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HEAD AND CUTICULAR STRUCTURES OF SOME SPECIES IN THE FAMILY STEINERNEMATIDAE (NEMATODA)

by

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Head and cuticular structures of the Steinernematidae: *Steinernema kraussei*, *Neoaplectana glaseri*, *N. carpocapsae* and *N. bibionis* were compared by scanning electron microscopy. In both genera and all species studied one ring of six labial papillae and one of four cephalic papillae were clearly visible, which shows that *Steinernema* and *Neoaplectana* cannot be separated on the number of head papillae alone. Details of the morphology of the lips, openings of secretory organs and of the surface of the cuticle are given. Invasive juveniles have only one ring of four cephalic papillae.

Members of the Steinernematidae are all obligate parasites of insects adapted to the way of life by the alternation of giant and small females and permanent association with specific strains of bacteria. The morphology and biochemistry of the associated bacteria do not help to differentiate the species or genera of the family. The first descriptions by Filipjev (1934) and others at a time when a new host indicated usually a new parasite lacked distinct taxonomic characters and relied on Cobb's indices in the hope that, later, these would make differentiation possible. For that reason the male spicula and gubernaculum were described without details of the chitinous and soft parts and their range of variability. The use of additional characters was therefore urged. Similarly characterization of the head papillae and the surface patterns of the rear end of the body was inadequate (Weiser & Koehler, 1955). Observation of the head papillae in the optical microscope is rather difficult due to their minute size and to circumoral distribution which allows only a lateral view in which the structures overlap each other. It was generally accepted (Steiner, 1929), that there are two rings of head papillae, six papillae each, that the development of anal papillae varied in individuals of the same species and that minor differences were unimportant as specific markers. Therefore only the head papillae persisted as characters of the family.

Using electron microscopy (SEM), Mráček & Weiser (1979) showed that in three species of the family Steinernematidae the second ring contains four papillae only, and made a revision of former morphological data necessary. Their work also showed that morphological variability of the genera in the

family was slight, making necessary a further search for taxonomic markers in adults and juveniles to separate genera and species.

We therefore used SEM to study available laboratory and field strains of members of the family.

MATERIALS AND METHODS

Steinernema kraussei (Steiner) was isolated from naturally infected *Cephaleia abietis* (L) collected in S. Bohemia and N. Moravia in 1974 and maintained on agar slants with sterile kidney or liver of mice thereafter. *Neoaplectana glaseri* Steiner was a strain isolated by Stoll (1959) on mouse liver on agar slants, a subculture of the strain maintained at the Rockefeller University, N.Y., U.S.A. *Neoaplectana carpocapsae* Weiser, strain DD-136 isolated by Dutky from infected *Cydia pomonella* (L) in 1955 and maintained in the laboratory as axenic culture. *Neoaplectana bibionis* Bovien, strain N8 isolated from forest soil in E. Slovakia and maintained on larvae of *Galleria mellonella* L. and on agar slants with mouse kidney with associated bacterium.

Nematodes for SEM were isolated from cultures or infected *G. mellonella* larvae and washed after storage for 12 h in 0.05% formalin solution and then transferred three times to fresh 0.05% NaCl at 2 h intervals. They were fixed in fresh 2.5% glutaraldehyde in kakodylate buffer at 4° for 3 × 2 h and 1 × 16 h and postfixed in 1% osmium tetroxide for 1.5-2 h. The nematodes were dehydrated for 1 h in 50% ethanol, overnight in 70% ethanol, 1 h in 80%, 90% and 95% ethanol and 3 × 45 min. in fresh absolute alcohol. After 1 h in ethanol/aceton mixtures of 50/50, 30/70 and 10/90 and 3 × 45 min. in fresh pure aceton they were dried at critical point. They were mounted onto stubs with adhesive tape and were dried at critical point. They were mounted onto stubs with adhesive tape and were gold-coated at 300 Å in a Jeol Sputter JFC-1100. The photographs were taken at 15 kV accelerating voltage in a Jeol JSM-35 SEM.

RESULTS

Mouth and buccal cavity. In Figs 1-6 of the heads of adults, the mouth opening was wide open, triangular shaped with rounded corners. The short buccal cavity broadens from the oral opening and is closed by three lips in the front end of the oesophagus. Its wall is smooth and the lips probably do not distort the shape of the mouth opening. Invasive juveniles have closed mouths and the buccal cavity is indistinct (Fig. 8).

Lips and head papillae. Head papillae (Fig. 1-6) are in two circles, the interior hexangular (corresponding with the triangular mouth opening). The outer circle is tetrangular with four papillae. The papillae in both circles differ slightly: the series of six papillae of the labial circle rest on slightly protruding labial