Description of Acroukrainicus sagittiferus gen. et sp. n. (Nematoda: Cephalobidae) from the area of an old sulphur industry in Ukraine

Oleksandr Holovachov*, Sven Boström** and Andrij Susulovsky***

*Department of Zoology, Biological Faculty, L'viv National University, Grushevsky str. 4, L'viv 79005, Ukraine, **Department of Invertebrate Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden, e-mail: sven.bostrom@nrm.se.

*** State Museum of Natural History, Theatralna str. 18, L'viv 79008, Ukraine.

Accepted for publication 3 April 2001

Summary. Acroukrainicus sagittiferus gen. et sp. n. from a loamy-sand soil in Ukraine is described on the basis of light and scanning electron microscopy. The new genus is placed in the Cephalobinae and distinguished from all other genera in the subfamily by the possession of three pairs of lips, each lip flat and three-lobed (asymmetrically triangular); primary axils narrow; secondary axils broad; and three pointed labial probolae, each probola shaped as an arrow-head with one rounded lateral tine on each side and a concave abaxial prominence.

Key-words: Acroukrainicus sagittiferus, morphology, new genus, SEM, taxonomy, Ukraine.

Soil samples were collected in the area of a former stagnant-water pond previously used for cooling and cleaning of technical water from a sulphur extraction industry in the Yavoriv District, L'viv Province, Ukraine. The bottom of the pond is comprised of sand, clay and some mineral sulphur, that precipitated from polluted water. After closure of the sulphur extraction, the water evaporated and the bottom of the former pond became covered with vegetation. The first plants appeared in 1995 and in 2000 about 60-65% of the area was dry and covered with mosses and higher plants. The pond is now a part of the "Cholginsky Ornithological Reserve", which was founded for protection and conservation of endangered migratory birds (Shydlovskyy et al., 1999). It includes dry, desert-like, eroded landscapes covered with xerophytes, mostly gramines and composites, and mosses, that grow on the thin humus layer. Samples were taken in the habitat with 1-2 years old vegetation, and were comprised of loamy-sand soil with some organic material such as roots and mosses.

The subfamily Cephalobinae of the family Cephalobidae Filipjev, 1934 consists of about 20 genera of which many have a worldwide distribution. The most recent genera described were *Cri*- bronema Siddiqi, 1993 and Chiloplacoides Heyns, 1994 from a rainforest in Cameroon and nunataks in Antarctica, respectively (Heyns, 1994; Siddiqi, 1993). A new species belonging to a new genus of the subfamily was found in soil samples from the "Cholginsky Ornithological Reserve" and is here described from studies by light and scanning electron microscopy.

MATERIALS AND METHODS

Nematodes were extracted by a modified Baermann funnel method, relaxed by gentle heat, fixed in cold TAF, transferred to pure glycerine by a slow evaporation method and mounted on permanent slides in glycerine with paraffin wax as support for the coverslip. After measuring and observations, four females were washed with gradually added distilled water, resuspended in TAF and prepared for SEM. They were postfixed in 1% osmium tetroxide (OsO₄) and transferred to pure acetone through an acetone/distilled water series. Specimens were critical point dried in liquid CO_2 , mounted on stubs, gold-plated under vacuum to a thickness of 200 Å in a sputter, and examined in a Zeiss Novascan 30 SEM at an accelerating voltage of 15 kV.

DESCRIPTION Acroukrainicus gen. n.

Diagnosis. Cephalobidae. Cephalobinae. Body length about 0.5 mm. Cuticle annulated, without longitudinal striation. Lateral field with five incisures. Three pairs of lips, each lip flat and threelobed (asymmetrically triangular); primary axils narrow, secondary axils broad. Guarding processes absent. Three pointed labial probolae, each probola flattened, conical, shaped like an arrowhead with one rounded lateral tine on each side and a concave abaxial prominence. Stoma cephalobid, subdivided into cheilo-, gymno- and stegostom. Pharyngeal corpus cylindrical, metacorpus fusiform, corpus 3-4 times longer than isthmus. Excretory pore at or near the level of corpus-isthmus junction. Female reproductive system cephalobid, spermatheca small and empty, postvulval uterine branch short. Tail conical, with acute tip.

Relationships. The new genus is characterized and separated from all other genera of the Cephalobinae by the possession of three pairs of lips, each lip flat and three-lobed (asymmetrically triangular), primary axils narrow, secondary axils broad, and three pointed labial probolae, each shaped as an arrow-head with one rounded lateral tine on each side and a concave abaxial prominence.

Type and only species: Acroukrainicus sagittiferus sp. n.

Acroukrainicus sagittiferus sp. n. (Figs. 1-2)

Measurements: Table 1.

Female. Body generally straight or slightly arcuate ventrad when heat relaxed, gradually narrowing anteriorly from nerve ring and in posterior part. Cuticle 1.7-2.4 µm thick, strongly annulated, annules 1.9-2.4 µm wide just anterior to vulva. Longitudinal striation absent. Lateral field with five incisures, outer two wings areolated, inner two wings smooth; originating as two incisures at level of anterior part of corpus, gradually increasing to five incisures at or just posterior to deirid, decreasing on the tail to three incisures at phasmid and a singe incisure almost to tail tip. Lip region with four cephalic and six labial papillae; amphid opening oval, surrounded by a cuticular ridge, located dorso-laterally on the base of each lateral lip. Three pairs of lips, one dorsal and two ventrolateral, each lip flat and three-lobed (asymmet-

rically triangular). Pairs of lips separated by narrow primary axils with one pair of large rounded lobes; bases of adjacent lips slightly overlapping. The two members of each pair separated by broad secondary axils with two pairs of smaller and rounded lobes. Guarding processes absent. Lips connected by radial ridges to external stoma wall. Labial probolae 0.7-0.9 times as long as labial region width, connected at bases by prominent tangential ridges. Each labial probola flattened, conical, shaped as an arrow-head with rounded lateral tines and a concave abaxial prominence. Apical parts of labial probolae curved inward under LM, inward or outward as seen in SEM. Stoma 1.1-1.4 times as long as labial region width, divided into cheilo-, gymno- and stegostom. Cheilorhabdia bacilliform in median section, other stomatal elements weakly sclerotized. Pharyngeal corpus cylindrical, metacorpus somewhat fusiform, isthmus clearly delineated from and narrower than corpus. Basal bulb with strongly developed valves. Excretory pore situated from 2 annules anterior to 8 annules posterior to nerve ring, near the corpus-isthmus junction. Deirids small but clearly visible, located 2-8 annules posterior to the excretory pore. Reproductive system monodelphic, prodelphic, ovary in the right part of body (dextral), reflexed anteriorly at oviduct, ovary posterior to vulva straight or with double flexure. Postvulval uterine sac half of vulval body width. Spermatheca small, 7-13 µm long, and empty. Vulva slightly depressed, situated at about two thirds of body length; vulval lips formed by cuticular ridges. Vagina about 0.3-0.4 times vulval body width. Rectum longer than anal body width, anus an arcuate slit. Phasmids somewhat anterior to middle of tail. Tail conoid, with 14-20 ventral annules, posteriormost annules fused and hardly discernible, tail tip hyaline, almost acute, but occasionally somewhat crumpled.

Male. Not found.

Type locality and habitat. Ukraine, L'viv Province, Yavoriv District, "Cholginsky Ornithological Reserve", 49°54'N, 23°27'E, former stagnant-water pond, loamy-sandy soil, mosses and gramines, May 10, 1999; legit O. Holovachov.

Type specimens. Holotype in the nematode collection of Wageningen Agricultural University, Wageningen, Netherlands. One paratype female in the nematode collection of the State Museum of Natural History, L'viv, Ukraine. Three paratype females in the type collection (access no. 5309) of the Department of Invertebrate Zoology, Swedish Museum of Natural History, Stockholm, Sweden.



Fig. 1. Acroukrainicus sagittiferus gen. et sp. n. Holotype (A, C, D, E, H). Paratype female (B, F, G). A: Anterior end, lateral median section; B: Schematic lip structure (abbreviations: I = primary axil, II = secondary axil, L = lateral lip, SD = subdorsal lip, SV = subventral lip); C: Entire body; D: Pharyngeal region; E: Typical tail; F-G: Aberrant tails; H: Reproductive system. Scale bars: A-B, 10 μ m; C, 100 μ m; D-H, 30 μ m.

Character	Holotype	Paratypes
Character	female	n = 8
L	610	598±43 (521-671)
Body width	33	30±2.0 (26-33)
Neck length	172	163±5.5 (152-171)
Tail length	49	45±2.5 (42-48)
ABW	18	18±1.2 (16-20)
à	18	20±1.8 (17-23)
b	3.6	3.7±0.2 (3.4-3.9)
С	12	13±0.9 (12-15)
c'	2.7	2.5±0.2 (2.3-2.9)
LRW	13	13±0.5 (12-13)
Labial probolae length	10	10±0.8 (9.5-12)
Stoma	16	16±1.0 (14-17)
Corpus	103	97±6.2 (83-109)
Isthmus	31	26±3.3 (21-30)
Bulbus	25	25±1.9 (22-27)
Corpus/isthmus ratio	3.4	3.7±0.4 (3.2-4.6)
Nerve ring (nr)	111	98±9.5 (85-114)
Excretory pore (ep)	106	108±9.3 (96-117)
Deirid (dei)	124	123±13.9 (105-138)
Nerve ring (% of neck)	65	61±5.8 (53-70)
Excretory pore (% of neck)	62	67±3.9 (63-72)
Deirid (% of neck)	72	76±6.7 (66-83)
V (%)	62	62±0.9 (61-64)
Vagina	11	11±1.1 (9.7-12)
PUS	12	16±1.9 (14-19)
PUS/BW	0.4	0.5±0.07 (0.4-0.6)
Rectum	26	25±2.5 (23-30)
Rectum/ABW	1.5	1.4±0.1 (1.2-1.6)
Phasmid from anus	21	18±2.3 (15-22)
Phasmid (% of tail)	43	40±4.6 (34-45)
Annule width	2.4	2.1±0.2 (1.9-2.4)
R _{nr} *	60	46±4 (42-54)
R _{ep} *	58	50±2 (48-52)
R _{dei} *	66	57±4 (53-61)
R _{neck} *	90	76±3 (70-78)
R _{vulva} *	179	171±9 (152-179)
R _{anus} *	260	252±13 (224-262)

Table 1. Measurements (in μ m) of *Acroukrainicus sagittiferus* gen. *et* sp. n. (the dimensions of paratype females are given as mean \pm standard deviation and range).

Etymology. The genus name refers to the provenance of the species and the specific epithet is derived from the Latin words *sagitta* (arrow) and *ferre* (to carry) and refers to the resemblance of the labial probolae to arrow-heads.

Diagnosis. With the characters of the genus, as described above.

Relationships. Acroukrainicus sagittiferus n. gen., n. sp. appears to have the closest affinity to species of Acrobeloides (Cobb, 1924) Steiner & Buhrer, 1933 by the following characters: labial probolae pointed, connected at bases by tangential ridges; three pairs of lips without guarding processes in the primary cephalic axils; pharyngeal metacorpus fusiform; lateral field extending posterior to phasmid. The new genus should also be compared with three other genera, viz. Cephalobus Bastian, 1865, Chiloplacus Thorne, 1937 and Acrobelophis Andrássy, 1984. These four genera each possess lips with cephalic probolae, at least in some of the species. The cephalic probolae vary from (i) small extensions along the primary axils as in e.g. Cephalobus persegnis Bastian, 1865 (Boström, 1993a: Fig. 1A), through (ii) rounded-pointed extensions as in e.g. Acrobeloides nanus (de Man, 1880) Anderson, 1968 (Boström, 1993a: Fig. 2D-F), Acrobeloides tricornis (Thorne, 1925) Thorne, 1937 (Boström, 1988: Fig. 1A; Boström, 1993b:



Fig. 2. Acroukrainicus sagittiferus gen. et sp. n. SEM micrographs of female (A-G). A: Anterior end, lateral view; B: Anterior end, ventral view; C: *en face* view, arrow points at ventral side; D: Excretory pore (arrow); E: Disrupted specimen showing anterior flexure of ovary and spermatheca (arrow); F: Vulva and lateral field (arrow); G: Tail region, arrow points at phasmid. Scale bars: A, B, D, F - 2 μ m; C - 1 μ m; E - 10 μ m; G - 4 μ m.

Fig. 1A), Acrobeloides sp. 3 (Sauer & Annells, 1985: Fig. 4I), Chiloplacus tenuis Rashid & Heyns, 1990 (Rashid & Heyns, 1990: Fig. 2A-B), and Acrobelophis minimus (Thorne, 1925) Andrássy, 1984 (Boström, 1985: Fig. 2A-D), to (iii) long and slender extensions as in e.g. Acrobeloides saeedi Siddiqi, De Ley & Khan, 1992 (Siddiqi et al., 1992: Fig. 2A), Acrobeloides sp. 2 (Sauer & An-

nells, 1985: Fig. 4H), and Acrobelophis lanceolatus Vinciguerra & Clausi, 1996 (Vinciguerra & Clausi, 1996: Fig. 2A-C). However, of these genera no species so far studied by SEM have labial probolae with tines and a concave abaxial prominence. A. sagittiferus gen. n., sp. n. differs from species of Acrobeloides and Cephalobus also by having lateral tines and a concave abaxial prominence on the labial probolae. It further differs from *Chiloplacus* and *Acrobelophis* by having simple, not bifurcate, labial probolae with a concave abaxial prominence. Some species of *Acrobeloides* have more elaborate anterior structures that appear to be part of a trend towards the anterior organisation in *A. sagittiferus* gen. n., sp. n.

More species and populations of these genera need to be studied with SEM to enable the taxonomy of this group to be revised and to provide information about the usefulness of different characters in the systematics of the family Cephalobidae.

REFERENCES

- Boström, S. 1985. Description and morphological variability of *Chiloplacus minimus* (Thorne, 1925) Andrássy, 1959 (Nematoda: Cephalobidae). *Nematologica* 30 (1984): 151-160.
- Boström, S. 1988. A scanning electron microscope study of some species of terrestrial nematodes from Spitzbergen. *Nematologica* 33 (1987): 366-374.
- Boström, S. 1993a. Some cephalobids from Ireland and Malaysia (Nematoda: Rhabditida). *Afro-Asian Journal of Nematology* 3: 128-134.
- Boström, S. 1993b. Some cephalobids from Turkey (Nematoda: Rhabditida). Nematologia mediterranea 21: 295-300.

- Heyns, J. 1994. Chiloplacoides antarcticus n. gen., n. sp. from western Dronning Maud Land, Antarctica (Nematoda: Cephalobidae). Fundamental and applied Nematology 17: 333-338.
- Rashid, F. & Heyns, J. 1990. Chiloplacus and Macrolaimellus species from South West Africa / Namibia (Nematoda: Cephalobidae). Phytophylactica 22: 189-199.
- Sauer, M.R. & Annells, C.M. 1985. Lip region structure in Acrobelinae (Nematoda: Cephalobidae). Nematologica 30 (1984): 140-150.
- Shydlovskyy, I.V., Lysachuk, T.I. & Holovachov, O.V. 1999. Western-Ukrainian ornithological station. Report on the results of five-year activity: 1995-1999. "Spolom", L'viv, 32 pp.
- Siddiqi, M.R. 1993. Nematodes of tropical rainforests: 2. Five new genera and eight new species of cephalobs. Afro-Asian Journal of Nematology 3: 212-225.
- Siddiqi, M.R., De Ley, P. & Khan, H.A. 1992. Acrobeloides saeedi sp. n. from Pakistan and redescription of A. bodenheimeri (Steiner) and Placodira lobata Thorne (Nematoda: Cephalobidae). Afro-Asian Journal of Nematology 2: 5-16.
- Vinciguerra, M.T. & Clausi, M. 1996. Two new species of *Acrobelophis* (Nematoda: Cephalobidae) from Subantarctica and notes on the genus. *Afro-Asian Journal of Nematology* 6: 104-109.

Holovachov O., Boström S., Susulovsky A. Описание Acroukrainicus sagittiferus gen. et sp. n. (Nematoda: Cephalobidae) из района бывших серных разработок в Украине.

Резюме. На основании данных световой и электронной микроскопии в подсемействе Cephalobinae описан Acroukrainicus sagittiferus gen. et sp. n., выделенный из песчано-глинистой почвы. Для нового рода характерно наличие трех пар плоских трехлопастных губ, глубоких и узких первичных выемок, неглубоких и широких вторичных выемок и трех губных пробол, по форме напоминающих наконечник стрелы с боковыми округленными нитевидными выступами, по одному с каждой стороны, и выпуклым абаксиальным утолщением. По этим признакам он может быть дифференцирован от всех известных родов цефалобид.