Note on morphology of two nematode species *Ascaridia hermaphrodita* and *Ascaridia platyceri* (Nematoda): scanning electron microscope study

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Summary

Morphological features of adult specimens of *Ascaridia hermaphrodita* and *A. platyceri* (Nematoda: Ascaridida), parasitizing parrots (Psittaciformes), were studied for the first time using scanning electron microscopy. The morphology of *A. hermaphrodita*, the type species of *Ascaridia*, coincides fully with the generic diagnosis. The shape of the labial inner structure (two spoon-like projections), cervical alae and spicule with cuticular wings were typical for this species. *A. hermaphrodita* has been found in the Czech Republic for the first time in a new host, *Amazona pretrei*, from Brazil. The morphology of *Ascaridia platyceri* does not correspond with the generic diagnosis in all aspects, as some variation was found, mainly in the presence of interlabia, small teeth in the inner ribbon of the lips and long lateral alae. These morphological characteristics in other species of *Ascaridia* are discussed. The generic diagnosis is to include the statement “interlabia very rarely present”.

Key words: *Ascaridia hermaphrodita*; *A. platyceri*; Psittaciformes; Czech Republic; morphology; differences

Introduction

Nematodes of the genus *Ascaridia* (Dujardin, 1845) infect many species of birds (Mozgovoj 1953, 1973; Yamaguti 1961). To date, only five species of ascaridiids have been recorded from parrots (Psittaciformes). Two species, *A. platyceri* Hartwich et Tscherner, 1979 (= *A. sprengi* Mines, 1979), and *A. hermaphrodita* (Froelich, 1789), are the species most commonly found in Europe (Hartwich & Tscherner, 1979; Kajerová *et al.*, 2004a) in parrots living in captivity.

A. platyceri in the Czech Republic was first reported by Kajerová *et al.* (2004b). In the present study, we report the first record of *A. hermaphrodita* in the Czech Republic.

The SEM study provides more details of the morphology of adult specimens of both species with new differentiation features.

Material and Methods

An infection with parasites showing the morphological characteristics of ascaridiid nematodes was observed in the small intestine of two species of parrots during a post-mortem examination:

1. *Psittacula longicauda* (Boddaert, 1783) imported in spring 2005 to Austria from the Philippines and then in summer 2005 transported to the Czech Republic. The parrot died after 1 week in captivity, due to heavy infection with ascaridiids (*A. platyceri*); in total, 242 specimens were found.

2. *Amazona pretrei* (Temminck, 1830) imported to the Czech Republic in 2005 from Brazil. Died after 3 months in captivity with symptoms of ascaridiosis. 37 nematodes, *Ascaridia hermaphrodita* (36 adults – 7 males and 29 females, 1 fourth-stage larva), were detected in the small intestine.

Specimens were fixed in 4 % formaldehyde solution and cleared in glycerine-water for morphological examination. A light microscope Olympus BX 50 equipped with a differential interference contrast (Nomarski DIC) and digital image analysis (analySIS) for measuring was used. Nematodes were identified to species according to Travassos (1913, 1930), Hartwich and Tscherner (1979), and Kajerová *et al.* (2004b).

Specimens used for scanning electron microscopy (5 males and 5 females of both species) were washed several times in water, fixed with hot 4 % formaldehyde and stored at room temperature. Prior to analysis, they were dehydrated through ethanol, dried in a CPD 030 critical point drying apparatus (Bal-tec, Balzers, Liechtenstein) using liquid

CO₂, mounted on aluminium stubs with double sided adhesive discs, coated with gold in a SCD 040 sputter coating unit (OC Oerlikon Balzers Coating, Balzers, Liechtenstein) and examined in a VEGA scanning electron microscope operating at 20 kV.

Reference material of *A. hermaphrodita* has been deposited in the collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. N-508.

Results

Selected morphological characteristics

Ascaridia hermaphrodita (Plate 1)

Mouth opening surrounded by one dorsal and two lateroventral lips (180 – 231 µm wide and 147 – 179 µm high), each of them divided into one medial and two lateral lobes (Fig. 1). One pair of elliptical papillae, width 32 – 38, is symmetrically situated in middle of dorsal lip (Fig. 2). One flat elliptical papilla situated near ventral margin of lateroventral lips and one round papilla with amphideal opening located near lateral margin of lip (Fig. 1). In addition to these papillae, a group (three to four) of small rounded papillae is present on lateroventral lips. Viewed from the lateral or dorsal surfaces, the margins of medial lobes are rounded, their top margins separated by a transverse shallow cut. Top margins of medial lobes overlap a laterally flat rounded projection (in length 3 – 5 and width 16 – 22). The surface between lips is smooth, without interlabia. Top margin and inner wall of medial lobes smooth. Two spoon-like flat elements (height 40 – 55 and width 18 – 25) extend from every lip, approximately from mid-point of inner wall of medial lobes (Fig. 3). The lateral alae reach maximum width 100, starting just below base of lips (80 – 92) and ending in first third of body length, above vaginal opening (Fig. 4). Cuticula in pre-cloacal and post-cloacal regions of ventral surface of male is without marked structures (almost smooth), forming a slight bulge on body (Fig. 5). Two spicules of same morphology and shape are formed by lower sclerotising reinforcement, to which cuticular wings (high 30 – 45) forming a smooth channel (Fig. 6). Viewed laterally, wing as appears to be unilateral. Wings end 42 – 51 from distal rounded tip of spicule (Fig. 7).

Ascaridia platyceri (Plate 2)

Mouth bordered by one dorsal and two lateroventral lips (84 – 137 wide and 84 – 120 high), each of them divided into one medial and two lateral lobes. Triangular interlabia do not exceed half height of lips (Fig. 8). Anterior edge of medial lobe forms narrow rounded ribbon separated from lip by shallow transverse slit. Two oval papillae (wide 8 – 14) are present on dorsal lip in middle of its height, separated by slit in cuticle of lip. One flat elliptical papilla present near ventral margin and one small rounded papilla

with amphideal opening located near lateral margin of lateroventral lips. In addition, group of three small rounded papillae (accessory labial papillae), distributed in line or in triangle, is present (Fig. 9, 10). Inner wall of medial lobe of lips formed by flat reinforcement separated by shallow slit from external wall of lip. Anterior margin of reinforcement has one line of 27 – 29 small teeth (height 1 – 2), in length of edge 49 – 70 (Fig. 11). Lateral alae (maximum width 20) decreased in cervical region and melt into low transverse tegumentary ridges to level of anus (Figs 12 and 13). Cuticle in pre-cloacal and post-cloacal regions of male has fine transverse serrations, without marked bulge (Fig. 14). Spicules without cuticular wings. Distal tip of spicules rounded.

Discussion

The genus *Ascaridia* (Dujardin, 1845) has a cosmopolitan distribution and incorporates more than 55 species in the intestine of mainly terrestrial birds (Mozgovoj, 1953, 1973; Yamaguti, 1961). Seven *Ascaridia* species have been found in birds of the order Psittaciformes. Two species have been detected most frequently in wild and captive parrots (survey in Kajerová *et al.*, 2004b). *A. platyceri* was found in the Czech Republic for the first time by Kajerová *et al.* (2004b).

In the present study, *A. hermaphrodita* is reported for the first time from a new host, *Amazona pretrei*, imported from Brazil. *Ascaridia hermaphrodita* is the type species of the genus and its morphological features reflect all the characters of the generic diagnosis (Mozgovoj, 1953; Yamaguti, 1961) as follows: Ascaridiinae with cuticular lateral flanges generally present; lips without interlabia; oesophagus club-shaped, without posterior bulb. Male: precloacal sucker with chitinous rim; caudal alae narrow, papillae relatively large; spicules equal or subequal; gubernaculum absent. Female: uteri opposed; vulva near middle of the body; oviparous; eggs thick shelled.

The morphology of the lips with inner spoon-like projections is a typical morphological feature of *A. hermaphrodita* recognized using SEM. This feature was described and documented by Travassos (1930). The morphology of the spicules was previously studied by Skrjabin (1917) but he did not mention the presence of two longitudinal cuticular alae, forming a channel for the purpose of directing the sperm, as suggested in the present study. The shape of the lips and the labial papillae and the absence of interlabia correspond with the diagnosis of the genus.

Hartwich and Tscherner (1979) and Mines (1979) noted discrepancies with the generic diagnosis in the original description of *A. platyceri*. It concerns the presence of the triangular interlabia having blunt tips and not exceeding one half of the height of the lips. This feature was documented also by Kajerová *et al.* (2004a) and by SEM in the present study.

In the lower part of the lateroventral lips, three small accessory papillae were observed for the first time for both *A. hermaphrodita* and *A. platyceri*. In our opinion, these

are sense organs - mechanoreceptors already described from other *Ascaridida* species (McLaren, 1976; Wright, 1980).

Another structure of taxonomic importance is a flat reinforcement on the inner wall of the medial lobe of the lips that is equipped with a line of small teeth at the edge. This structure is not mentioned in the generic diagnosis and it is most likely a rare feature in the genus. Among the members of *Ascaridia*, it is present only in a few species. The presence of large and finger-like interlabia and line of small teeth on the inner margin of the lips has been observed in *A. australis* (Linstow, 1879), from the host *Macropygia nigrirostris* (Columbiformes, Columbidae) common in New Guinea and NW Islands. Mozgovoj (1953) noted that presence of the interlabia in this species

does not correspond with the diagnosis of the genus. Unlike Yamaguti (1961), he also mentioned the birds *Aviceda subcristata bismarckii* (Accipitridae, Falconiformes) from New Britain, New Ireland and New Hanover and *Ninox odiosa* (Strigiformes, Strigidae) from New Britain as the hosts of *Ascaridia australis*. However, we believed that these two host records need verification.

Lips with a dentate-like wall were described in *Ascaridia compressa* (Schneider, 1866) from *Gallus gallus* f. *domestica*, in North Australia. Also, Skrjabin (1920) described the lips as having a toothlike wall on the upper margin. A flexure with small teeth in the middle of the outer margin is absent on the lateroventral lips; the front margin of flexure with teeth comes immediately to the margin of the own lip. In an illustration by Schneider (1866), the tooth-

Plate 1. *Ascaridia hermaphrodita* – scanning electron micrographs. Fig. 1. Head (apical view); Fig. 2. Dorsal lip (total view); Fig. 3. Two inner finger-like elements in the medial lobe of the lateroventral lip; Fig. 4. Anterior extremity (dorsal view); Fig. 5. Postcloacal region of male (ventral view); Fig. 6. Spicule with two alae (ventral view); Fig. 7. Distal end of the spicule (lateral view); (Scale – bars: Fig. 2 = 40 μ m; Fig. 1, 3 = 50 μ m; Fig. 5, 7 = 100 μ m; Fig. 4, 6 = 200 μ m).

Plate 2. *Ascaridia platyceri* – scanning electron micrographs. Fig. 8. Head (lateroventral view) with interlabia; Fig. 9, Fig. 10. Lateroventral lip (total view); Fig. 11. Flat reinforcement of the medial lobe of lip with small teeth (detail); Fig. 12. Anterior extremity (ventral view); Fig. 13. Lateral ala at vulvar region (lateral view); Fig. 14. Postcloacal region of male (ventral view); (Scale-bars: Fig. 11 = 20 μ m; Fig. 10 = 30 μ m; Fig. 9 = 40 μ m; Fig. 8 = 50 μ m; Fig. 13, 14 = 100 μ m; Fig. 12 = 200 μ m).

like ribbon of the lip overlaps only the upper and lateral parts of the medial lobe, which, however, corresponds with the description of *A. platyceri* rather than *A. compressa*. Therefore, it is impossible to consider *A. compressa* as a species conspecific with *A. galli* (Schränk, 1788) as suggested by Frenzen (1955).

Also in the description of *A. serrata* (Schneider, 1866) from *Penelope humeralis* and *Pipile jacutinga* (Cracidae, Galliformes) in Brazil, the presence of anterior and posterior tooth lamellae is mentioned. One row of eight small teeth is present in the form of tooth lamellae. In a redescription of *A. francolina* (Linstow, 1899) from *Pternistis bicalcaratus* (Phasianidae, Galliformes) from Africa, Khalil (1932) described the presence of three tooth-like combs on the inner surface of the lips. Gushanskaya (1950) also mentioned fine small teeth on the margins of the lips

in *Ascaridia* sp. from *Tetrastes bonasia viciniatus* (Galliformes, Tetraonidae) from Sakhalin Island (Russia).

Lateral alae on the spicules of *A. platyceri* were not observed by Hartwich and Tscherner (1979), Mines (1979) or Kajerová *et al.* (2004b). The presence of the interlabia and small teeth on the inner wall of the lips does not correspond to the generic diagnosis of *Ascaridia* (as given by Mozgovoj, 1953, 1973 and Yamaguti, 1961).

The occurrence of two spoon-like flat elements on the inner wall of the medial lobes of the lips, which we observed in *A. hermaphrodita*, is rare in *Ascaridia*. This feature was reported in the original description and illustration of *A. cristata* by Linstow (1901) from *Balearica pavonina* and *B. regulorum* (Gruidae, Gruiformes) from Africa (Kongo). Also, Kreis (1938) mentioned the presence of three toothlike plates on every lip in *Ascaridia* sp. from

Gallus sp. (locality unlisted).

The presence or absence of cuticular bosses in the cloacal region of the males is one of the main features used for the differentiation of species by Mozgovoĵ (1953, 1973) and Freitas and Ibanez (1965). In both species studied here, the cuticle is only finely transversely striated (in *A. platyceri* it is more strikingly marked, but the striation in *A. hermaphrodita* is finer). Therefore, it markedly differs from ascaridiids with cloacal regions ornamented with thickened cuticular bosses. This feature, typical for *A. galli*, was emphasized by Baruř (1966) and Baruř *et al.* (1975) in numerous other species of *Ascaridia* (see Mozgovoĵ, 1953, 1973; Freitas & Ibanez, 1965).

A. hermaphrodita and *A. platyceri* belong to the large group of species of *Ascaridia* with lateral cuticular alae; in the case of *A. platyceri*, they end near the anus. The morphological features of *A. hermaphrodita* documented in the present study fully correspond with the diagnosis of *Ascaridia*. In the case of *A. platyceri*, only the presence of the interlabia does not correspond with the generic diagnosis. As an appropriate resolution, we amendation the generic diagnosis to include the statement: “in the genus *Ascaridia*, interlabia are very rarely present”.

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