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On the vulval morphology of some species of *Bursaphelenchus* (Nematoda: Parasitaphelenchinae)

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Summary – The vulval pattern of six species of the genus *Bursaphelenchus* (*B. abruptus*, *B. conicaudatus*, *B. fraudulentus*, *B. luxuriosae*, *B. mucronatus* and *B. xylophilus*) was studied using scanning electron microscopy. A terminology for the vulval region structures observed is proposed herein and illustrated by micrographs and line drawings. It was shown that, of the studied species, only *B. mucronatus* and *B. xylophilus* share an identical morphology of the vulval region, all other species differing significantly from each other and from both *B. mucronatus* and *B. xylophilus*. This study indicates the diagnostic potential for variation in vulval morphology within *Bursaphelenchus* and it is recommended that such features are recorded in all future descriptions.

Keywords – *Bursaphelenchus abruptus*, *Bursaphelenchus conicaudatus*, *Bursaphelenchus fraudulentus*, *Bursaphelenchus luxuriosae*, *Bursaphelenchus mucronatus*, *Bursaphelenchus xylophilus*, SEM.

Due to the economic importance of the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970), *Bursaphelenchus* Fuchs, 1937 is one of the most intensively studied genera within the superfamily Aphelenchoidea and currently contains about 100 valid species (Hunt, 2008). Although molecular techniques are now widely used to identify *Bursaphelenchus* species (Hoyer *et al.*, 1998; Ye *et al.*, 2007; Kanzaki *et al.*, 2008), morphology continues to be an important tool in nematode systematics (Hunt, 1993, 2008; Braasch, 2001; Ryss *et al.*, 2005; Giblin-Davis *et al.*, 2006). Until now, the vulval pattern variation of *Bursaphelenchus* spp. has not been studied in great detail (*e.g.*, Yik & Birchfield, 1981), although for some species the presence of vulval papillae have been reported and illustrated by SEM (Palmisano & Ambrogioni, 1994; Carletti *et al.*, 2005). It would appear that for species of the *xylophilus*-group, and also for other species in the genus, this region offers potentially important morphological features for species identification (*e.g.*, presence of vulval flap, vulval flap shape).

Materials and methods

In this study, six species were compared (Table 1), namely *B. abruptus* Giblin-Davis, Mundo-Ocampo, Baldwin, Norden & Batra, 1993, *B. conicaudatus* Kanzaki, Tsuda & Futai, 2000, *B. fraudulentus* Rühm, 1956, *B. luxuriosae* Kanzaki & Futai, 2003, *B. mucronatus* Mamiya & Enda, 1979 and *B. xylophilus*. Nematodes of each species were collected from established cultures of *Botrytis cinerea* Pars. using the Baermann funnel technique and then washed several times in distilled water.

For SEM observations live, mature, inseminated females were collected separately for each species and placed in a few drops of cold tap water in a glass vial. They were fixed in a mixture of 4% gluteraldehyde-2% formaldehyde for 48 h, post-fixed in 2% OsO_4 overnight, dehydrated in an ethanol series and then critical point drying and sputter coated with gold (Eisenback, 1985) and observed with a Jeol 35 SEM. For each species, six to ten individuals were observed and photographed at different positions for comparison. Line drawings of the vulval region are provided to illustrate each species. The drawings were prepared from the SEM images obtained for the species used in this study.

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Table 1. Bursaphelenchus *isolates used in this study.*

Nematode species	*n*	Country	Isolate	Plant or insect host
B. abruptus	6	USA	Ne12/98	*Anthophora abrupta* Say (Hymenoptera: Anthophoridae)
B. conicaudautus	6	Japan	BconJ	*Ficus carica* L. (Urticales: Moraceae)
B. fraudulentus	8	Germany	DE10w	*Pinus* sp. and *Picea* sp. (Pinales: Pinaceae)
B. luxuriosae	6	Japan	BluxJ	*Acalolepta luxuriosae* Bates (Coleoptera: Cerambycidae)
B. mucronatus	10	Germany	DE4w	*Pinus sylvestris* L. (Pinales: Pinaceae)
B. xylophilus	10	Portugal	Troia	*Pinus pinaster* Aiton (Pinales: Pinaceae)

For detailed plant, insect host and species distribution, see Ryss *et al.* (2005). n = number of mature inseminated females photographed and observed.

Results

Terminology of vulval region morphology

In order to describe the diversity of the vulval region it is necessary to establish new terms for the observed structures as the vulval morphology has not been studied in detail to date (Fig. 1). The proposed terms for the various vulval structures are listed in Figure 2. The only structure hitherto widely used in diagnostics of *Bursaphelenchus* species is the vulval flap, *i.e.*, a posteriorly directed cuticular extension of the anterior vulval lip. The flap shape varies within different species. The posterior margin of the flap is herein referred to as the vulval flap limb. The posterior vulval lip (= vulval labium) may bear a pair of vulval papillae. Between the anterior lip (vulval flap) and the posterior lip (vulval labium) of some species there is a zone which we call the vulval prelabium as it is located anterior to the labium yet posterior to the slit which leads into the vaginal vestibulum. The latter continues (underneath the vulval flap) into the vagina, its entrance being named the gonotreme. The vulval area is bordered laterally by the vulval lateral alae. The vulval lateral alae may be straight or strongly curved.

Vulval morphology in the studied species

The details of the vulval morphology of all six *Bursaphelenchus* species are given below and are illustrated in Figures 1 and 2.

i) *B. abruptus* (Figs 1A; 2A). Vulval papillae absent. Vulval region asymmetrical with vulval flap limb inclined posteriad from right to left, *i.e.*, entrance directed to the right (Note: in the original description of *B. abruptus* (Giblin-Davis *et al.*, 1993) the vulval area appears symmetrical. However, in all six specimens observed in this study, the vulval area displays the same pattern as shown in Figure 1A). Vulval flap massive with somatic annulation running across it anteriorly. Vulval lateral alae absent. Prelabium indistinct, not separated from labium.

ii) *B. luxuriosae* (Figs 1B; 2B). Vulval papillae absent. Vulval area symmetrical. Vulval flap moderately long, semicircular. Its limb arch-shaped, conically-rounded. Vulval flap thin with annulation parallel (areolated) to vulval flap limb; annulation not contiguous with somatic annulation. Vulval lateral alae well developed, straight to slightly curved ventrally. Prelabium distinct, not separated from labium by incisure.

iii) *B. fraudulentus* (Figs 1C; 2C). Vulval papillae present, prominent. Vulval area symmetrical. Vulval flap moderately long, limb arch-shaped to rectangular. Vulval flap thin, annulation parallel to vulval flap limb, not contiguous with somatic annulation. Vulval lateral alae indistinct, fused with vulval flap. Prelabium not separated from labium by incisure, prelabium at least twice length of labium.

iv) *B. conicaudatus* (Figs 1D; 2D). Vulval papillae present, low and flattened. Vulval area symmetrical. Vulval flap moderately long, semicircular. Its limb arch-shaped, conically rounded. Vulval flap thin, with annulation parallel to vulval flap limb, not contiguous with somatic annulation. Vulval lateral alae well developed, straight to slightly curved ventrally. Prelabium separated from labium by incisure; prelabium at least twice labium length.

v) *B. mucronatus* (Figs 1E; 2E) and *vi*) *B. xylophilus* (Figs 1F; 2F). Vulval papillae present, prominent. Vulval area symmetrical. Vulval flap extremely long. Its limb arch-shaped to rectangular; lateral edges of flap not reaching labium and papillae. Vulval flap thin with annulation parallel (areolated) to vulval flap limb, not contiguous with somatic annulation. Vulval lateral alae well developed, curved. Labium enclosed by incisure. Prelabium

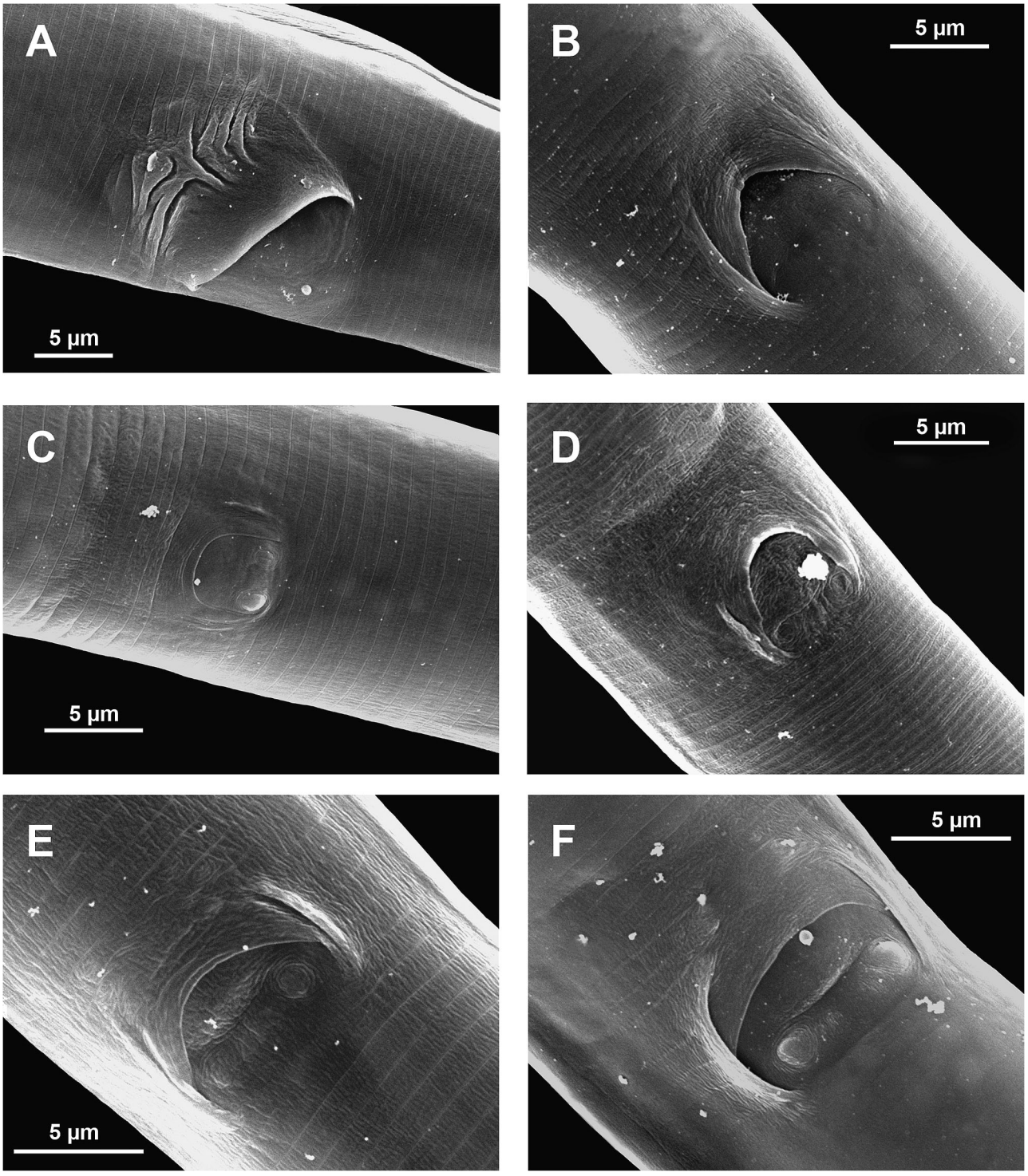

Fig. 1. *Scanning electronic microscope observations of the female vulva region of six different* Bursaphelenchus *species. A:* Bursaphelenchus abruptus*; B:* B. luxuriosae*; C:* B. fraudulentus*; D:* B. conicaudatus*; E:* B. mucronatus*; F:* B. xylophilus.

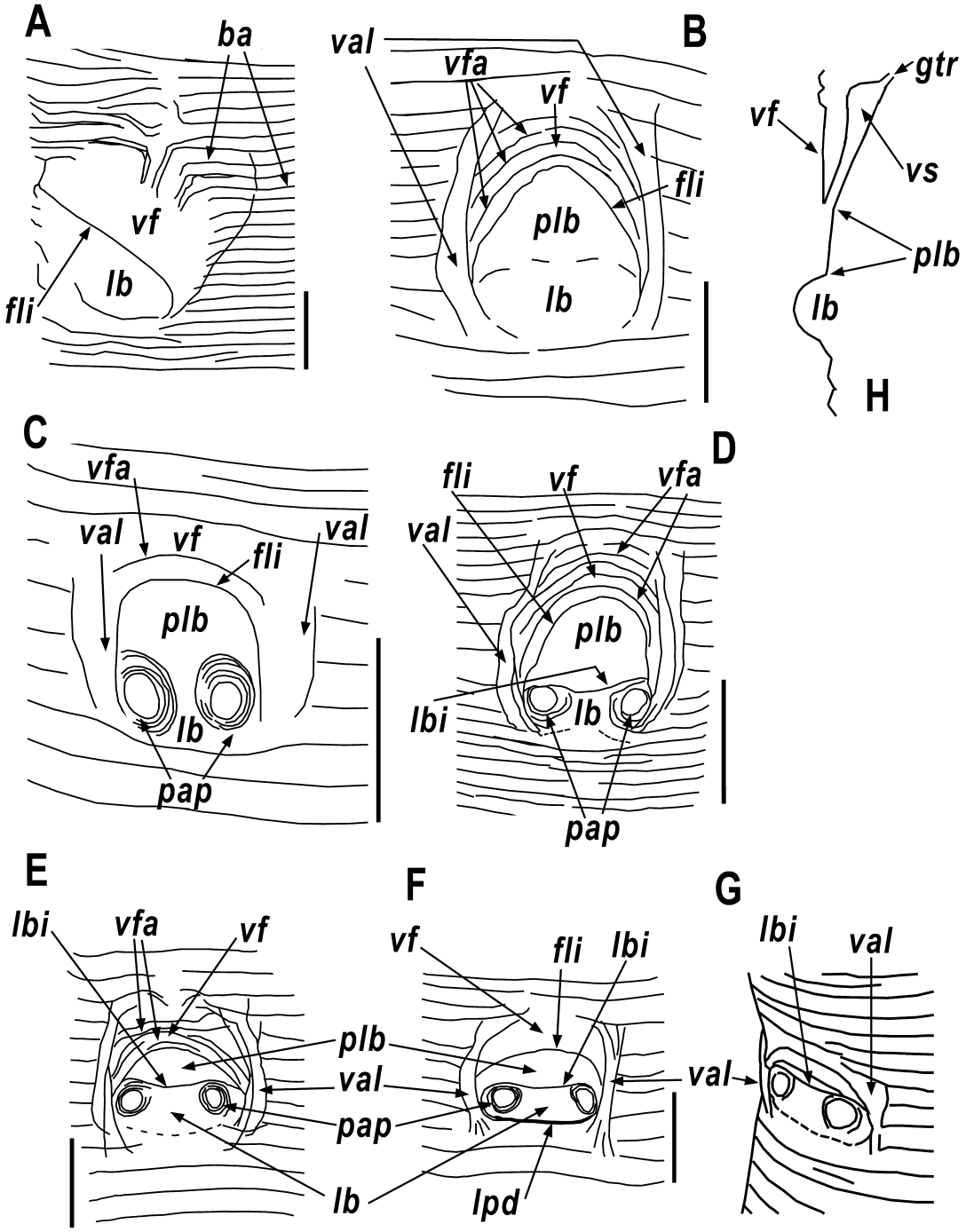

Fig. 2. *Female vulval region (ventral view). A:* Bursaphelenchus abruptus*; B:* B. luxuriosae*; C:* B. fraudulentus*; D:* B. conicaudatus*; E, G:* B. mucronatus*; F:* B. xylophilus*; G, H: Female vulva region (ventral and lateral view) showing constituent parts. Abbreviations: ba = body annules; fli = vulval flap limb (labrum limb); gtr = gonotreme; lb = vulval labium (posterior lip); lbi = vulval labium incisure; lpd = vulval labium posterior depression; pap = vulval papillae; plb = vulval prelabium; val = vulval lateral alae; vf = vulval flap* (i.e.*, vulval labrum or anterior lip of vulva); vfa = vulval flap annuli; vs = vaginal vestibulum. (Scale bars = 5 μm.)*

separated from labium by incisure. Prelabium length not greater than labium length.

Discussion

Most of the species studied differ from each other in vulval morphology. Females of *B. abruptus* and *B. luxuriosae* lack vulval papillae on the labium and *B. abruptus* has no vulval lateral alae, the somatic annulation therefore continuing across the anterior region of the vulval flap. Conversely, lateral alae are present in *B. luxuriosae*. Such alae are present in the other studied species of the *xylophilus*-group (although indistinct in *B. fraudulentus*) and separate the somatic annulation from the vulval area. In *B. fraudulentus* and *B. conicaudatus* the prelabium is very large, its length being at least twice that of the labium, whereas in *B. xylophilus* and *B. mucronatus* the prelabium is less well developed and is shorter than the labium length. The vulval area of *B. fraudulentus* is very small, the lateral alae being fused with the vulval flap. Conversely, in *B. conicaudatus*, the vulval region is distinctly larger with the lateral alae separated from the vulval flap. The prelabium in *B. fraudulentus* is not separated from the labium by an incisure, whereas such an incisure is present between the labium and prelabium in *B. conicaudatus*. Distinct differences in vulval morphology between *B. xylophilus* and *B. mucronatus* females were not found.

This study, although confined to only six species, indicates the diagnostic potential for variation in vulval area morphology and it is therefore recommended that future descriptions illustrate this area using the SEM.

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