

***Aprutides guidettii* Scognamiglio, 1974 (Nematoda: Aphelenchoididae) and *Subanguina picridis* (Kirjanova, 1944) Brzeski, 1981 (Nematoda: Anguinidae) from Iran**

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ABSTRACT

Several soil samples were taken from western parts of Iran. One of the samples collected from the rhizosphere of nectarine trees (*Prunus persica* L.) in Moghan, Ardabil Province, contained *Aprutides guidettii*. The species *Subanguina picridis* was recovered from leaf gall of *Serratula latifolia* Boiss (Asteraceae) in the vicinity of Mashhad, Khorasan Province. Although this species showed remarkable differences from the original description, it was considered to be *S. picridis* considering the high level of variability described in the literature. Both species are described in detail and documented with drawings. Additional morphological data are illustrated by means of SEM. *Subanguina picridis* is described here for the first time from Iran.

Keywords: Anguinids, *Aprutides*, Iran, *Prunus*, *Serratula*, *Subanguina*.

INTRODUCTION

The genus *Aprutides* was proposed in 1970 with the description of the type species *Aprutides martuccii* by Scognamiglio *et al.* (1970). Later on, Scognamiglio (1974) described another species of the genus namely *A. guidettii*, from Italy. In Turkey Saltukoglu *et al.* (1976) described another population of the latter species from the Istanbul area and stated that "this small nematode has a digestive and female reproductive system comparable to that of the genus *Aphelenchoides* Fischer, 1894 but the head provided with the remarkable cap and the clavate tail are both unusual characteristics in that genus". A similar head structure has been described for *Omemea maxbassiensis* (Massey, 1971). A clavate tail was also described for *Aprutides martuccii* Scognamiglio *et al.* (1970) but with the usual *Aphelenchoides* head. *Aprutides guidettii* stands apart from other species of the genus *Aprutides* and is morphologically closer to the genus *Omemea*

through having a similar head structure. A completely similar population of *Aprutides guidettii* was collected from the rhizosphere of nectarine trees in Moghan and studied in detail by light and scanning electron microscopy (SEM).

Many species of the family Anguinidae are well known parasites of aerial parts of the monocots or dicots. Leaves of a wild plant, namely *Serratula latifolia* Boiss, collected from Khorasan Province were found to be infected with a nematode species belonging to the family Anguinidae. The host plant is a dicot belonging to the family Asteraceae (= Compositae) found in Iran, Afghanistan and Turkmenistan. The nematode induced galls which are produced on the leaves, especially on the basal leaves. Each leaf may contain several, round or ellipsoid galls, measuring 2-7 mm. with a central vesicle, sometimes formed in groups nearby and causing leaf deformation. The galls may appear pale green to gray or even black in colour with age. Nematodes from galls of *Serratula latifolia* were identified as *Subanguina picridis*

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although they display some morphometric differences with the type population. We can expect that these dissimilarities are influenced by the host plants or environmental conditions.

MATERIALS AND METHODS

Soil nematodes were extracted from the soil by sieving and a centrifugal sugar-flotation method. To isolate leaf nematodes, fresh galls were dissected and immersed in distilled water for several hours. Nematodes were removed from the water and concentrated in a small volume of distilled water. The specimens were killed and fixed with a hot solution of FGA 4:1:1 (formaldehyde, glycerin and acetic acid) and processed to anhydrous glycerin using a modified Seinhorst's method (De Grisse, 1969). The nematodes were then mounted on aluminium slides with double cover slips and examined under a light microscope. Some of the specimens studied and measured were selected for SEM studies. After measuring, the slides were opened, the glycerine embedded nematodes transferred to a drop of glycerine in a small embryo dish and then distilled water was added drop by drop until the nematodes were in almost pure distilled water. The nematodes were then dehydrated in a series of 25, 50, 75, 95, and 100% solutions of ethanol. The nematodes in the last concentration were left overnight. For drying, the standard critical point drying procedure with CO₂ was used. Finally the nematodes were put on the stubs and coated with gold for SEM examination. The nematodes were observed with a Scanning Electron Microscope JMS 840 15 KV.

Aprutides guidettii Scognamiglio, 1974 (Figures 1 and 2)

Measurements: see Table 1

Female

Body slightly curved to C-shape when relaxed by gentle heat. Annules fine, almost 1 µm wide, sometimes not clearly distinctive.

Lateral field with three lines (Figures 1: D and 2: D-E), middle one is fine and somewhat difficult to see by light microscopy. Lip region high, not offset with body contour. Head narrowed toward anterior end and bearing a very distinct cap, basal part of head framework and cheilorhabdia well sclerotized. Amphid pore like, situated at lateral base of head cap. Stylet with small thickening at its base, anterior part shorter than posterior and provided with a large, ventrally situated opening. Oesophagus with narrow procorpus and a prominent metacarpus, very short isthmus and oesophago-intestinal junction starting immediately after metacarpus. Oesophagus gland lobes extending 57 µm posteriorly over intestine. Nerve ring immediately behind the median bulb. Excretory pore slightly posterior to the median bulb and almost at the same level with the nerve ring. Tail subcylindrical, with a clavate tip and longer than twice the body width at anal region. Phasmids pore like, near to the tail end. Vagina at right angle to the body axis. Genital apparatus monodelphic-prodelphic, straight, females do not contain sperms, the spermatheca is empty, non functional and inconspicuous. PUS not so long (see Table 1) and without undifferentiated cells.

Scanning electron microscopy (SEM, Figure 2) study demonstrated three lines on lateral field (instead of two as seen by light microscope), outer lines are distinct but middle one is very thin as also mentioned by Saltuoglu *et al.* (1976). Lateral bands are finely areolated with some distances (Figure 2: E). Head high, bearing two annules, anterior annule resembles to mushroom cap. The posterior annule is wider and thicker than the anterior one and, from the middle toward the anterior end, it has a truncate cone shape. Amphid aperture pore like, situated at the base of head cap, in the junction zone of two head annules (Figure 2: B, C). Labial papillae situated in two circles on labial disk. Oral disk small, almost hexangular with small, pore like, oral aperture (Figure 2: A).

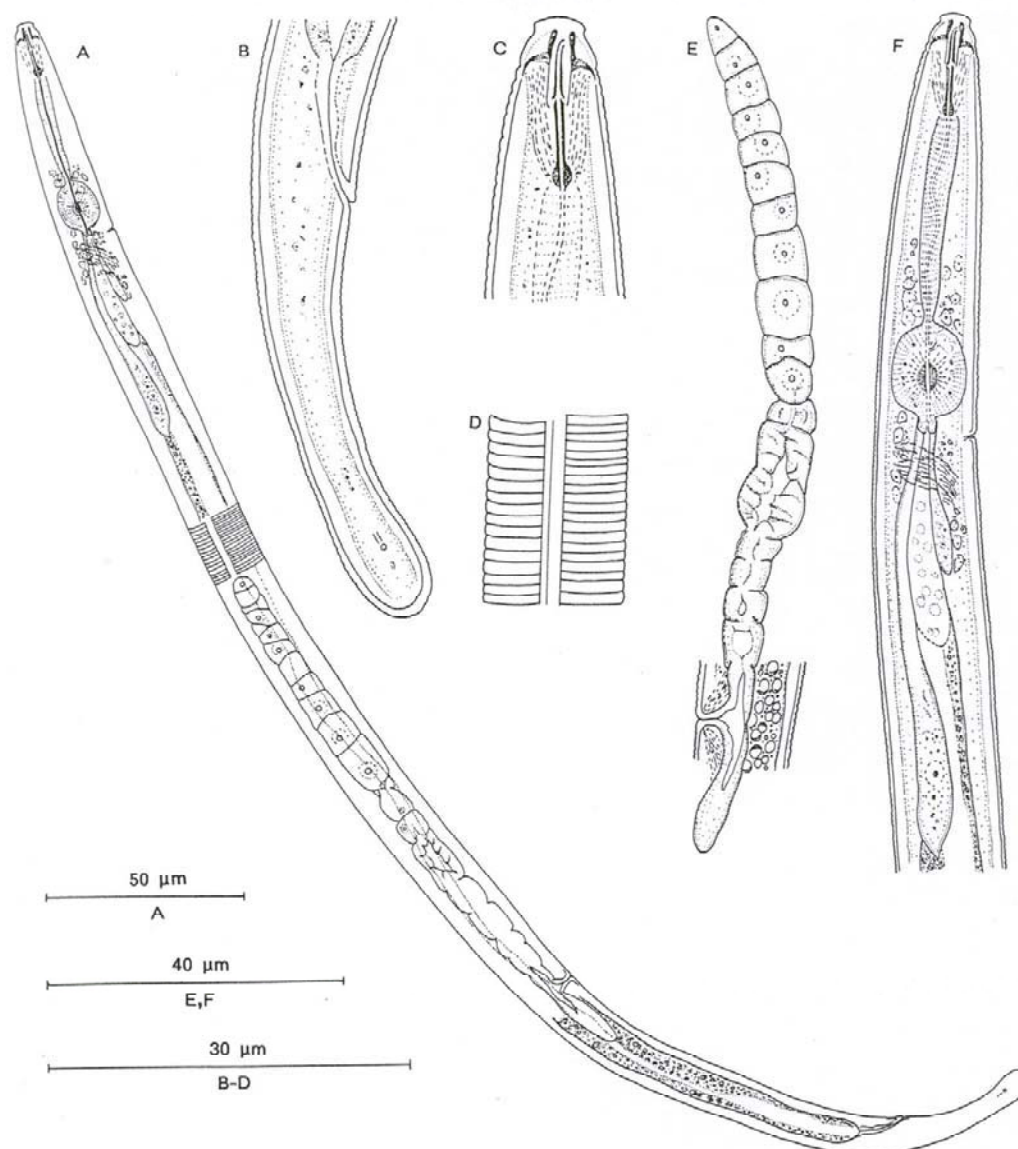


Figure 1. *Aprutides guidettii*. Female. A: Entire body; B: Tail shape and phasmid; C: Anterior region; D: Lateral field; E: Genital tract; F: Oesophageal region.

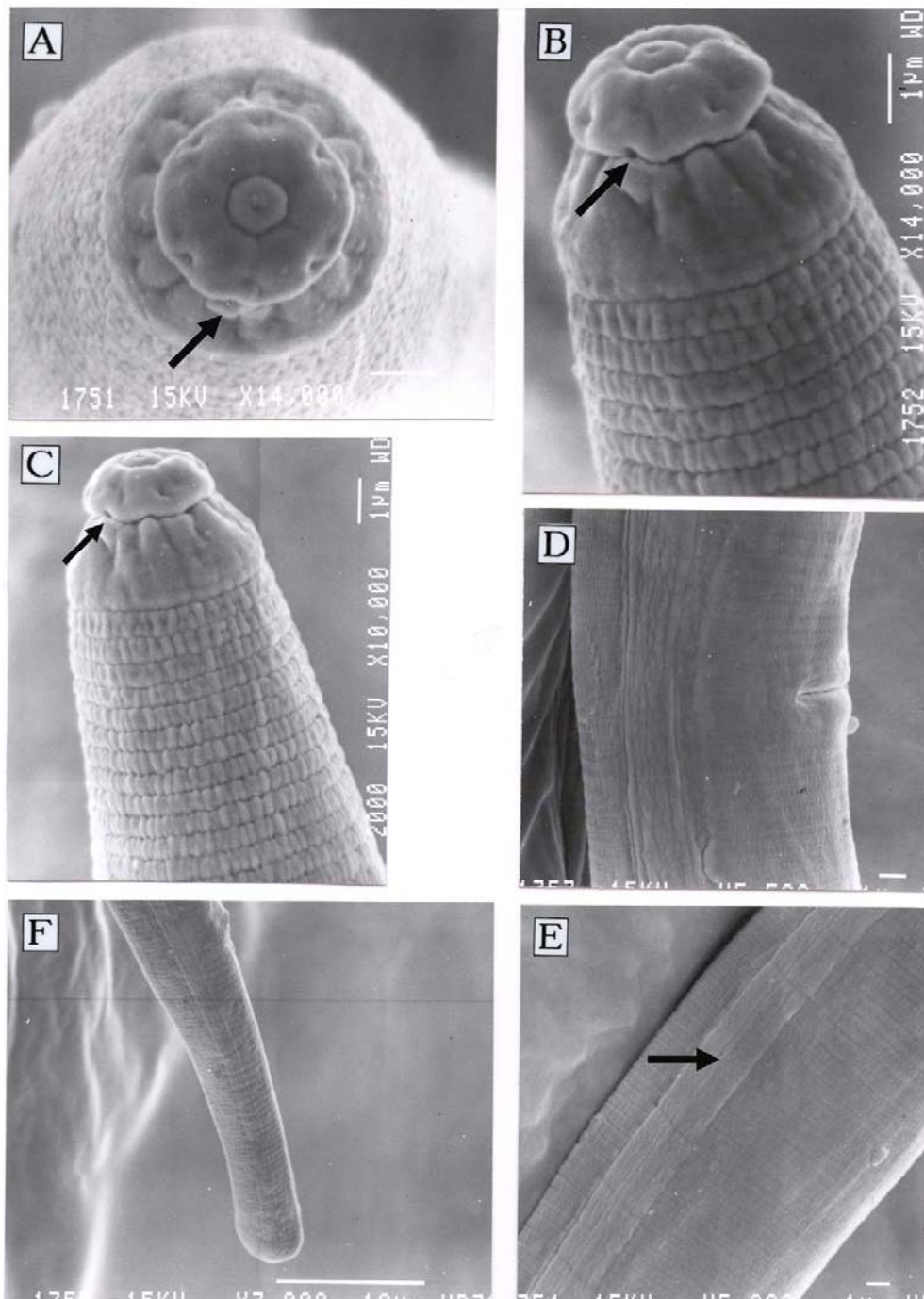


Figure 2. SEM photography of *Aprutides guidettii*. A: Head en face view; B, C: Head lateral view; D: Vulva and lateral field; E: Lateral field at mid body (arrow point at third line); F: Tail. Arrows in A-C point at amphid. Scale bar, 1 μ m for A-E and 10 μ m for F.

Table 1. Morphometric data of some populations of *Aprutides guidettii* (all measurements in μm).

Population	Iran (Moghan)	Saltukoglu <i>et al.</i> , (1976)	Scognamiglio, (1974).
Habitat	<i>Prunus persica</i>	Grassland	<i>Ficus carica</i>
n	10	9	15
L	423 $\pm$ 44 (365-475)	330-510	379-469
L'	385 $\pm$ 41 (330-434)	-	-
a	27.2 $\pm$ 2.9 (23-31.5)	24-32	28-33.8
b	7.5 $\pm$ 0.7 (6.4-8.3)	7.2-9.6	5.4-6.8
b'	3.9 $\pm$ 0.5 (3.3-4.5)	-	-
c	11 $\pm$ 0.7 (10-12)	9-12.2	11-13.5
c'	4.2 $\pm$ 0.3 (3.7-4.9)	4.2-4.7	-
V	66 $\pm$ 2 (63-69)	65-69	66.4-70.5
Stylet	12.3 $\pm$ 0.4 (12-13)	12.5-13.5	11.9-13.6
Median bulb	49 $\pm$ 2.3 (45-52)	-	-
MB	87 $\pm$ 0.3 (82-91)	-	-
Oesophagus	56 $\pm$ 1.9 (52-58)	47-58	-
Excretory pore	59 $\pm$ 8 (49-76)	51-64	-
Body width	16 $\pm$ 0.9 (14-17)	-	-
Head-vulva	279 $\pm$ 32.5 (232-328)	-	-
G1	123 $\pm$ 28 (99-187)	78-116	-
G1%	29 $\pm$ 5.1 (24-39)	-	-
PUS	20 $\pm$ 3.4 (15-25)	14-27	-
PUS/BW	1.3 $\pm$ 0.3 (0.9-1.7)	-	-
Vulva-anus	102 $\pm$ 10.8 (84-121)	-	-
Tail	38 $\pm$ 3.5 (33-42)	-	-
Anal body width	9.1 $\pm$ 0.6 (8-10)	-	-
Vulva-anus/Tail	2.7 $\pm$ 0.2 (2.4-2.9)	-	-

Male

Not found. So far males have not been reported for this species.

Locality

The soil sample was collected from the rhizosphere of nectarine trees (*Prunus persica* L.) in Moghan (Ardabil Province), Iran.

DISCUSSION

Since the introduction of *A. guidettii* by Scognamiglio in 1974, this nematode has been reported from Australia (Bird and

Yeates, 1993) and Iran (Barooti, 1998).

Our population of *A. guidettii* has shown close relationship to original description of the species (Scognamiglio, 1974) and also that of Turkish population (Saltukoglu *et al.*, 1976). There are no discernible morphological and morphometrical differences among the three populations. In contrast to most other nematode species, little natural variation was observed within populations of this species (Table 1). Despite the large number of soil and plant samples which were collected and examined by various investigators from all over the country, it appears that this species is confined to a restricted region of northwest Iran. Similarity in the climatic conditions of the regions in which *A. guidet-*



tii has been recorded (Italy and Turkey) may indicate that the species possibly adapted to a specific type of ecosystem. Almost all the plant parasitic species of nematodes belonging to aphelenchids reported so far are associated with the above ground (aerial) parts of their host plants (Franklin and Siddiqi, 1972; Mamiya, 1984; Griffith, 1987; Yin *et al.*, 1988; Hunt, 1993; De Waele, 2002). There has been no report in the literature indicating discernible damage on the roots caused by these nematodes including *A. guidettii*. On the other hand, there are adequate reports indicating fungal feeding habits of these groups (Hunt, 1993; Nickle and Hooper, 1991; Franklin and Siddiqi, 1972; Goodey,

1960).

***Subanguina picridis* (Kirjanova, 1944)
Brzeski, 1981 (Figures 3 and 4)**

Measurements: see Table 2

Female

Body 1.9-2.9 mm long, slightly curved ventrally to a C shape (Figure 3: G). Cuticle thin, finely annulated. Lip region slightly set off, flattened with five annules (as seen by SEM). Oral disc not conspicuous, oral aperture small and rounded, surrounded by six small papillae. Amphidial aperture pore like,

Table 2. Morphometric data of Iranian population of *Subanguina picridis* (all measurements in μm).

Characters	Female	Male	J2
n	17	8	10
L	2400 $\pm$ 294 (1925-2885)	1775 $\pm$ 183 (1450-2040)	954 $\pm$ 108 (775-1135)
a	22.9 $\pm$ 2.8 (18.3-27.2)	32 $\pm$ 4 (27-37)	34.8 $\pm$ 3.9 (29.7-42)
b	9.7 $\pm$ 1.6 (7.5-13.6)	8 $\pm$ 1 (7-9)	5.9 $\pm$ 0.9 (4-7.4)
b'	9 $\pm$ 1.3 (7-12.4)	7 $\pm$ 1 (6-8)	5.4 $\pm$ 0.8 (3.9-6.8)
c	33 $\pm$ 4.2 (26-40.4)	23 $\pm$ 1 (22-26)	15.3 $\pm$ 2.3 (13.6-20)
c'	2.9 $\pm$ 0.3 (2.4-3.4)	3 $\pm$ 0 (3-4)	3.2 $\pm$ 0.1 (2.9-3.3)
V	87.5 $\pm$ 1.8 (82.5-89.5)	-	9.6 $\pm$ 0.7 (9-11)
Stylet	9.9 $\pm$ 0.4 (9.5-11)	10 $\pm$ 0 (9-10)	1.8 $\pm$ 0.3 (1.5-2)
Orifice	1.8 $\pm$ 0.2 (1.5-2)	2 $\pm$ 0 (2-2)	63 $\pm$ 3.1 (57-68)
Median bulb	78 $\pm$ 4.4 (70-86)	76 $\pm$ 7 (65-90)	38 $\pm$ 5.3 (24-41)
MB	30 $\pm$ 5.2 (25-36)	32 $\pm$ 3 (27-38)	169 $\pm$ 42.6 (140-280)
Oesophagus	243 $\pm$ 33.1 (175-290)	217 $\pm$ 23 (175-240)	183 $\pm$ 43 (151-294)
End of glands	265 $\pm$ 31.5 (200-310)	238 $\pm$ 25 (190-275)	14.2 $\pm$ 4.3 (7-20)
Overlapping	20 $\pm$ 5 (15-33)	21 $\pm$ 9 (10-35)	126 $\pm$ 7.7 (115-132)
Excretory pore	200 $\pm$ 41 (145-260)	150 $\pm$ 15.3 (125 - 175)	27.5 $\pm$ 2.3 (24-32)
Body width	105 $\pm$ 14.6 (85-135)	56 $\pm$ 6 (50-67)	-
Head-vulva	2103 $\pm$ 279 (1580-2575)	-	-
PUS	130 $\pm$ 27 (80-190)	-	-
PUS/BW	1.5 $\pm$ 0.5 (1-2.9)	-	-
Vulva-anus	225 $\pm$ 33 (175-285)	-	-
Tail	73 $\pm$ 6.1 (65-85)	76 $\pm$ 8 (65-85)	61 $\pm$ 6.6 (50-72)
Anal body width	24.8 $\pm$ 2.3 (21-30)	24 $\pm$ 2 (21-28)	18.9 $\pm$ 2 (16-22)
PUS/Vulva-anus	58 $\pm$ 10.8 (39-78)	-	-
Egg length	62.3 $\pm$ 6.7 (55-74)	-	-
Egg width	36.3 $\pm$ 2.7 (33-40)	-	-
Spicule	-	32 $\pm$ 2 (30-37)	-
Gubernaculum	-	10 $\pm$ 1 (10-11)	-

situated at the base of cephalic disc (Figure 4: B). Cephalic frame work weakly developed. Stylet short, with two approximately equal parts and well developed rounded knobs. Oesophagus well developed, procorpus enlarged, usually separated from median bulb by a constriction. Median bulb broadly oval with crescentic plates, anteriorly situated. Isthmus long and narrow. Glandular basal bulb massive, spatulate, sometimes oblique and dorsally overlapping the intestine, dorsal oesophageal gland opening just posterior to the stylet knobs, its nucleus distinct and posterior to the nuclei of the subventral glands which are smaller. Nerve ring at the middle of isthmus or more posterior. Hemizonid not sharply distinct, two to three annules anterior to the excretory pore. Cephalids and hemizonion not conspicuous. Excretory secretory pore situated at level of glandular part of oesophagus, excretory duct normal. Lateral fields with several longitudinal and oblique incisures (Figure 4: F). Ovary generally with one or two flexures, it may extend and terminate near the basal bulb. Oocytes arranged around a rachis, posterior growth zone with large Oocytes in a single file. Spermatheca elongate and filled with round sperms. Crustaformeria consisting of 24-48 cells (6 -12 cells in four rows). Up to five synchronous eggs may be present in the genital tract. Postvulval uterine sac long (58 % of vulva-anus distance in mean), a few rudimentary cells attached to its posterior end. Tail conical with pointed terminus. Deirids and phasmids were not seen, however a phasmid-like structure (Figure 4: E) was observed on the dorsal side (posterior to anus, 50 µm from tail tip).

Male

Body straight to slightly curved ventrally, smaller and narrower than female. Head structure and *en face* view (by SEM) similar to female. Excretory pore at the level of terminal bulb. Hemizonid not sharply distinct, 3-5 µm anterior to the excretory pore. Stylet with well developed knobs. Oesophagus well developed, with wide procorpus. Me-

dian bulb large and ovate with crecentic plates, just anterior to the centre. Isthmus long and narrow. Basal oesophageal gland large, spatulate, with conspicuous dorsal gland nucleus, overlapping the intestine for a short distance. Dorsal gland opens near the stylet knobs. Testis outstretched; it may extend and terminate near the basal bulb, occasionally with one flexure. Spicule massive and curved (Figure 3: J). Gubernaculum stout. Bursa extends almost to the tip of the tail. Cloaca protruding from body (Figure 4: G). Deirids and phasmids not seen.

J₂

Body straight, length less than 1 mm (0.95 mm in mean) and narrow (27 µm at mid body). Head shape and stylet (9.6 µm) similar to the adults. Oesophagus well developed. Oesophageal gland long, rounded or dorsally overlapping the anterior part of intestine. Tail conoid with bluntly pointed to round terminus.

Locality

The sample was collected from the leaf galls of *Serratula latifolia* (Asteraceae) in Mashhad vicinity, Khorasan Province, Iran.

DISCUSSION

Few changes have been made in the taxonomy of anguinid nematodes for a long period, since Brzeski (1981), Chizhov and Subbotin (1985), Siddiqi (1986) and Fortuner and Maggenti (1987) proposed their original revisions of the group. Krall (1991) accepted Siddiqi's proposal (1986) of dividing anguinids into subfamilies and that of Chizhov and Subbotin (1985) in a generic taxonomy of the most important subfamily, Anguininae. He mentioned that the latter system reflects some evolutionary trends in the group and would be regarded as the most promising for future discussion. Siddiqi (2000) has made several changes to the taxonomy of anguinidae. He considered the

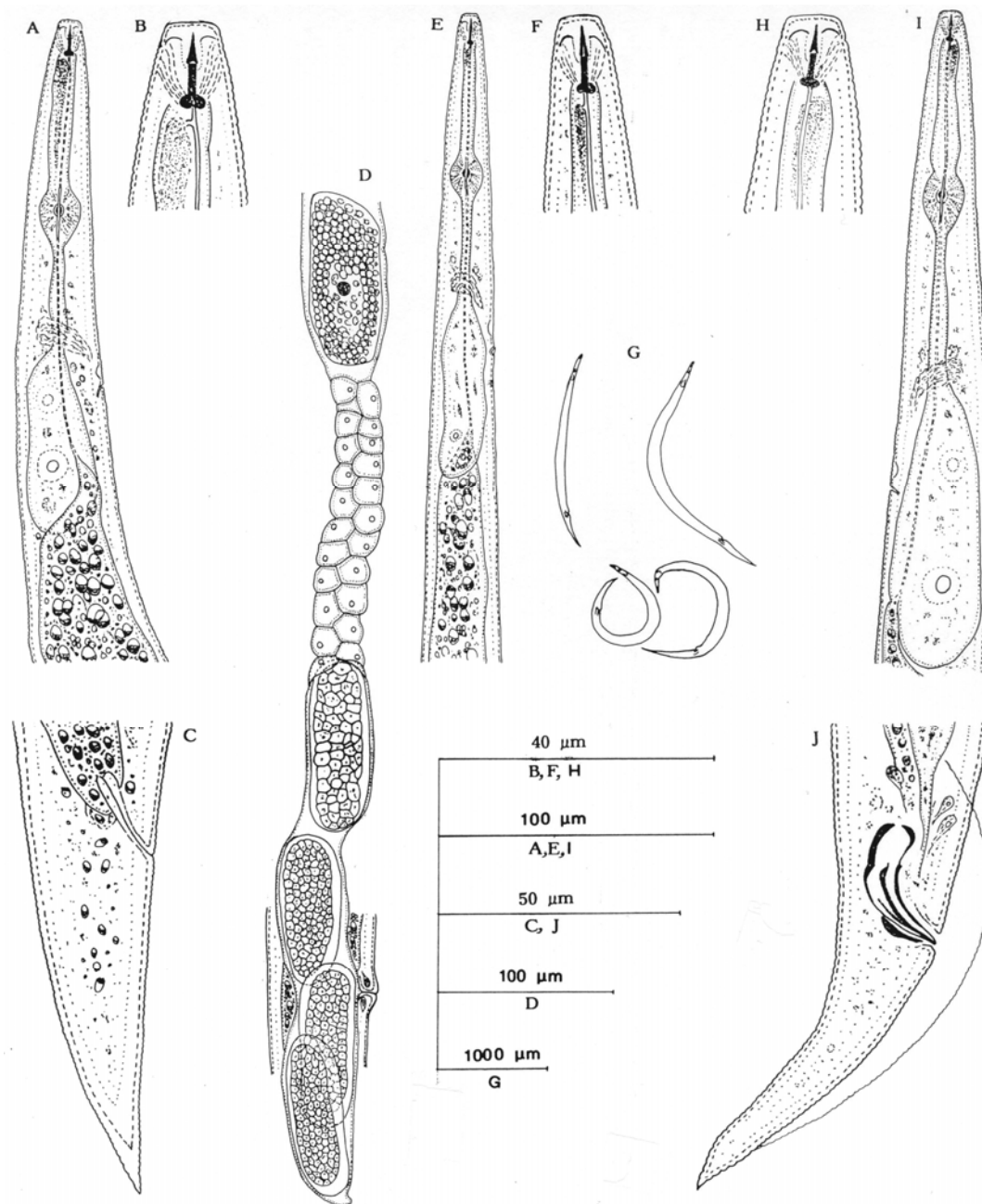


Figure 3. *Subanguina picridis*, Female (A-D, G); J₂ (E, F); Male (H - J). A, E, I: Oesophageal region; B, F, H: Anterior region; D: Reproductive apparatus; G: Entire body; C, J: Tail.

genera *Heteranguina* Chizhov, 1980, *Mesoanguina* Chizhov and Subbotin, 1985 and *Afrina* Brzeski, 1981 as synonyms for *Subanguina* Paramanov, 1967. He also transferred *S. amsinckiae* to the genus *An-*

guina and some species of *Anguina* to the *Subanguina*.

We believe that further studies are needed in the classification of anguinid nematodes, especially concerning the generic taxonomy.

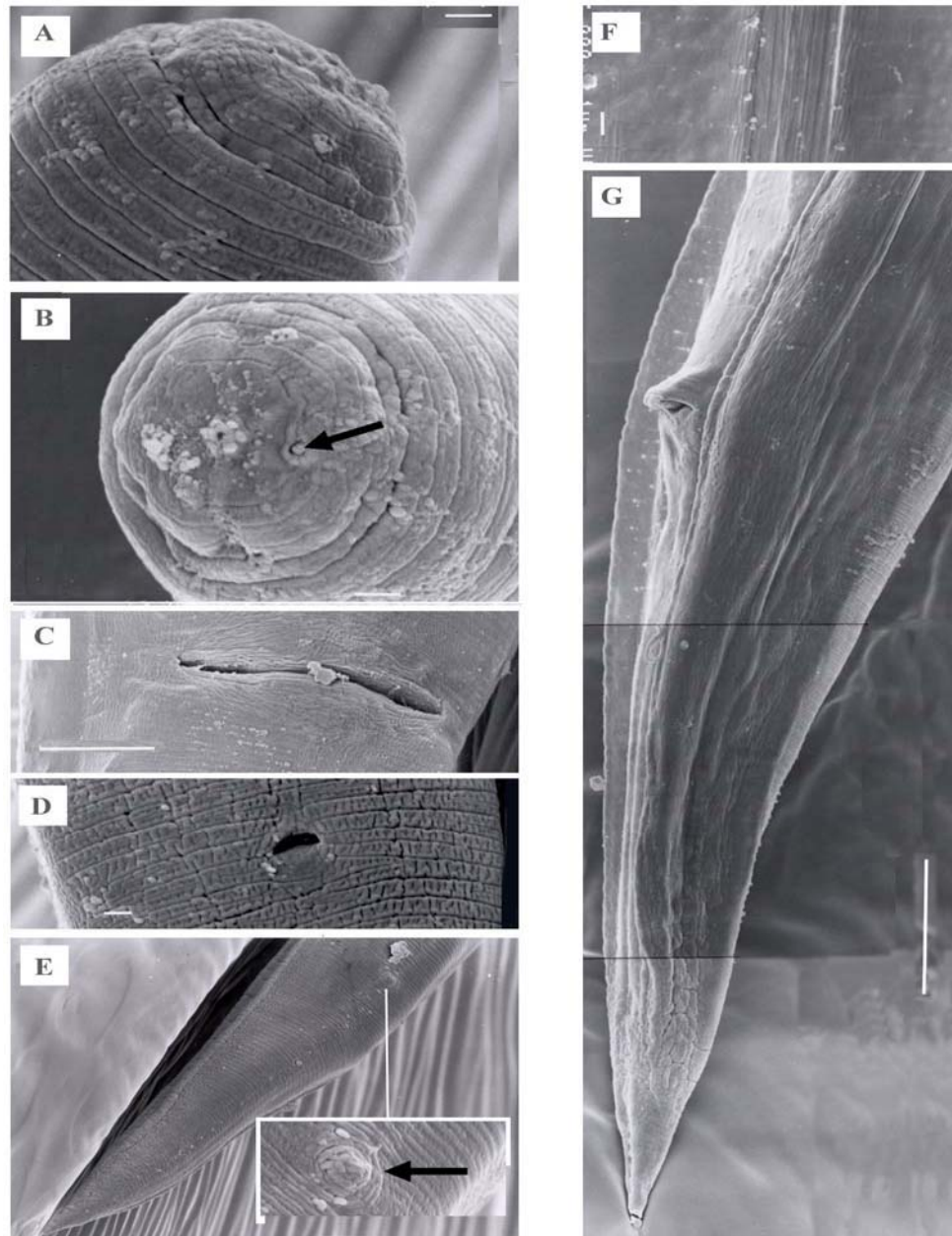


Figure 4. SEM photography of *Subanguina picridis*. Female (A-F). Male (G). A: Head lateral view; B: Head *en face* view (Arrow point at amphid); C: Vulva; D: Anus; E: Female tail and phasmid like structure (showed by arrow); F: Lateral field anterior to anus; G: Posterior part of male. Scale bar, 1 μ m for A, B, D, F and 10 μ m for C, E, G.

Our *Subanguina picridis* population resembles *S. plantaginis* (Hirschmann, 1977) Brzeski, 1981, but differs from it in terms of a longer body size (1.9-2.9 vs 1.2-2.1 mm), higher *b* value (7.5-12 vs 5.9-8.8), higher *c*

value (26-40 vs 15-26), longer PUS (80-190 vs 45-93 μ m), different host plant (*Asteraceae* vs *Plantaginaceae*) and with no vs conspicuous deirid reported for *S. plantaginis* (Hirschmann, 1977).



The population studied also resembles *S. mobilis* (Chit and Fisher, 1975) Brzeski, 1981, but it differs from the latter by a higher V value (87.5 vs 82.7), higher b value (9.7 vs 7.7), longer spicules (30-37 vs 23-25 μm), longer gubernaculum (10-11 vs 7-8 μm) and longer body size of J₂ {0.78-1.14 vs 0.26-0.27 mm (Krall, 1991)}, shorter PUS (58 vs 70% of the vulva-anus distance), smaller egg sizes (55-74 $\times$ 33-40 vs 82-114 $\times$ 50-57 μm) and different host plant (*Serratula* vs *Arctotheca*). In *S. mobilis*, the third-stage juveniles withstand desiccation and attack plants when the first true leaves appear in the second half of April (Chit and Fisher, 1975), but only J₂ was extracted from dried gals in our samples.

Our population is most similar to *S. picridis* but differs from the original description in having a shorter stylet (10 vs 12 μm), shorter spicule (30-37 vs 40-43 μm) and gubernaculum (10-11 vs 10-15 μm), higher c value (29-40 vs 14-25) and more or less different head *en face* view (see Watson, 1986 and Figure 4: A). Despite these remarkable differences, we consider, for the time being, our population to be *S. picridis* because of the high morphometric variation described in the literature. Watson (1986) described a short stylet (8-10 μm) in some preserved specimens and some variations in the gubernaculum (9.01-16.51 μm) and spicula (28.5-45 μm) length and a head *en face* view variation depending on the host plant. Our data agree with these variations and we can expect that the differences we observed here are probably influenced by the host. The host, *Serratula*, belongs to the tribe Cardueae (Family Asteraceae), which is usually the host for the *S. picridis*.

In this population, a phasmid-like structure (dorsal and only one) was observed on the tail; similar structures were observed for anguinids by Sturhan and Rahi (1996). These structures, however, were observed on a different position (dorsal to the lateral fields, in the postmedian portion of the body). We do not consider the structure on the dorsal position in our population as homologous with real phasids.

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توصیف دو گونه نماتد *Aprotides guidettii* Scognamiglio, 1974 و *Subanguina picridis*
جمع آوری شده از ایران (Kirjanova, 1944) Brzeski, 1981

۱. پورجم و و. برت

چکیده

به منظور شناسایی نماتدهای انگل گیاهی، تعدادی نمونه خاک از مناطق غرب کشور جمع آوری شد. در یکی از نمونه ها که از اطراف ریشه درخت هلو (*Prunus persica*) در ناحیه مغان (استان اردبیل) جمع آوری شده بود، یک گونه نماتد موسوم به *Aprotides guidettii* مورد شناسایی قرار گرفت. همچنین یک گونه نماتد انگل گیاهی موسوم به *Subanguina picridis* از گالهای ایجاد شده بر روی برگهای گیاه وحشی (*Serratula latifolia* (Asteraceae) که از اطراف مشهد (استان خراسان) جمع آوری شده بود، مورد شناسایی قرار گرفت. هر چند گونه *Subanguina picridis* جمع آوری شده از ایران تطابق کامل با شرح اصلی گونه نداشت، لیکن با توجه به اختلاف آن با سایر گونه های مشابه و تغییرات نسبی که در جمعیت های مختلف *Subanguina picridis* در منابع موجود گزارش شده است، به عنوان *Subanguina picridis* شناسایی گردید. شرح خصوصیات کامل هر دو نماتد با استفاده از میکروسکپ نوری و میکروسکپ الکترونی نگاره (SEM) در این مقاله آمده است.