# Some Cephalobidae (Nematoda : Rhabditida) from Crete, Greece

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Summary — Soil samples from Rethymnon, Crete, Greece, yielded some species of the nematode family Cephalobidae (Rhabditida). Seleborca complexa (Thorne, 1925) Andrássy, 1985, Zeldia punctata (Thorne, 1925) Thorne, 1937 and Acrobeles ciliatus von Linstow, 1877 were found in sand close to the beach. Acrobeles singulus Heyns, 1969, Cervidellus serratus (Thorne, 1925) Thorne, 1937 and Acromoldavicus skrjabini (Nesterov & Lisetzkaja, 1965) Nesterov, 1970 were found in coarse limestone soil of a pine forest. The synonymisation of Seleborca complexa with Acrobeles crossotus Steiner, 1929 by Andrássy (1984) was supported. The retention of A. ciliatus and A. singulus as separate species was supported, thus rejecting their synonymisation by Andrássy (1984). The population of C. serratus was found to narrow the gap between this species and C. neftasiensis Boström, 1986.

Résumé – Quelques Cephalobidae (Nematoda : Rhabditida) provenant de Crête, Grèce – Des échantillons de sol provenant de Rethymnon, Crête, Grèce, contenaient quelques espèces de Cephalobidae (Rhabditida). Seleborca complexa (Thorne, 1925) Andrássy, 1985, Zeldia punctata (Thorne, 1925) Thorne, 1937 et Acrobeles ciliatus von Linstow, 1877 ont été rencontrés dans des échantillons sableux, proches des plages. Acrobeles singulus Heyns, 1969, Cervidellus serratus (Thorne, 1925) Thorne, 1937 et Acromoldavicus skrjabini (Nesterov & Lisetzkaja, 1965) Nesterov, 1970 ont été trouvés dans le sol calcaire grossier d'une forêt de pins. La synonymisation (Andrássy, 1984) de Seleborca complexa avec Acrobeles crossotus Steiner, 1929 est acceptée. A. ciliatus et A. singulus sont considérés comme des espèces distinctes et leur synonymisation (Andrássy, 1984) n'est pas retenue. Les caractères observés sur la population de C. serratus amenuisent les différences entre cette espèce et C. neftasiensis Boström, 1986.

Key-words : Cephalobidae.

During a visit to the island of Crete, Greece, in May 1989, soil samples were taken at various localities for nematode studies. Samples from Rethymnon yielded some interesting species of cephalobids. Seleborca complexa (Thorne, 1925) Andrássy, 1985, Zeldia punctata (Thorne, 1925) Thorne, 1937 and Acrobeles ciliatus von Linstow, 1877 were found in sand close to the beach. Acrobeles singulus Heyns, 1969, Cervidellus serratus (Thorne, 1925) Thorne, 1937 and Acromoldavicus skrjabini (Nesterov & Lisetzkaja, 1965) Nesterov, 1970 were found in rather coarse limestone soil.

The species were studied by light microscopy (LM) and scanning electron microscopy (SEM) and are described below.

# Material and methods

Samples were taken on the 2nd May 1989 from a coarse limestone soil from a pine forest up-hill Rethymnon. The soil was dry and contained some organic material. On the 3rd May 1989 samples were taken from a sandy patch of the Rethymnon beach. The sand was dry and contained no organic material.

Nematodes were extracted by a wet funnel method (Sohlenius, 1979). They were killed by heat, fixed in cold TAF and transferred to anhydrous glycerine by a slow evaporation method (Hooper, 1970). For light microscopy (LM) nematodes were mounted in glycerine as described in Boström and Gydemo (1983). Specimens for scanning electron microscopy (SEM) were processed as described in Boström (1989*a*).

Nematodes were identified to species level in the LM.

# Seleborca complexa (Thorne, 1925) Andrássy, 1985 (Fig. 1)

#### **M**EASUREMENTS

Female (n = 8) : L = 863 ± 13 (817-920)  $\mu$ m; width = 53 ± 2 (46-61)  $\mu$ m; a = 16 ± 0.4 (14-18); pharynx = 199 ± 3 (188-208)  $\mu$ m; b = 4.3 ± 0.03 (4.2-4.5); tail = 78 ± 1 (75-84)  $\mu$ m; c = 11 ± 0.2 (11-12); c' = 2.8 ± 0.1 (2.4-3.0); V = 60 ± 0.3 (59-62); V-A/T = 3.5 ± 0.1 (3.3-3.9); egg = 59-73 × 27-38  $\mu$ m.

 $\begin{aligned} &Male\,(n=9): L=784\,\pm\,9\,(751\text{-}825)\,\mu\text{m}; \,\text{width}=\\ &48\,\pm\,1\,(43\text{-}52)\,\mu\text{m}; \,a=17\,\pm\,0.4\,(15\text{-}19); \,\text{pharynx}=\\ &191\,\pm\,2\,(188\text{-}203)\,\mu\text{m}; \,b=4.1\,\pm\,0.04\,(4.0\text{-}4.3); \,\text{tail}=\\ &56\,\pm\,0.4\,(53\text{-}58)\,\mu\text{m}; \,c=14\,\pm\,0.2\,(14\text{-}15); \,c'=\\ &1.9\,\pm\,0.1\,(1.7\text{-}2.3); \,T=58\,\pm\,1.5\,(52\text{-}64); \,\text{spicules}=\\ &40\,\pm\,0.6\,(37\text{-}41)\,\mu\text{m}; \,\,\text{gubernaculum}=24\,\pm\,0.6\\ &(22\text{-}26)\,\mu\text{m}. \end{aligned}$ 

#### DESCRIPTION

Adult : When relaxed by heat body posture variable, usually ventrally arcuate, sometimes slightly C-shaped. Cuticle "double", annules 2.8-3.1 µm wide at midbody, transverse striae with small tubercles. Lateral field

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obscure in LM; two incisures were seen, beginning at level of isthmus, extending to some annules posterior to phasmid in females and almost to tail tip in males. Six cephalic probolae with membraneous fringes; 6-8 tines of different lengths on each side, middle tines longer in secondary cephalic axils, single apical tine; long guarding pieces. Three deeply bifurcate labial probolae, 14-18 µm high, with fringes; ten to twelve tines on outer side and seven or eight tines on inner side; proximal tines rounded, distal and apical tines longer and thinner. Stoma cephaloboid; cheilostom wide; cheilorhabdions round, 1.7-2.5 µm wide; rest of stoma narrow, 12.5-15 µm long. Pharynx cephaloboid; procorpus cylindrical, metacorpus fusiform, isthmus narrow; corpus about four times longer than isthmus. Bulb oval or pear-shaped with valves,  $32-38 \times 24-32 \ \mu m$  in females,  $31-36 \times 22-27 \ \mu m$  in males. Cardia not prominent, enveloped by intestinal cells. Nerve-ring surrounds isthmus; hemizonid at isthmus or isthmus-bulb junction; excretory pore just anterior to hemizonid. Deirid at level of bulb.

*Female* : Reproductive system monodelphic, prodelphic; ovary reflexed at oviduct and straight or with double flexure posterior to vulva. Spermatheca 71-79  $\mu$ m long, 17-26  $\mu$ m broad. Post-uterine branch (PUB) 8-19  $\mu$ m long. Vulval lips not or slightly protruding; cement plug covering vulva seen in five specimens (three in LM, two in SEM). Rectum 22-27  $\mu$ m or 0.7-1.1 anal body width (ABW) long. Anus a transverse slit. Tail conoid with acute terminus. Phasmids at 22-30 % of tail length.

*Male* : Reproductive system monorchic, testis reflexed anteriorly. Cement producing gland not observed. Spicules cephaloboid, ventrally arcuate. Three pairs of ventrolateral precloacal papillae; two pairs of subventral caudal papillae; two pairs of lateral caudal papillae; one pair of subdorsal caudal papillae; a single ventromedian papilla on anterior cloacal lip; precloacal grooves extending about 35 annules anterior to cloaca. Tail ventrally curved, conoid with acute terminus. Phasmids at 39-46 % of tail length.

# DISCUSSION

This population is close to both Seleborca complexa and Acrobeles crossotus Steiner, 1929, as redescribed by Heyns (1969). Steiner's (1929) original description of A. crossotus did not contain any measurements, and it is doubtful if type material exists (Heyns, 1969). The two species were synonymised by Andrássy (1984), and for this reason A. crossotus was never transferred to the genus Seleborca, which was erected later by Andrássy (1985). Steiner (1929) stated that A. crossotus most closely resembled A. complexus Thorne, 1925, but did not explicitly point to the characters separating them. Heyns (1969) in redescribing A. crossotus neither differentiated it from A. complexus. There may be some characters, viz. the length of the PUB in females and presence of cement producing glands in males, separating the two species. Thorne (1925) did not indicate the length of the PUB in *A. complexus*. He later (1961) stated it to be " about one-third as long as body width ", but his figure (16-17.A) showed it to be about 1.75 body widths (BW) long. Heyns (1969) described the PUB of *A. crossotus* to be 1.5-2.5 BW. In a Brazilian population of *A. crossotus*, described by Rashid *et al.* (1985), the PUB measured 34-141  $\mu$ m (corresponding to about 1-4 BW). They also recorded some females having a shorter PUB (18-21  $\mu$ m).

A special character, described by Steiner (1929) for A. crossotus males, was glands producing a cement to plug the vulva after copulation. He also stated that they are clearly distinguishable only in living specimens. Heyns (1969) also indicated presence of such glands and a prominent cement mass covering the vulva in older females of A. crossotus. Rashid et al. (1985) found neither glands in the males nor any cement mass covering the vulva of the females in their Brazilian population. Rashid et al. (1990b) described populations of S. complexa from Namibia and recorded the vulva usually covered with cement mass, but did not mention any cement producing gland in the males. Thomas (1965), in describing the morphology of A. complexus, commonly found cement masses covering the vulva of gravid females, but he saw no secretory glands in males or females. He proposed the gelatinous material to emanate from the uterus as in some heteroderids. It seems possible that Steiner (1929) and also Heyns (1969) have misinterpreted parts of the vas deferens, especially the bilobed region, to constitute cement-producing glands.

Females of the present population have a very short PUB (8-19  $\mu$ m) and some have cement plugs covering the vulva. The length of the PUB appears to have a wide range in *A. crossotus* and thus to be of less taxonomic significance. Presence of cement producing glands in males of *A. crossotus* seems doubtful and cement plugs were seen in females of both *S. complexa* and *A. crossotus*. The synonymisation of the two species is supported by this study.

The specimens of this population agree with a Senegalese population of S. complexa described by De Ley et al. (1990) and with the external morphology of the species as presented by Sauer et al. (1979).

# Zeldia punctata (Thorne, 1925) Thorne, 1937 (Fig. 2 A-B)

### **M**EASUREMENTS

Female (n = 3) : L = 755 ± 34 (689-800)  $\mu$ m; width = 37 ± 3 (31-40)  $\mu$ m; a = 21 ± 1 (20-22); pharynx = 195 ± 7 (182-203)  $\mu$ m; b = 3.9 ± 0.03 (3.8-3.9); tail = 41 ± 2 (38-44)  $\mu$ m; c = 18 ± 0.3



**Fig. 1.** Seleborca complexa. A : Lateral view of head; B : Slightly tilted, ventral view of head; C : Lateral field; D : Vulva with remnants of cement plug; E : Lateral view of female tail; F-G : Ventro-lateral view of male tail. (Arrows point to phasmids). (Bar equivalents :  $A-C = 2 \mu m$ ;  $D-F = 4 \mu m$ ;  $G = 10 \mu m$ .)

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(18-19);  $c' = 2.0 \pm 0.06$  (1.9-2.1);  $V = 66 \pm 0.3$ (65-66);  $V-A/T = 5.3 \pm 0.1$  (5.0-5.5).

# DESCRIPTION

*Female* : Body almost straight or slightly ventrally arcuate upon fixation. Cuticle annulated, annules 2.1-2.4  $\mu$ m wide at midbody. Subcuticle ornamented with two rows of punctations per annule. Lateral field with three incisures extending to phasmid. Three pairs of cephalic probolae; anterior margins with short membraneous projections, long projections along primary cephalic axils; apically pointed guarding pieces in primary cephalic axils. Cephalic axils about 1-1.5  $\mu$ m wide. Three broad labial probolae, bifurcated about one quarter of their length. Stoma cephaloboid; cheilostom wide; cheilorhabdions bacilliform, about 2  $\mu$ m high; rest of stoma narrow, 12-13 µm long. Pharynx cephaloboid; corpus cylindrical, about nine to eleven times longer than isthmus; bulb with valves, 26-29 µm long, 19-21 µm broad. Cardia prominent, enveloped by intestinal cells. Nerve-ring surrounds metacorpus; hemizonid opposite nerve-ring; excretory pore just anterior to hemizonid. Deirid not seen. Reproductive system monodelphic, prodelphic; ovary reflexed at oviduct and straight or somewhat reflexed posterior to vulva. Vulval lips protruding. Spermatheca 12-17 µm long, empty; PUB 12-23 µm long. Rectum 18-25 µm or about one ABW long. Anus a transverse slit. Tail conoid with pointed terminus, slightly ventrally arcuate, terminus strongly arcuate. Phasmids at about one third of tail length.

Male : not found.



**Fig. 2.** Zeldia punctata. A : Ventro-lateral view of head; B : Lateral field. Cervidellus serratus. C : Ventro-lateral view of head. Acromoldavicus skrjabini. D : Slightly tilted, ventral view of head. (Bar equivalents : A, C,  $D = 1 \ \mu m$ ;  $B = 2 \ \mu m$ ).

Fundam. appl. Nematol.

#### DISCUSSION

Zeldia punctata showed a wide variability in anterior structures (labial and cephalic probolae) and tail shape, as described by Allen and Noffsinger (1972). These authors also synonymised several species, viz. Z. glaphyra (Steiner, 1935) Thorne, 1937, Z. serrata Heyns, 1962 and Z. paucipunctata Andrássy, 1967, with Z. punctata. Andrássy (1984) suspected two more species, i.e. Z. minor Allen & Noffsinger, 1972 and Z. thornei Nesterov, 1979, to be synonymous with Z. punctata. Rashid et al. (1985) also indicated Z. minor as a possible synonym of Z. punctata and De Ley et al. (1990) considered them as synonyms. The present population agrees well with the descriptions of Z. punctata (including its junior synonyms) and with the external morphology of the species as shown by Sauer et al. (1979).

#### Acrobeles ciliatus von Linstow, 1877

#### MEASUREMENTS

Female (n = 4) : L = 533 ± 16 (508-566)  $\mu$ m; width = 32 ± 1 (30-34)  $\mu$ m; a = 17 ± 0.3 (16-17); pharynx = 144 ± 5 (130-152)  $\mu$ m; b = 3.7 ± 0.1 (3.6-3.9); tail = 54 ± 1 (52-56)  $\mu$ m; c = 10; c' = 3.2 ± 0.2 (2.9-3.4); V = 63.5 ± 0.3 (63-64); V-A/T = 2.75 ± 0.05 (2.7-2.8)

#### DESCRIPTION

Female : Body almost straight when relaxed by heat. Cuticle coarsely annulated, annules about 2.4-3.3 µm at midbody. Lateral field with two incisures extending to phasmid. Six cephalic probolae with membraneous fringes; seven or eight tines of various length on each side; middle tines longer in secondary cephalic axils; long guarding pieces in all cephalic axils. Three deeply bifurcate labial probolae, 12-15 µm high, about eight tines on inner and ten to twelve tines on outer side; proximal tines short and rounded, middle and distal tines long and slender, apical tines very fine. Pharynx cephaloboid; corpus cylindroid, lumen often distended; metacorpus somewhat fusiform; isthmus narrow. Bulb with valves, 25-27 µm long, 20-22 µm broad. Cardia prominent. Nerve ring surrounds isthmus. Hemizonid opposite. Excretory pore at metacorpus, 80-97 µm or 23-24 annules from base of head. Deirid not seen. Reproductive system monodelphic, prodelphic; ovary reflexed at oviduct, postvulval flexure not discernable. Spermatheca not observed. PUB 29-37 µm or about 1 BW long. Vulval lips not protruding. Rectum 15-16 µm long. Anus a transverse slit. Tail conoid with acute terminus. Phasmids at about one quarter of tail length.

Male : not found.

# Acrobeles singulus Heyns, 1969

#### Measurements

*Female* (n = 6) : L =  $403 \pm 11$  (380-449) µm; width =  $26 \pm 0.3$  (25-27) µm; a =  $16 \pm 0.3$ 

#### DESCRIPTION

Female : Body slightly ventrally arcuate when relaxed by heat. Cuticle annulated, annules 1.9-2.4 µm wide at midbody. Lateral field with two incisures, stippled, extending to phasmid. Six cephalic probolae with membraneous fringes; six or seven tines of various length on each side; middle tines longer in secondary cephalic axils; long guarding pieces in all cephalic axils. Three deeply bifurcate labial probolae, 12-13 µm high, with fringes; about seven or eight tines on inner and nine or ten on outer side; proximal tines short and rounded, middle and distal tines long and slender, apical tines very fine. Stoma cephaloboid; cheilostom broad, cheilorhabdions 2.5-3 µm high; rest of stoma narrow, 10-11 µm long. Pharynx cephaloboid; corpus cylindrical, isthmus narrow; bulb with valves, 21-23 µm long, 16-18 µm wide. Cardia prominent. Nerve ring surrounds isthmus or metacorpus-isthmus junction. Hemizonid at isthmus. Excretory pore at metacorpus, 54-66 µm or 19-23 annules from base of head. Deirid at level of bulb. Reproductive system cephaloboid, monodelphic, prodelphic; ovary reflexed at oviduct, variable posterior to vulva : from straight to double flexure. Spermatheca small, 7-8 µm long, empty. PUB short, 3-12 µm or 0.1-0.5 BW long. Vulval lips protruding. Rectum 12-14 µm long. Anus a transverse slit. Tail conoid with acute, minutely rounded terminus. Phasmids at 16-24 % of tail length.

Male : not found.

#### DISCUSSION

Heyns (1969) differentiated A. singulus from A. ciliatus (as redescribed by Thomas and Allen, 1965) " in the formation of the labial and cephalic probolae, and in tail shape ". Andrássy (1984) synonymised the two species. Rashid et al. (1985) were unable to distinguish the two species on basis of probolae shape. They used differences in tail shape (terminus subacute in A. singulus vs acute in A. ciliatus), position of excretory pore (15-23 annules vs 20-25 annules from anterior end), length of PUB (very short vs 1.5 BW) and development of spermatheca (not always distinct and usually empty vs well developed and usually filled) to identify their Brazilian population of A. singulus. Rashid et al. (1990a) compared type specimens of A. singulus with Namibian specimens of A. ciliatus and found distinct differences between the two species, *i.e.* PUB short against long, spermatheca indistinct and empty against well developed and filled, and presence against absence of a long hooked tine on the cephalic probolae.

The two populations from Crete could not be unequivocally separated by the shape of probolae and tail, position of the excretory pore or development of the spermatheca. They were, however, differentiated by total body length (508-566  $\mu$ m vs 380-449  $\mu$ m), length of PUB (0.9-1.1 BW vs 0.1-0.5 BW) and width of body annules (2.4-3.3  $\mu$ m vs 1.9-2.4  $\mu$ m). Based mainly on these characters, although not very strong, the two populations were identified as *A. ciliatus* and *A. singulus*, respectively.

# Cervidellus serratus (Thorne, 1925) Thorne, 1937 (Fig. 2 C)

#### MEASUREMENTS

Female (n = 9) : L =  $303 \pm 4$  (285-319) µm; width =  $19 \pm 0.6$  (16-21) µm; a =  $16 \pm 0.4$ (15-18); pharynx =  $92 \pm 1$  (88-96) µm; b =  $3.3 \pm 0.02$ (3.2-3.4); tail =  $27 \pm 0.3$  (27-29) µm; c =  $11 \pm 0.2$ (11-12); c' =  $2.4 \pm 0.02$  (2.4-2.5); V =  $64 \pm 0.2$ (63-65); V-A/T =  $3.0 \pm 0.1$  (2.7-3.2).

#### DESCRIPTION

Female : Body slightly ventrally arcuate when relaxed by heat. Cuticle annulated, annules 1.1-1.7 µm broad at midbody; longitudinal striation seen in the SEM. Lateral field with three incisures extending to phasmid, the two outer extend some annules posterior to phasmid. Six cephalic probolae consisting of five leaf-like elements each (as described in Boström, 1986). Three slender labial probolae about 4-5 µm high, bifurcate about 0.25 of their length. Stoma cephaloboid, 6.7-7.5 µm long; cheilostom broad, rest of stoma narrow. Pharynx cephaloboid; procorpus somewhat fusiform, isthmus narrow. Bulb oval with valves, 15-17 µm long, 12-13 µm broad. Cardia prominent, enveloped by intestinal cells. Nerve ring surrounds isthmus or metacorpus-isthmus junction. Hemizonid at isthmus. Excretory pore just anterior to hemizonid, 63-69 µm from anterior end. Deirid at level of bulb. Reproductive system cephaloboid, monodelphic, prodelphic; ovary reflexed at oviduct, variable posterior to vulva. Spermatheca 17-25 µm long, empty. PUB 17-32 or 0.9-1.7 BW long. Vulval lips slightly protruding. Rectum 11-13 µm long. Anus a transverse slit. Tail conoid with acute terminus. Phasmid in a pit at 42-47 % of tail length.

Male : not found.

#### DISCUSSION

These specimens are intermediate in size between C. serratus and C. neftasiensis Boström, 1986, just like a population recorded from Samos (Boström, 1991). The latter was identified as C. neftasiensis on basis of its small body length – 289  $\pm$  4 (271-307) µm – and short PUB (0.5-0.7 BW). The specimens of the present population are larger than the Samos specimens and also have a longer PUB. But they are on average smaller than C. serratus (L = 300-420 µm; PUB = 1.5-2 BW; Andrássy, 1984). They are thus narrowing the gap between

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the two species, but for the time being identified as *C. serratus.* 

# Acromoldavicus skrjabini (Nesterov & Lisetzkaja, 1965) Nesterov, 1970 (Fig. 2 D)

# **M**EASUREMENTS

*Female* (n = 1) : L = 512  $\mu$ m; width = 30  $\mu$ m; a = 17; pharynx = 116  $\mu$ m; b = 4.4; tail = 26  $\mu$ m; c = 20; c' = 1.8; V = 65; V-A/T = 6.1.

#### DESCRIPTION

Female : Body strongly ventrally arcuate when killed by heat. Cuticle coarsely annulated, annules about 3 um wide at midbody; longitudinally striated, about 30 striae at midbody. Lateral field with three incisures, starting just anterior to bulb and extending almost to tail tip. Six leaf-like cephalic probolae with extensions along primary cephalic axils; in ventral cephalic axil a large pointed guarding piece. Three thin, broad labial probolae, triangular-rounded, bending outwards, connected basally. Stoma about 7 µm long, without differentiations or sclerotizations, enveloped by a somewhat expanded pharyngeal collar. Pharynx cephaloboid; procorpus cylindrical, metacorpus fusiform; isthmus narrow; bulb  $22 \times 18 \,\mu\text{m}$ , with valves. Nerve ring surrounds is thmus. Hemizonid at isthmus-bulb junction. Excretory pore just anterior to hemizonid. Deirid at level of bulb. Reproductive system cephaloboid, monodelphic, prodelphic; ovary reflexed at oviduct, straight posterior to vulva. Spermatheca about 42 um long. PUB 27 um or 0.9 BW long. Vulval lips protruding. Rectum 21 µm or 1.5 ABW long. Anus a transverse slit. Tail ventrally arcuate, conoid with acute terminus. Phasmids at 32 % of tail length.

Male : not found.

#### DISCUSSION

Several juveniles were found of this species. The single adult specimen is smaller than those of *A. skrjabini* described by Nesterov (1970), but it agrees in most characters with that species. The external structures also agree with those of *Acromoldavicus* juveniles reported by Boström (1989*b*).

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