1,000-1,180); right spicule length 152 ± 10 (155-160); gubernaculum length 78 ± 25 (48-100); phasmids 57 ± 5 (52-60) from tail tip.

• Specimens from the cercopithecid *Erythrocebus patas*, Jacksonville

Paratypes of *O. (O.) youngi* Addison *et al.,* 1986, storage number MT78-G, U.S. National Parasite collection, Beltsville. Two males and two females were examined. No ventral view of males observed.
General morphology similar to other samples. In vulvar region cuticular bosses well developed, extending more anteriorly than posteriorly to vulva (85 µm *vs* 40 µm, in a female Fig. 1M; 50 µm *vs* no bosses in the other one). Membranous extremity of left spicule short. Gubernaculum of simple (in one specimen) or complex (in the other one) shape. Ring of the buccal capsule of a female with a double tooth on a lateral side, the anterior one large and the posterior one of usual size. Ovejector of a female 1,600 µm long; pouch with embryonated eggs and spermatozoa.

Measurements
Female. Body length 11,055 & 12,055; width at mid-body 360 & 400; nerve ring 200 & 235 and excretory pore 320 & 350 from apex; deirids 260 & 265 from apex; buccal capsule length 32 & 40; oesophagus length 720 & 860; tail length 280 & 325; vulva 720 & 775 from tail tip; phasmids ND & 87 from tail tip.
Male. Body length 6,700 & 8,000; width at mid-body 250 & 255; nerve ring and excretory pore 190 & 195 and 270 & 280 from apex; deirids 217 & 265 from apex; buccal capsule length 30 & 30; oesophagus length 675 & 720; tail length 265 & ND (cut); phasmids 72 & ND from tail tip; left spicule length 1,130 & 1,332 long; right spicule length 165-200 long; gubernaculum length 50 & 115.

• Infective larvae from *Nauphoete cinerea*, Moscow

Live larvae pinkish in colour, white when fixed. Body tapering in posterior two thirds (Fig. 2A, B). Cuticle: longitudinal crests present, 6-8 per quadrant (Fig. 2G). Head (Fig. 2C, D): six protruding lips; each of four submedian ones bearing one internal labial papilla and smaller external labial papilla; lateral lips each with an internal labial papilla only. Outer circle of button-like cephalic papillae and amphids. Deirid, a simple point. Mouth aperture: hexagonal, slightly flattened laterally.

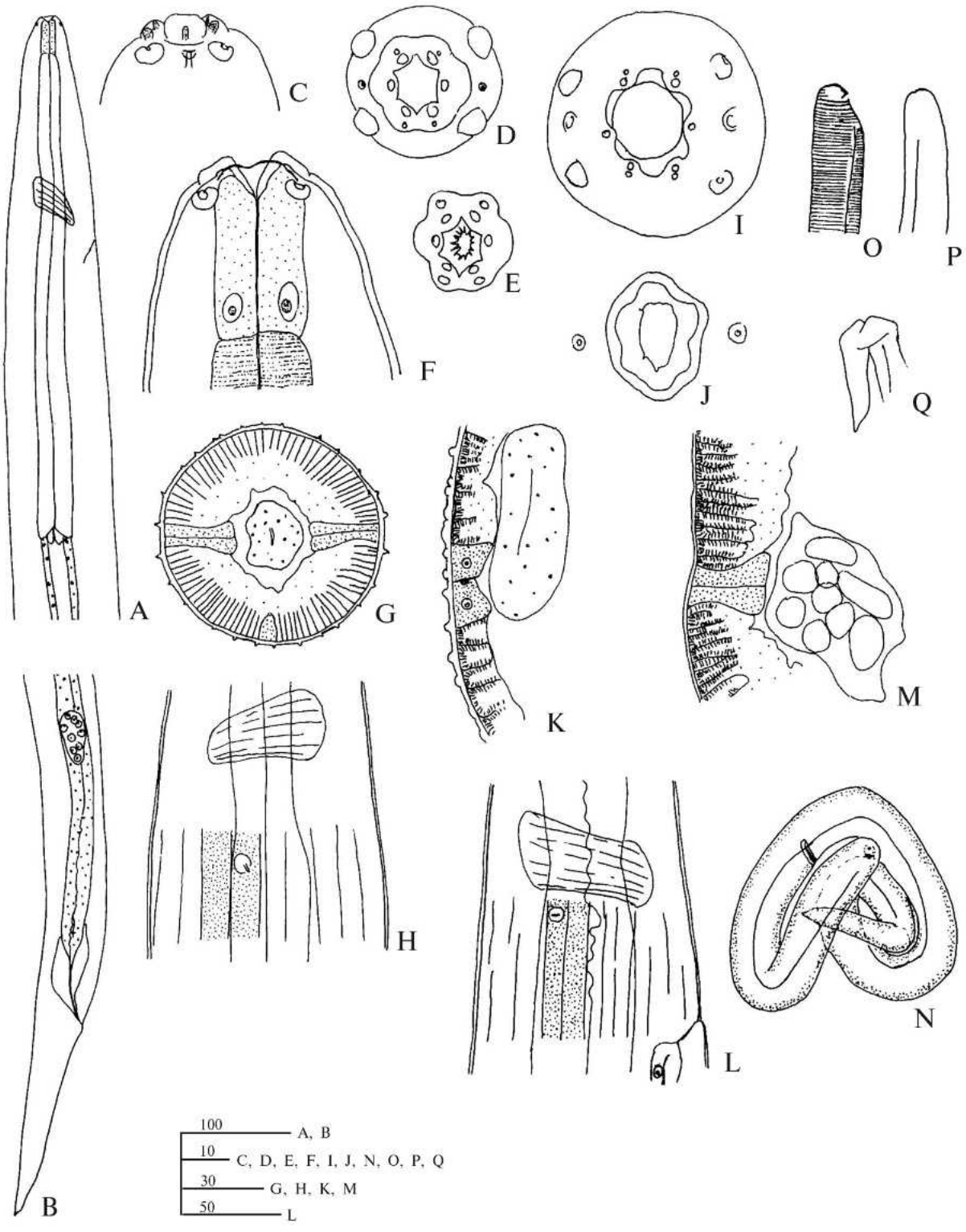

Fig. 2. – *Oxyspirura (O.)* spp. A-I. *O. (O.) conjunctivalis* infective larva from *Nauphoete cinerea*. A. Anterior part, right lateral view. B. Posterior part, right lateral view, same larva. C. Head, lips and papillae, lateral superficial view. D. Head, apical view. E. Same head, at a deeper level, to show the structure of buccal cavity. F. Cephalic end, median view. G. Transverse section, at mid-body. H. Deirid, lateral chord and longitudinal crests at level of nerve ring, left lateral view. I-L. *O. (O.) conjunctivalis* male, from *Nycticebus c. coucang*, Moscow. I. Head, apical view. J. Head, at level of anterior/posterior chambers of buccal cavity, apical view. K. Lateral region of a transverse section at mid-body, with longitudinal crest and intestine. L. Nerve ring, deirid, lateral chord, longitudinal crests and excretory pore, right lateral view. M. *O. (O.) mansoni* from *Gallus gallus:* lateral region of a transverse section at mid-body, and a uterus. N-Q. First stage larva extracted from egg of *O. (O.) conjunctivalis* from *N. c. coucang*. N. Larva. O & P. Anterior part and lateral alae, with head in dorsal view and in right lateral view, respectively (transverse striae not drawn in P). Q. Tail region. Scale bars in µm.

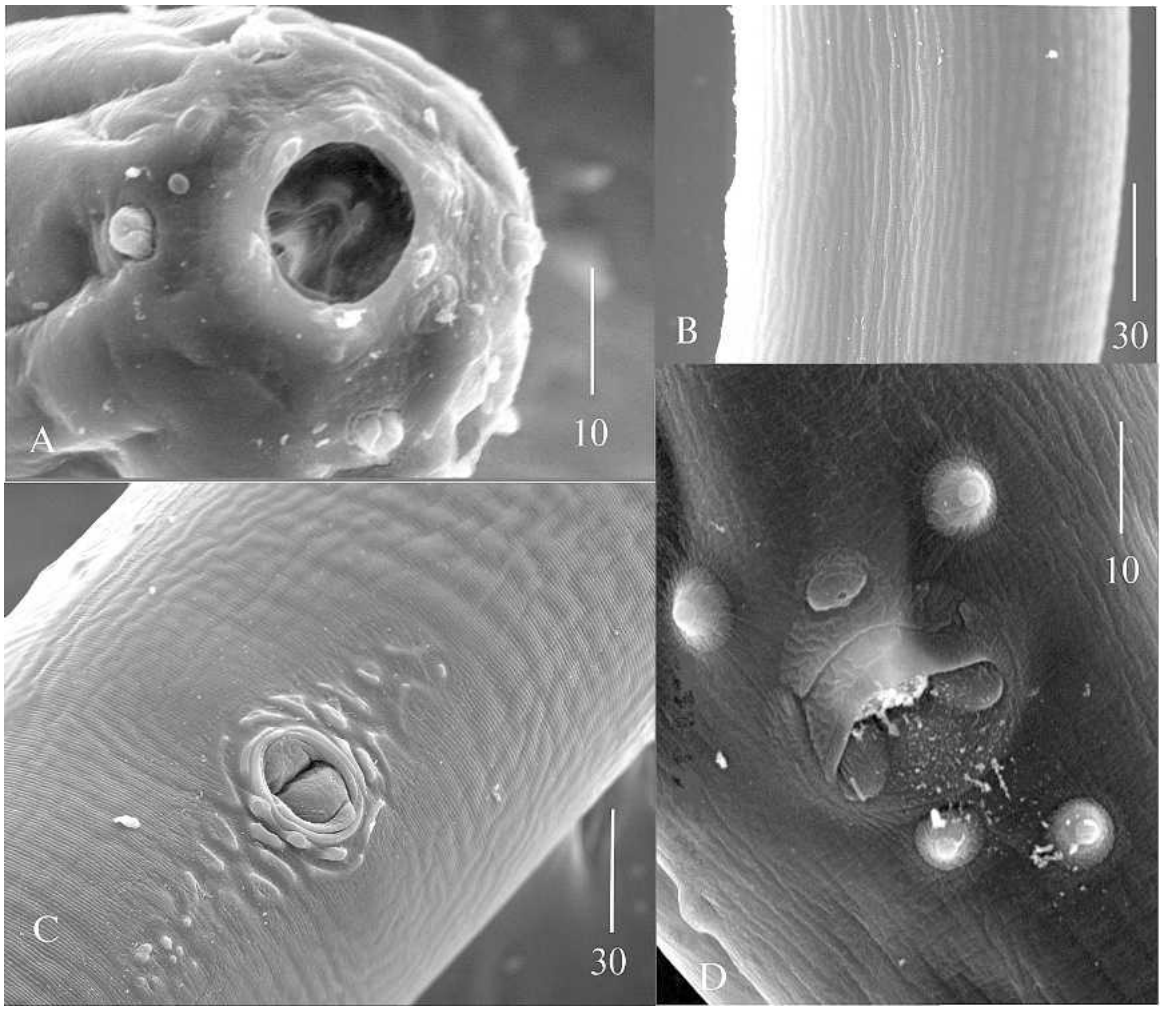

Fig. 3. – *Oxyspirura (O.) conjunctivalis* from *Nycticebus c. coucang*. SEM photographs: A. Female, anterior end. B. Longitudinal crests at mid-body of a female, lateral view. C. Bosses of the vulvar region (direction of worm apex upper right). D. Unpaired precloacal papilla, cloacal aperture and the two nearest pairs, ventral view (direction of tail tip lower left). Scales in µm.

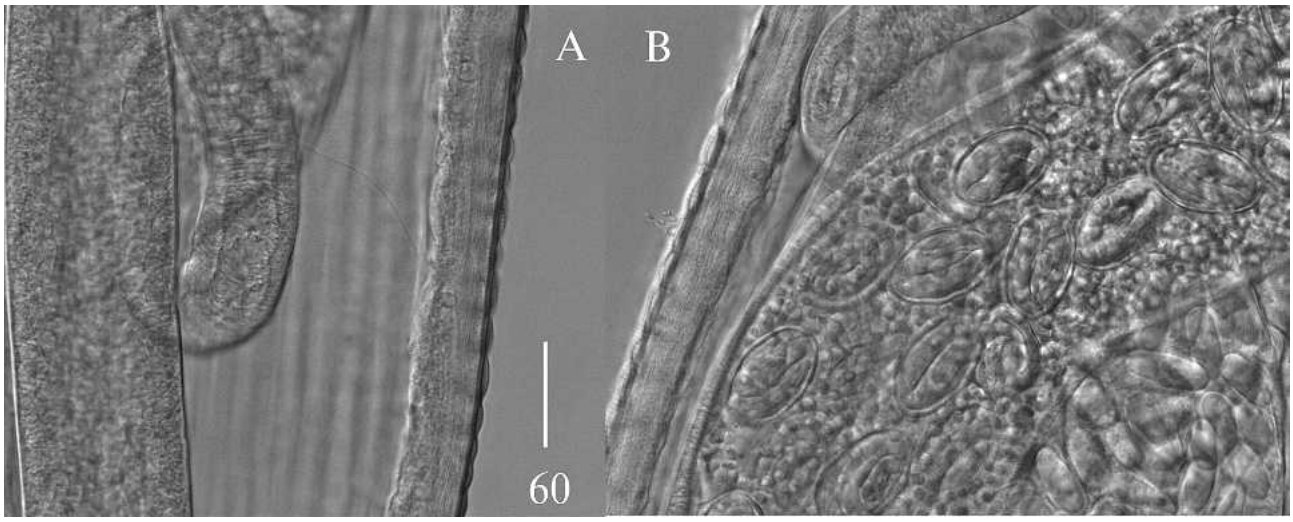

Fig. 4. – *Oxyspirura (O.) conjunctivalis*, female from *Nycticebus c. coucang*. DIC photographs. A. Transverse annulations and longitudinal crests of body cuticle in worm posterior third. B. Embryonated eggs and spermatozoa in the uterine pouch. Scales in µm.

Buccal cavity (Fig. 2E, F): funnel-shaped sclerotized anterior portion, internal ridges posteriorly; longer posterior part not sclerotized, with wall made by few large cells; no tooth-like thickenings present. Oesophagus without glandular posterior part, 2-3 times longer than distance nerve ring to apex. Tail conical with constricted blunt extremity. Genital primordium 28.5 ± 3.2 (21-34) µm long, situated 662 ± 423 (410-1,150) µm from tail tip.

Measurements (n = 7): body length 3,536 ± 105 (3,400-3,700); maximum width at the end of anterior body third 95 ± 15 (90-120);width at the middle of posterior third 73 ± 5 (68-80), nerve ring, deirids and excretory pore 166 ± 12 (150-185), 204 ± 6 (195-210) and 231 ± 13 (190-225) from apex, respectively; buccal cavity length 27 ± 2 (25-30); oesophagus length 476 ± 21 (440-500); tail length 194 ± 11 (180-195).

DISCUSSION

This study has revealed the mode of transmission of oxyspirurosis in primates. Cockroaches served as intermediate hosts of the nematode agents. Since these insects are known as vectors of diverse spirurids (Anderson, 2000), a molecular analysis of adult worms from *Nycticebus c. coucang* eye orbit and infective larvae was performed to assess the correct specific identification of the larvae. In Moscow zoo, from the five species of cockroaches examined, only two had infective larvae. Those from *B. germanica* corresponded to an unidentified spirurid, but those from *N. cinerea* showed a complete similarity with the ITS rDNA sequences of adult *Oxyspirura* and proved their conspecificity.

In the infective larvae found in *N. cinerea*, the number and arrangement of head papillae was that of the spirurid type (Chabaud, 1975): characteristically, the lateral labial internal papillae were absent. In adults, lips are reduced but six well developed, internal labial papillae are present whereas the four external labial papillae are partly atrophied. The mouth and buccal cavity are of the primitive hexagonal shape though the latter is laterally flattened in adults. An important new feature is the cuticular ornamentation of the larva made of longitudinal crests. These were also observed in adult worms, but in greater number.

The life cycle of only a single species of *Oxypsirura* was previously known, that of *O. (O.) mansoni*, a parasite of poultry transmitted by a cockroach. No detailed morphological analysis of the infective larva of this species is available. Moreover the posterior region figured by Fielding (1928) is likely to belong to another spirurid since the caudal extremity has several digitiform processes. Thus, it is not known if *O. (O.) mansoni* infective larvae have cuticular longitudinal crests; we observed adult worms and found no crests (Fig. 2M), but no conclusion can be made concerning the infective stage, since crests might become atrophied in later stages.

In the *Oxypsirura* infective larvae that we studied, the cuticle ornamentation contrasts with that of the L3 of *Thelazia* (*Thelazia*) Bosc, 1819. In the well-studied species parasitic in cattle and dogs, it is formed of transverse overlapping segments (Krastin, 1957 and Kozlov, 1965, in Skrjabin *et al.*, 1967; Khromova, 1979). The cuticle ornamentation has a phyletic value. It supported, together with the very short oesophagus and tail, the close relationship between *Parafilaria* Yorke & Maplestone, 1926, regarded as a filarial worm in the past, and the Thelaziinae (Bain, 1981; Bain, 2002). In the present study, it emphasizes the divergence between *T.* (*Thelazia*) and *Oxyspirura,* suggesting that the parasitism of orbital cavities by both is due to convergence. The sequence of the ITS-1 region of *Oxyspirura (O.) conjunctivalis* was aligned with corresponding domains of five *Thelazia* species (Fig. 5). Very few areas of unambiguous alignment between *O. (O.) conjunctivalis* sequence and those of *Thelazia* were revealed. It was also noted that sequences of different *Thelazia* species demonstrated few areas with obvious correspondence. It seems, that ITS1 domain evolved very quickly in spirurid nematodes.

Morphological study of the four nematode samples recovered from diverse primates from different zoos shows that the gubernaculum is not an appropriate character to distinguish *O. (O.) conjunctivalis* and *O. (O.) youngi*, since the dimensions are not precise and overlap. The set of characters including the ventral cuticle ornamentation near the vulva and the extremity of the left spicule seems more appropriate. The morphological differences do not seem to be linked to the host species in the zoos: nematodes from two Berlin samples were morphologically similar though one sample came from a Malagasy lemur, *Microcebus murinus*, and the other from an Asiatic lori, *Loris gracilis* (= *Stenops gracilis*). In fact, the four samples studied represent three morphological models, each corresponding to one zoo: *i*) Berlin: no or very few perivulvar bosses; membranous extremity of left spicule elongated; gubernaculum length from 42 to 100 µm; salient conical papillae; *ii*) Jacksonville: prominent prevulvar extension of bosses; membranous extremity of left spicule short; gubernaculum length from 50 to 150 µm (our data and Addison *et al*, 1986). In addition, the first two post-cloacal ventral papillae are joined on the median line, according to Addison *et al.* (1986); *iii*) Moscow: extension of bosses more prominent posterior to vulva, or equal; membranous extremity of left spicule short; gubernaculum from 40 to 120 µm long; the first two post-cloacal ventral papillae not joined on the median line.

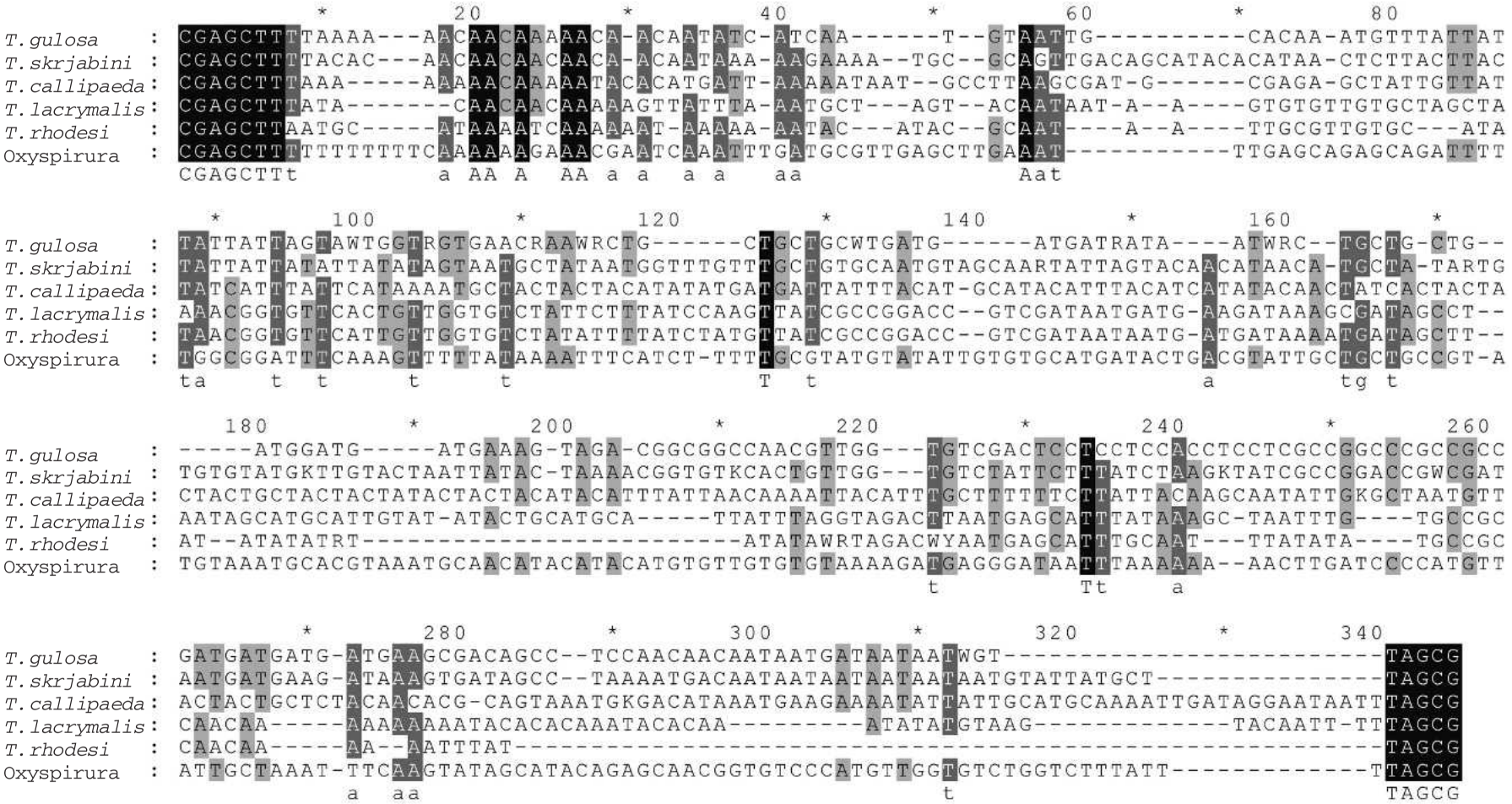

Fig. 5. – Most informative part of ITS-1 rDNA alignment of *Oxyspirura (O.) conjunctivalis* and five species of *Thelazia* parasites of orbital cavity of mammals

Because of the particular situation existing in zoos, the hosts recorded may not be the natural ones. For this reason all three slightly different lots, which suggest a natural diversity, are provisionally placed in the same taxon, *O. (O.) conjunctivalis* (Linstow, 1907) (= *O. (O.) youngi* Addison *et al.*, 1986).

In Moscow zoo the range of hosts is large and zoologically not coherent. The infected animals were the lorisiforms *Nycticebus pygmaeus* Bonhote, 1907 and *N. c. coucang* (lesser and larger slow loris), from the Asiatic region, *Perodicticus potto* Müller, 1766 (potto), *Galago crassicaudatis* È. Geoffroy, 1812 and *G. sengalensis* È. Geoffroy, 1796, from the Ethiopian region; the lemuriforms *Cheirogaleus medius* È. Geoffroy, 1812 and *Microcebus murinus* J. Miller, 1777 (fat tailed and lesser mouse lemurs), from the Ethiopian region; and the platyrhinian monkeys, *Saguinus fuscicollis* Spix, 1823 and *Callithrix* (*Cebuella) pygmaea* Spix, 1823 (brown-headed tamarin and pygmy marmoset), from the Neotropical region. *Nyticebus c. coucang* was the most seriously affected by the disease and total destruction of the eye ball was recorded in some cases. The origin of the infection in Moscow has been traced. The problems with primates in the zoo started shortly after the arrival of *N. pigmaeus* from Vietnam in 1995, when several specimens were confiscated by the Moscow customs officials and delivered to the zoo. As the Moscow zoo was being reconstructed at this time, the animals were housed in the old buildings together with several other species of primates, though in separate pens. The building was infested with cockroaches (originally with *B. germanica*, later also with *N. cinerea* and *P. americana* which had been propagated for feeding to monkeys and are known to escape easily from cages and become established). The infection soon spread among the insectivorous primate species. This history seems to be in favour of *N. pygmaeus* being the original host. No material had been collected from this animal to compare with worms from *N. c. coucang*, but the molecular analysis showed that one single species was present in the Moscow zoo, in 2004-2005.

As in Moscow, the natural hosts of the Berlin and Jacksonville cases of oxyspirurosis are probably Oriental lorisiforms, because of the great similarity of the adult worms. However, the small but constant differences detected suggest some diversity of the nematodes in these geographical areas.

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