Studying of Nematode Morphology with Three Kinds of Methods: LM, CLSM and SEM

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Scanning electron microscopy (SEM), light microscopy (LM) and confocal laser scanning microscopy (CLSM) were used to study the morphology and anatomy of the reptile parasitic nematode *Strongyluris brevicaudata* (Mueller, 1894). Specimens were obtained from the intestine of African species of agama, *Agama agama* (Linnaeus, 1758) (Agamidae), collected in Niokolo Koba National Park, East Senegal. *S. brevicaudata* was examined and micrographed by a scanning electron microscope for the first time. Special attention was paid to morphological aspects of males and females such as cephalic structures, number and distribution of caudal papillae. Cuticular patterns were analyzed too.

The nematode specimens were fixed and preserved in 96% ethyl alcohol. For examination of fixed nematodes it was necessary to clear their bodies in glycerin-water solution to enable better examination of their internal organs, which are important features used in determination. The clearing was performed by gradual evaporation of the water from the glycerin-water mixture (ratio 1:20–1:5) for several hours [1]. After the clearing, they were examined under a light Olympus BX51 microscope equipped with differential interference contrast (DIC), digital camera Olympus DP70 and digital image analysis system (analySIS auto 5.0). Drawings were made with aid of a drawing attachment. Nematodes were also viewed and documented using an Olympus IX80 microscope equipped with laser-scanning FluoView 500 confocal unit (Olympus FluoView 4.3 and Imaris 3.0 software). We observed their auto fluorescence. Prior to SEM study, parasites were subsequently dehydrated through ethanol series (30%, 50%, 70%, 80%, 90%, 2x96%), dried in a critical point drying apparatus using liquid CO_2 (CPD 030, Bal-tec), mounted on aluminum stubs with double sided adhesive disc, coated with gold in a SCD 040 sputter coating unit (Balzers) and examined with a MIRA TESCAN scanning electron microscope operating at an accelerating voltage of 15 kV.

With all these methods we were able to observe the details of the nematode bodies, e.g. three massive triangular cephalic lips with papillae, oesophagus without valves, circular preanal sucker surrounded by a cuticularized ring, caudal papillae and granulate spicules.

Careful examination using methods of light and scanning electron microscopy allowed us to uncover a number of morphological characters to which little attention has been paid by former researchers. Some of our outputs will be demonstrated in a poster section of the conference.

1. F. Moravec, Nematodes of Freshwater Fishes of the Neotropical Region (1998) 475 pp.

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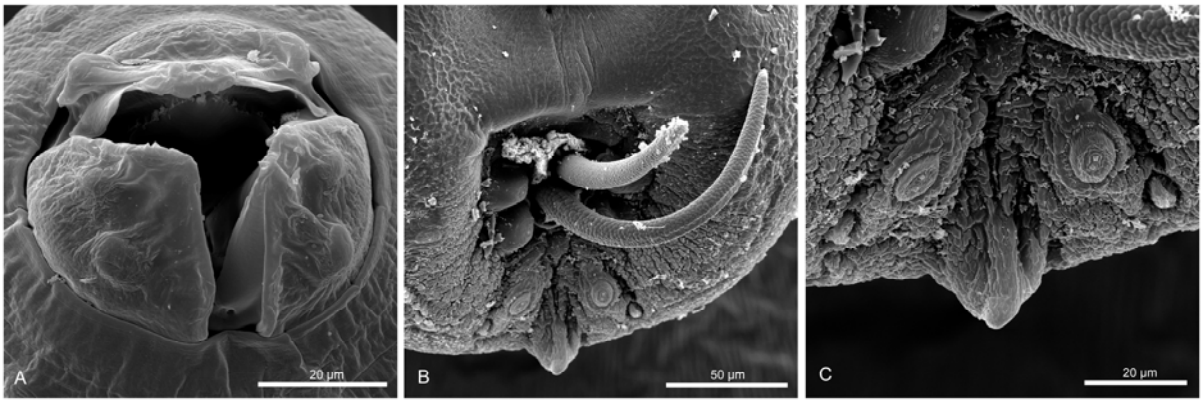

Figure 1. SEM of *Strongyluris brevicaudata*, male. A. Apical view of mouth opening. B. Ventral view of caudal part with spiculae. C. Ventral view of caudal part with detail of papillae.

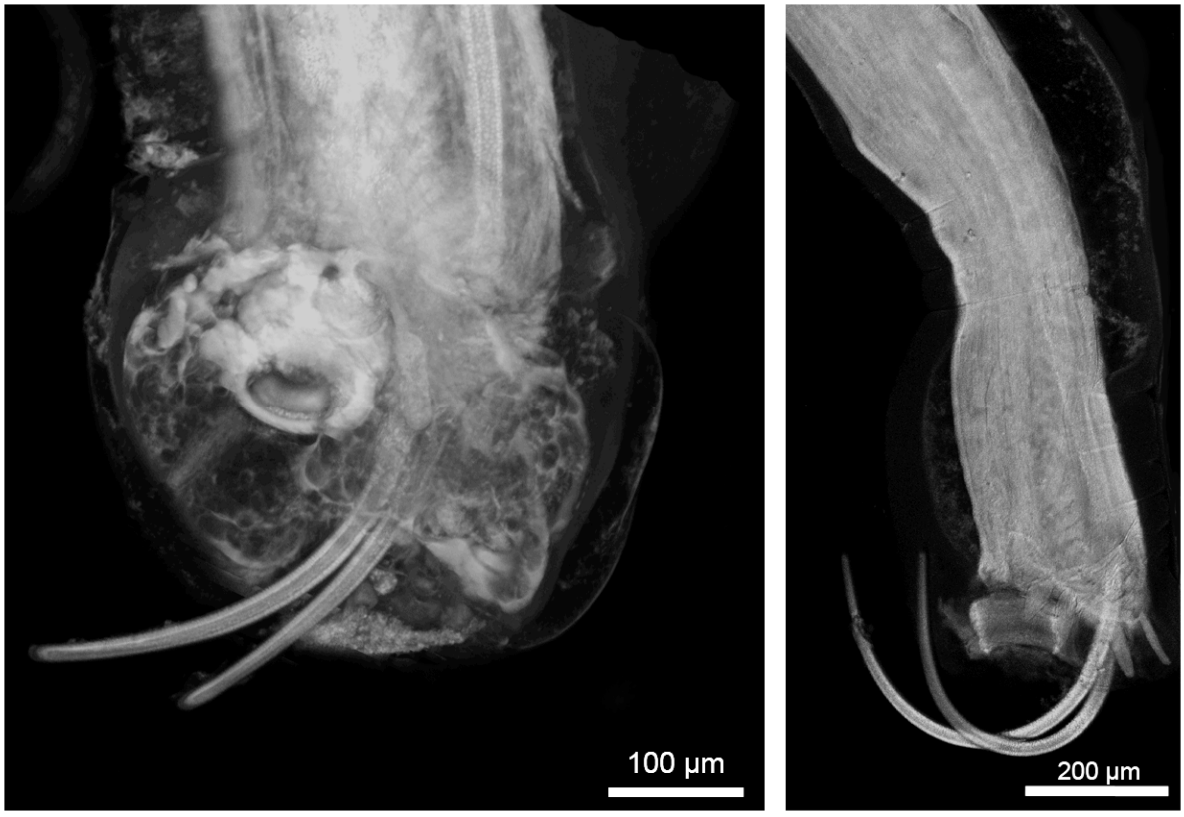

Figure 2. CLSM of *Strongyluris brevicaudata*, caudal parts of male.