Comparative morphology of *Globodera, Cactodera,* and *Punctodera* spp. (Heteroderidae) with scanning electron microscopy[1]

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SUMMARY

Second-stage juveniles (J2), males, and females of *Globodera rostochiensis, G. solanacearum, G. virginiae, G. tabacum, G. pallida, Cactodera cacti, C. eremica, C. weissi, C. betulae, Punctodera punctata* and *P. chalcoensis* were examined with the scanning electron microscope (SEM) to discover and clarify characters which may be useful for phylogenetic analysis of Heteroderidae. Certain features of lip patterns are useful for defining all three genera. However, intraspecific variation may have resulted in previous misrepresentation of lip characters for some taxa. Monophyly of *Globodera, Cactodera,* and *Punctodera* is supported by the relatively small labial disc and trend toward fusion of all lips in males. Monophyly of *Globodera* may be supported by protuberances on the female head region, *Cactodera* by reduced lateral lips in J2, and *Punctodera* by fusion of submedial lips with the labial disc and adjacent head annules. The unique *en face* pattern of J2 of *C. betulae* may indicate a transformation series between *Cactodera* or *Punctodera,* and *Heterodera.* The position of phasmids in J2 and posterior termination of lateral lines in J2 and males indicate differences among heteroderid species, but may not be useful for defining genera or phylogenetic analysis of the family. Comparative morphological-developmental investigations of overall cuticular and perineal patterns of females and cysts include promising characters for interpreting phylogeny but will best be evaluated with insight into changes with age.

RÉSUMÉ

Morphologie comparée d'espèces des genres Globodera, Cactodera *et* Punctodera *au microscope électronique à balayage*

Des juvéniles de deuxième stade (J2), des mâles et des femelles de *Globodera rostochiensis, G. solanacearum, G. virginiae, G. tabacum, G. pallida, Cactodera cacti, C. eremica, C. weissi, C. betulae, Punctodera punctata* et *P. chalcoensis* ont été examinés au microscope électronique à balayage pour découvrir et préciser les caractères qui pourraient être utilisés pour une analyse phylogénique des Heteroderidae. Certains caractères de la « figure labiale » (en vue de face) sont utilisables pour la définition de chacun de ces trois genres. Toutefois, la variation intraspécifique a parfois antérieurement conduit à une représentation erronée des caractères labiaux chez certains taxa. La monophylie de *Globodera, Cactodera* et *Punctodera* est suggérée par un disque labial relativement petit et une tendance à la fusion des lèvres, chez le mâle. La monophylie de *Globodera* peut s'appuyer sur les protubérances de la région céphalique des femelles; celle de *Cactodera* sur la réduction des lèvres latérales chez les J2; et celle de *Punctodera* sur la fusion des lèvres submédianes avec le disque labial et les anneaux céphaliques adjacents. La « figure labiale », unique, des J2 de *C. betulae* pourrait indiquer une variation en chaîne entre *Cactodera,* ou *Punctodera,* et *Heterodera.* La position des phasmides chez les J2, et la terminaison postérieure des lignes latérales chez les J2 et les mâles sont révélatrices de différences parmi les espèces d'Heteroderidae, mais ne peuvent être utilisées, ni pour la définition des genres, ni pour une analyse phylogénique de la famille. Des observations comparatives sur la morphologie et le développement de la cuticule et des caractères périnéaux des femelles et des kystes a révélé des caractères prometteurs pour l'interprétation de la phylogénie, mais qui seront mieux évalués à la lumière des changements liés à l'âge.

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Globodera Skarbilovich, 1959 (Behrens, 1975) was introduced to accommodate cyst-forming heteroderids with females that are spherical, lack a terminal protrusion, and lack an egg matrix. Subsequently, *Punctodera* Mulvey and Stone, 1976 was erected to accommodate species with anal and vulval fenestrae in the cysts, followed by *Cactodera* Krall & Krall, 1978 with only vulval circumfenestrae.

Several phylogenetic relationships have been proposed among these genera but only a little progress has been made since Luc, Taylor and Cadet (1978) suggested that Heteroderidae classification is artificial and inconsistent, partially because it is based on insufficient data including morphology. There are limited comparative SEM morphological observations of *Globodera*, *Punctodera* and *Cactodera* (Green, 1971; 1975; Mulvey, 1973*a,b;* Stone, 1975) and some studies restricted to description of a single species (Wouts, 1984; Baldwin & Bell, 1985); however, use of these findings for interpreting phylogeny is limited by incompleteness. For example, details may be obscured by poor quality of preparation, and often too few specimens have been examined to discuss variability. In this study we report detailed SEM morphological comparison of characters including *en face* patterns, lateral fields, tails, and sensory openings of second-stage juveniles (J2) and males as well as *en face* patterns of females. These characters, which may be useful for phylogenetic analysis (Stone, 1975, 1979; Ferris, 1979, 1985; Wouts, 1985), are described for eleven species representing *Globodera*, *Cactodera*, and *Punctodera*. Cuticular and perineal patterns of females and cysts are excluded from the present report because preliminary observations indicated detailed comparisons among taxa were confounded by changes with development and age of individuals (Othman, 1985). Terminology for *en face* patterns and character states is as previously described (Baldwin, 1986). When these new observations are considered with previous studies of cyst and noncyst-forming Heteroderidae (Green, 1971; Mulvey, 1973*a,b;* Stone, 1975; Momota & Oshima, 1976; Baldwin, Mundo-Ocampo & Othman, 1983; Baldwin & Bell, 1985; Othman & Baldwin, 1985, 1986; Othman, Baldwin & Bell, 1986) the combined data can be used for further testing and refining hypotheses of phylogeny of the family.

Materials and methods

Second-stage juveniles, males, and females of *Globodera rostochiensis, G. solanacearum*, G. virginiae*, G. tabacum*, G. pallida, Cactodera cacti, C. eremica, C. weissi, C. betulae, Punctodera punctata,* and *P. chalcoensis* were collected from the sources indicated (Tab. 1). The stages were processed for SEM by glycerin infiltration (Sher & Bell, 1975) and critical point drying (Othman & Baldwin, 1985); figures 1-5 are all of specimens prepared by glycerin.

* Although Stone (1983) proposed these as subspecies of *G. rostochiensis*, the recommendation has not yet been widely adopted.

Results

Head morphology

En face patterns of J2, males, and females of *Globodera, Cactodera,* and *Punctodera* are variable among species and stages; however, in all species, the pattern is based on a labial disc surrounded by six lips (two lateral and four submedial). *En face* patterns of J2 of all three genera have on ovoid labial disc and lateral lips may fuse with the labial disc. *Globodera rostochiensis, G. solanacearum, G. virginiae, G. tabacum,* and *G. pallida* each include variable J2s with *en face* patterns having both separated and fused lips (Fig. 1 A-I). However, all six lips are typically separate in J2 of *G. rostochiensis* (Fig. 1 A), whereas most commonly submedial lips are fused in J2 of *G. solanacearum, G. virginiae* and *G. tabacum* and *G. pallida* (Fig. 1 D, F, H, I).

In J2 of *C. cacti, C. weissi,* and *C. eremica* all lips are generally separate and lateral lips are reduced in size (Fig. 1 K-M). Rare individual J2 of *C. cacti* exist however, in which submedial lips are fused on one side of the labial disc (Fig. 1 K). Lip patterns of J2 of *C. betulae* are similar to those of *P. punctata* and *P. chalcoensis.* These consist of adjacent submedial lips that fuse completely with each other and partially with the labial disc; lateral lips are distinct and may also partially fuse with the labial disc (Fig. 1 N-P). Typically, fusion also occurs between submedial lips and the adjacent head annule in *C. betulae,* but not in *Punctodera* spp.

En face patterns of males of *Globodera, Cactodera,* and *Punctodera* also vary among species. Unlike J2, however, the labial disc is circular (*vs* elongate) and typically separated from lips (Fig. 2). In males of *G. rostochiensis* adjacent submedial lips partially or completely fuse with each other and with lateral lips tending to form a continuous annule (Fig. 2 A). Individuals exist, however, in which adjacent submedial lips are separate (Fig. 2 B). The lateral lips, which encircle clearly marked amphid openings, are also clearly demarcated (Fig. 2 A). In males of *G. solanacearum, G. virginiae,* and *G. tabacum* adjacent submedial lips are either separate or fused on one or both sides of the labial disc. The lateral lips are typically separate from submedial lips (Fig. 2 C-H), and there may be some tendency for lateral lips to fuse with the labial disc in *G. virginiae* (Fig. 2 E, F). Males of *G. pallida* typically have fused adjacent submedial lips with separate lateral lips (Fig. 2 I). *Cactodera cacti* and *C. weissi* have adjacent submedial lips separate from each other, but in *C. weissi* they are partially fused with lateral lips (Fig. 2 J-K). *Cactodera betulae* is unique among the three genera examined with partial to com-

Table 1

Species and number of *Globodera* spp., *Cactodera* spp., and *Punctodera* spp. examined and their source.

Nematode	*Host*	*Source*	*Number Examined*
Globodera rostochiensis (Wollenweber, 1923) Behrens, 1975	Potato *Solanum tuberosum* L.	Cornell University Ithaca, NY	50 juveniles 19 males 18 females
G. solanacearum (Miller & Gray, 1972) Behrens, 1975	Horse nettle *Solanum carolinense* L.	North Carolina State University, Raleigh, NC; Virginia Polytechnical Institute, Blacksburg, VA	100 juveniles 10 males 100 females
G. virginiae (Miller & Gray, 1968) Behrens, 1975	Horse nettle *Solanum carolinense* L.	North Carolina State University, Raleigh, NC; Virginia Polytechnical Institute, Blacksburg, VA	100 juveniles 10 males 80 females
G. tabacum (Lownsbery & Lownsbery, 1954) Behrens, 1975	Tobacco *Nicotiana tabacum* L.	Virginia Polytechnical Institute, Blacksburg, VA	50 males 20 juveniles 150 females
G. pallida (Stone, 1973) Behrens, 1975	Potato *Solanum dulcamara* L. *S. tuberosum* L.	Virginia Polytechnical Institute, Blacksburg, VA; Rothamsted Experimental Station, England; International Potato Center, Lima, Peru	40 males 45 juveniles 25 females
Cactodera cacti (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978	Cactus *Cereus speciosissimus* DC.	Division of Plant Industry, Gainesville, FL (UC Riverside greenhouse culture)	350 juveniles 1 male 20 females
C. eremica Baldwin & Bell, 1985	Shadscale *Atriplex confertifolia* (Torr. & Frem.) Wats	Cedar Valley, Utah (UC Riverside greenhouse culture)	60 juveniles 10 males 2 females
C. weissi (Steiner, 1949) Krall & Krall, 1978	*Polygonum hydropiperoides* Michx.	BARC-W Beltsville, MD	60 juveniles 8 males 20 females
C. betulae (Hirschmann & Riggs, 1969) Krall & Krall, 1978	*Betulae pubescens* Ehrh.	University of Arkansas Fayetteville, AR (UC Riverside greenhouse culture)	320 juveniles 10 males 40 females
Punctodera punctata (Thorne, 1928) Mulvey & Stone, 1976	Annual bluegrass *Poa annua* L.	New Jersey	30 juveniles 8 males 15 females
P. chalcoensis Stone, Sosa-Moss & Mulvey, 1976	Maize *Zea mays* L.	Michoacan, Mexico	30 juveniles 9 males 60 females

plete fusion of nearly all lip parts with each other and adjacent head annules (Fig. 2 L-M). In *en face* patterns of males of *P. punctata* and *P. chalcoensis* adjacent submedial lips are completely fused with each other as well as with lateral lips (in most individuals) to form a continuous annule surrounding the labial disc (Fig. 2 N-P).

En face patterns of females of *Globodera, Cactodera,* and *Punctodera* are quite similar in all species. The labial disc is oblong to rectangular. It is elevated above and surrounded by a circular plate formed by fusion of lips (Fig. 3 A, B). Lateral lips however, are clearly demarcated in *G. solanacearum, G. virginiae, G. tabacum,* and *P. chalcoensis* (Fig. 3 B). In *G. rostochiensis, C. eremica,* and *P. punctata* the lateral lips are not clearly demarcated (Fig. 3 A). In females of all *Globodera* spp. transverse rows of protuberances occur posterior to the lip region (Fig. 3 C, D); these protuberances were not observed in other genera.

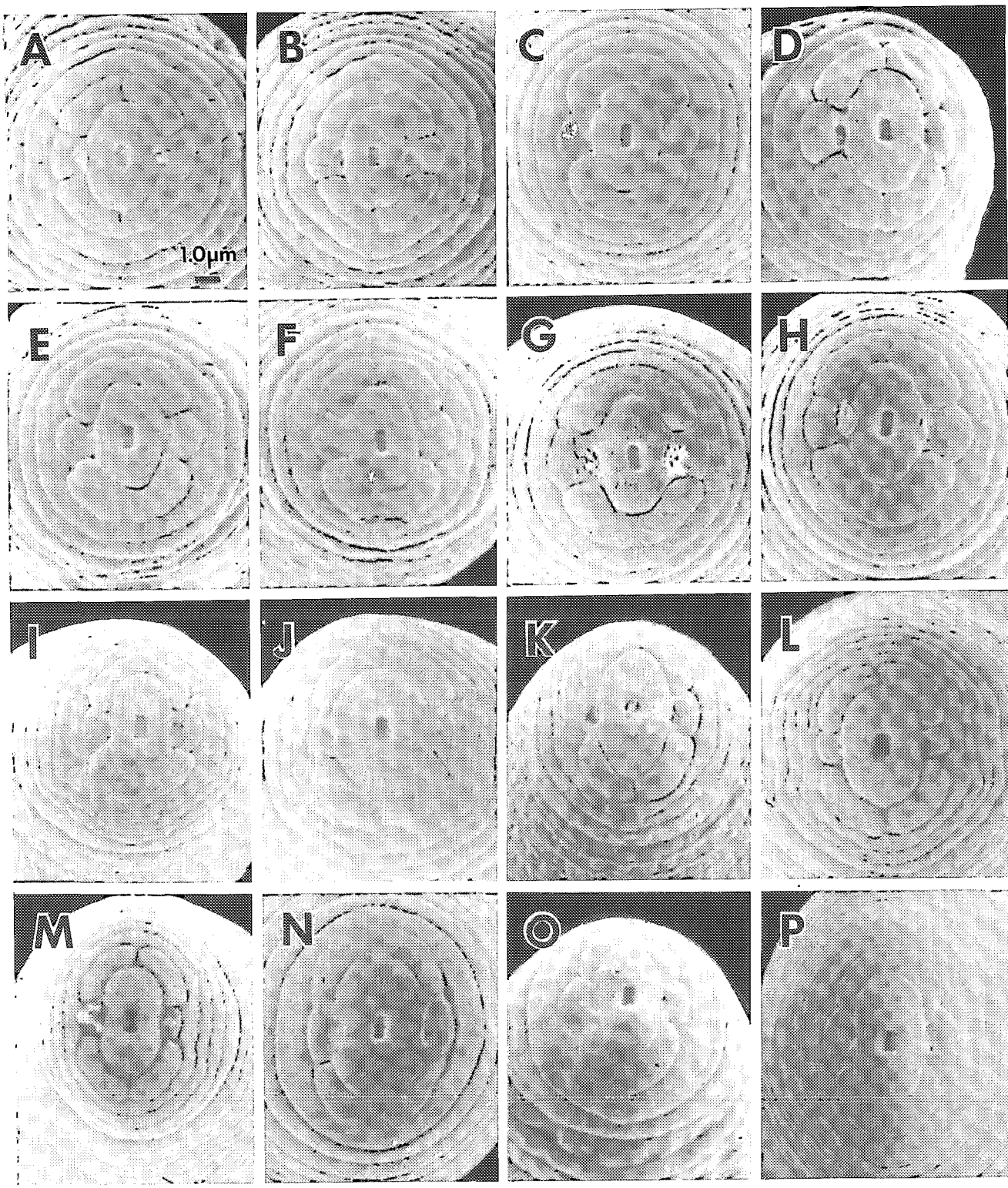

Fig. 1. *En face* pattern of second-stage juveniles of *Globodera* spp., *Cactodera* spp., and *Punctodera* spp. A : *G. rostochiensis,* common; B : *G. rostochiensis,* varient; C : *G. solanacearum,* common; D : *G. solanacearum,* varient; E : *G. virginiae* common; F : *G. virginiae,* varient; G : *G. tabacum,* common; H : *G. tabacum,* varient; I : *G. pallida;* J : *C. cacti,* common; K : *C. cacti,* varient; L : *C. eremica;* M : *C. weissi;* N : *C. betulae;* O : *P. punctata;* P : *P. chalcoensis.*

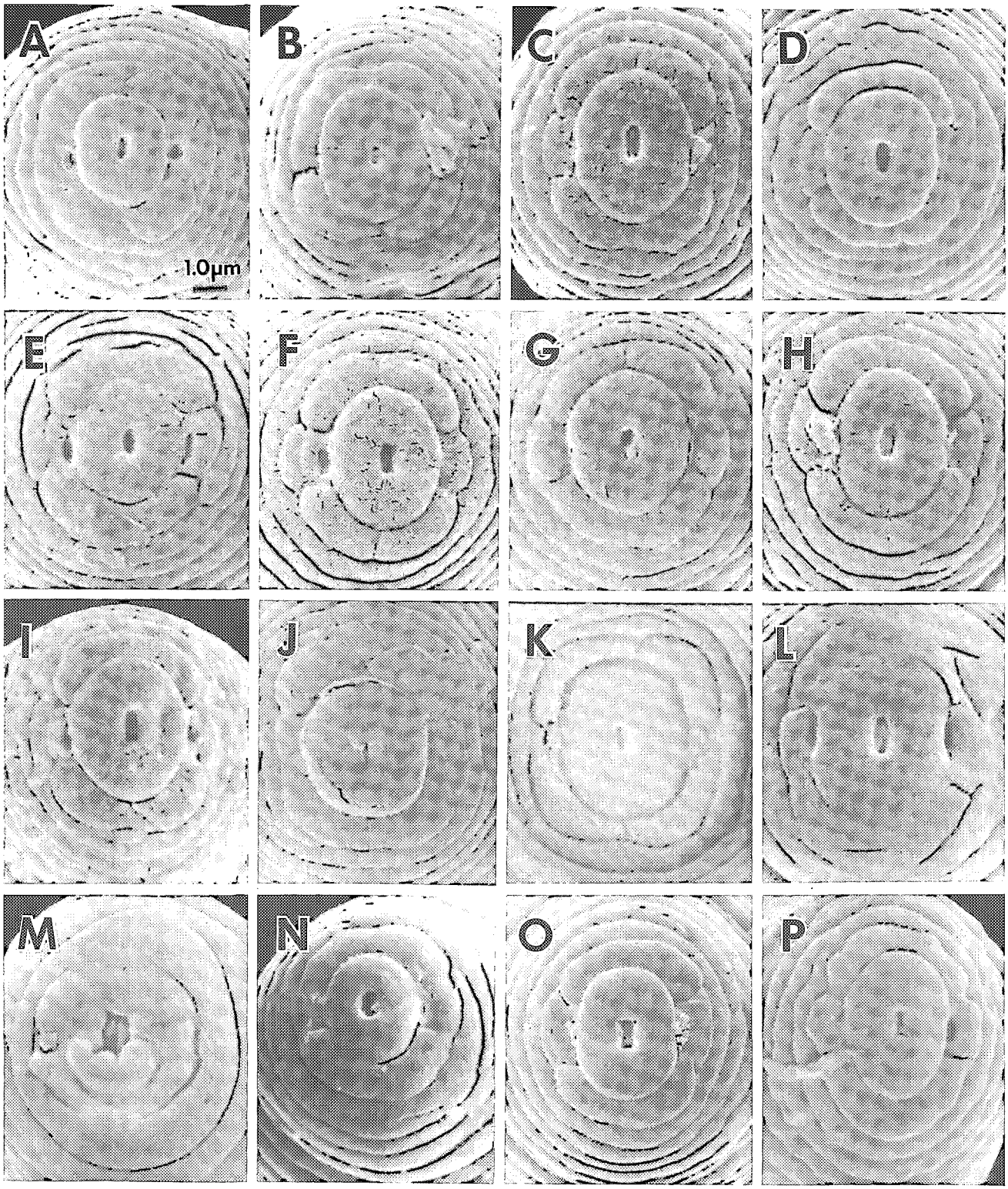

Fig. 2. *En face* pattern of males of *Globodera* spp., and *Punctodera* spp. A : *G. rostochiensis,* common; B : *G. rostochiensis,* varient; C : *G. solanacearum,* common; D : *G. solanacearum,* varient; E : *G. virginiae,* common; F : *G. virginiae,* varient; G : *G. tabacum,* common; H : *G. tabacum,* varient; I : *G. pallida;* J : *C. cacti;* K : *C. weissi;* L : *C. betulae,* common; M : *C. betulae,* varient; N : *P. punctata; O : P. chalcoensis,* common; P : *P. chalcoensis,* varient.

LATERAL FIELD AND PHASMIDS

The lateral field of J2 of *Globodera*, *Cactodera*, and *Punctodera* species examined originates as one, two, or three areolated bands, about nine to thirteen annules posterior to the labial disc. At midbody it consists of four incisures delineating three longitudinal bands. The middle band is smooth whereas the outer two bands are areolated, although in some cases areolations are faint. The lateral field changes little throughout the length of the nematode except in the area near the phasmid openings. In this region the middle band ends about twelve to twenty annules anterior to the tail end (Fig. 3 E-O); in *C. cacti*, *C. eremica* and *C. weissi* the middle band tends to persist very near to the terminus of the lateral field (Fig. 3 J-L). Phasmid openings typically are about five to seven annules anterior to the end of the lateral field (Fig. 3 E-O). Where the phasmid opening occurs with three bands in the lateral field, it typically is asymmetric between bands (Fig. 3 J-K), whereas with two bands it is more likely to occur centrally in the lateral field on the incisure (Fig. 3 E, F, M). The tail tip is narrower and more attenuated in J2 of *Punctodera* than in *Cactodera* (Fig. 3 J-N).

The lateral field in males of *Globodera*, *Cactodera*, and *Punctodera* is similar among species and typically consists of three longitudinal bands. The middle band is smooth, whereas the outer two bands are faintly areolated; there is little change throughout the length of the nematode. The lateral field ends near the tail terminus and in this region variation in details occur, even within populations; however, some tendencies seem to be taxon specific (Figs 4, 5).

In *Globodera* the incisures of the lateral field usually converge immediately anterior to the level of the cloacal opening and the pattern is rounded in lateral view (Fig. 4 B-H, J). The end view pattern between the two lateral fields is typically V-shaped lines in *G. rostochiensis* and *G. solanacearum*, whereas irregular tubercles occur in *G. virginiae*, *G. tabacum*, and *G. pallida* (Fig. 4 A-G, I). In *Cactodera* and *Punctodera* the terminus of the lateral field often fades or continues around the terminus by merging with lines of the end view pattern (Fig. 5 B, D, F, I, K, M). *Cactodera cacti*, *C. weissi*, and *C. betulae* tend to have lines in end view, whereas *C. eremica* and *P. chalcoensis* are relatively smooth (Fig. 5 A-G, J, L). The terminus of the lateral field of *P. punctata* is unusual by its modification to many fine tubercles which continue around the end of the tail, connecting the two fields (Fig. 5 J, K).

The tail end view of all species is triangular in shape extending to include the smooth spicule sheath; however, the sheath is greatly reduced or absent in *G. rostochiensis*, *G. pallida*, *C. eremica*, *C. betulae* and *P. punctata* (Figs 4 A, I; 5 C, G, J). Spicule tips are bifid in all *Cactodera* spp. but not in *Globodera* or *Punctodera* (Fig. 5 H, I). Phasmid openings were not confirmed in males of any of the six species (Figs 4, 5).

Discussion

SEM examination of J2 and adults of eleven species of *Globodera*, *Cactodera*, and *Punctodera* revealed new characters that can be used in conjunction with other characters for testing phylogenetic relationships among Heteroderidae genera. For example, the *en face* region of males of *Globodera*, *Cactodera* (including lip patterns of *C. eremica* as described in Baldwin and Bell, 1985), and *Punctodera* are variable within and among species and genera and yet they share some tendencies which make their patterns distinctive among Heteroderidae. These tendencies include a relatively small labial disc (e. g. *vs* males of *Sarisodera hydrophila* and *Rhizonema sequoiae*, discussed in Othman and Baldwin, 1986), and a trend toward fusion of all lips to form a relatively thick continuous lip annule, as expressed in *G. rostochiensis* and most completely in *C. betulae*. These derived patterns are unlike the highly specialized patterns of the other cyst-forming genus, *Heterodera*, and noncyst forming heteroderids (Othman, 1985; Othman & Baldwin, 1985, 1986; Othman, Baldwin, & Bell, 1986) with the possible exception of *Verutus volvingentis*, a genus of uncertain taxonomic position (Othman & Baldwin, 1985). Characters of the male lip patterns alone, may only offer weak support for monophyly of *Globodera*, *Cactodera*, and *Punctodera;* however, in combination with other derived characters, such as circumfenestrae the hypothesis is strengthened.

Lip patterns of J2 of *Globodera* and *Cactodera* are interpreted as relatively primitive, sometimes having six separate lips, or more often with fusion of adjacent submedial lips and fusion of lateral lips with the labial disc. The similarity of these features with J2 of *Atalodera* and *Thecavermiculatus* is striking (Othman, Baldwin & Bell, 1986), and must be considered in light of other possible shared derived features. These include the D layer of the female body wall in *Globodera-Cactodera-Punctodera*, as well as *Atalodera-Thecavermiculatus* (Cliff & Baldwin, 1985), and certain aspects of the syncytial host response. Although Mundo and Baldwin (1984) report evidence that the syncytial host response evolved independently in the two groups, this hypothesis must be further tested by a phylogenetic analysis of the family which considers additional characters including lip patterns.

Cliff and Baldwin (1985) noted that the taxonomic position of *C. betulae* is uncertain with several characters, including absence of a D layer, inconsistent with other species of the genus. Similarly, the lip pattern of J2 of *C. betulae* is distinct from other *Cactodera* spp. by the fusion of the labial disc with submedial lips; this character is shared with *Punctodera* spp. In addition, submedial lips are fused with adjacent head annules;

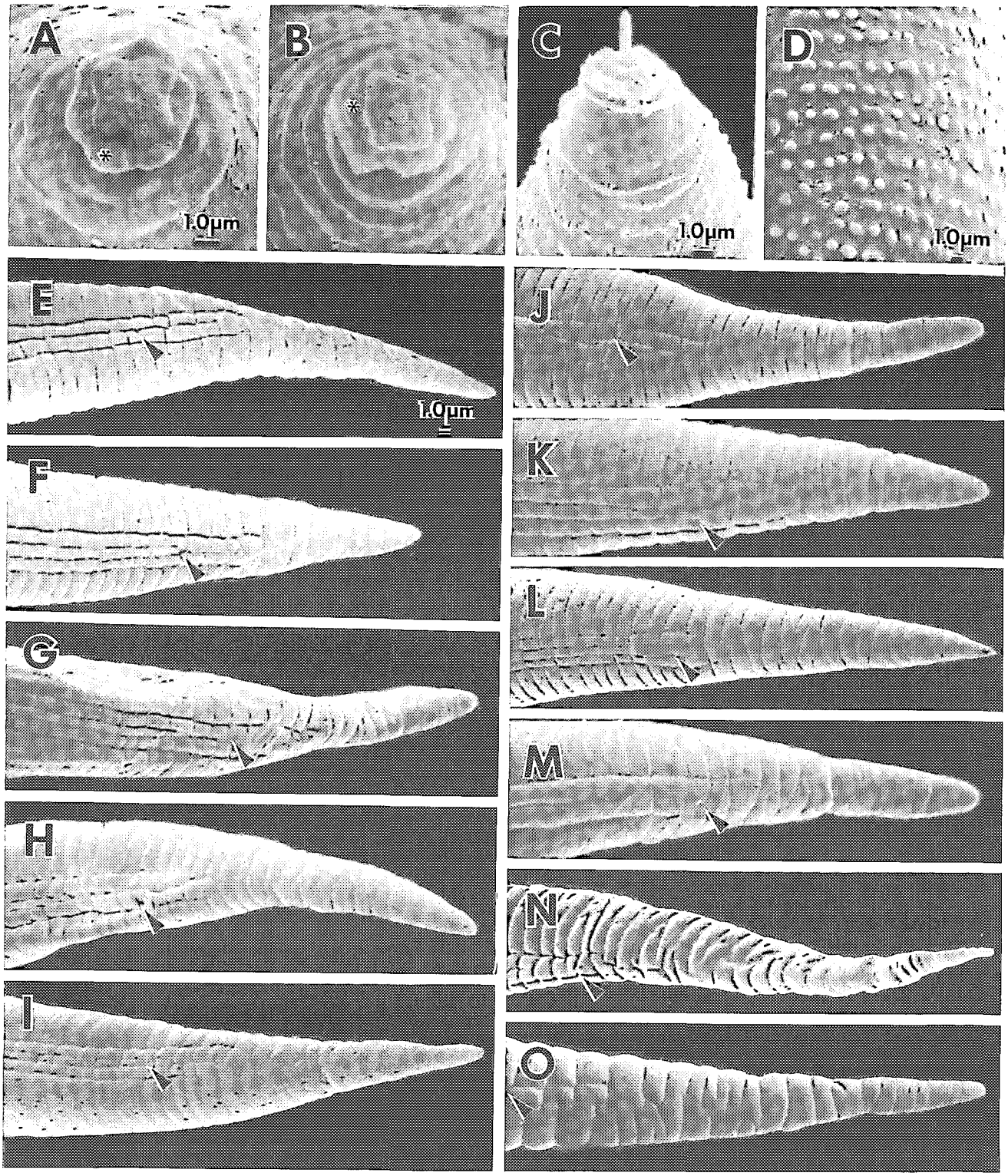

Fig. 3. Head region of females and tails of second-stage juveniles of *Globodera* spp., *Cactodera* spp., and *Punctodera* spp. A : *En face* pattern of *C. eremica* without distinct lateral lips; B : *En face* pattern of *G. solanacearum* with distinct lateral lip. Scale as in A; C : Dorsal view of *G. rostochiensis* with tubercles; D : *G. rostochiensis* with tubercles; E : Tail of *G. rostochiensis;* F : Tail of *G. solanacearum;* G : Tail of *G. virginiae;* H : Tail of *G. tabacum;* I : Tail of *G. pallida;* J : Tail of *C. cacti;* K : Tail of *C. eremica;* L : Tail of *C. weissi;* M : Tail of *C. betulae;* N : Tail of *P. punctata;* O : Tail of *P. chalcoensis* (* = lateral; arrowhead = phasmid opening).

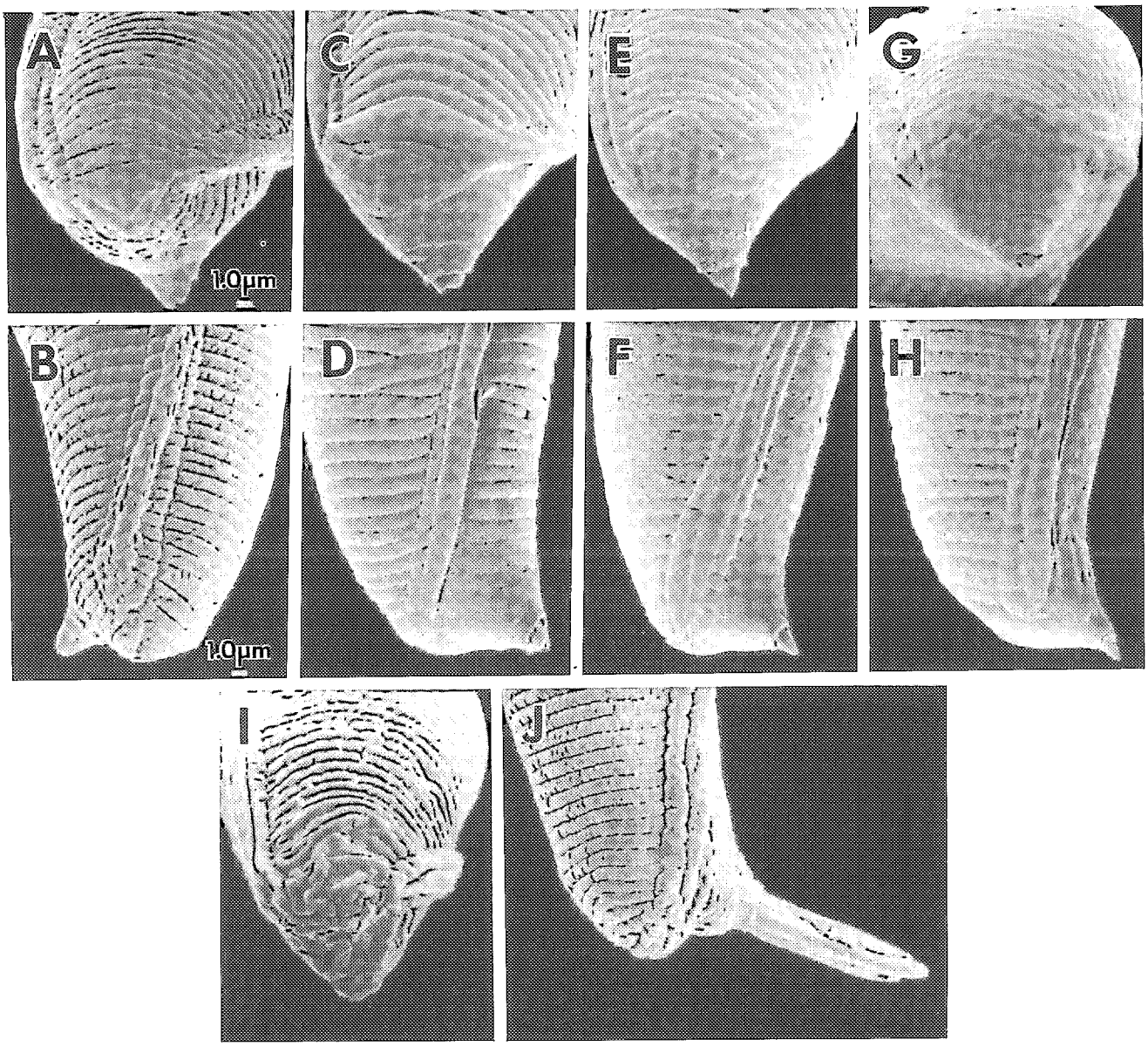

Fig. 4. Tail region of males of *Globodera* spp. A : *G. rostochiensis*, end view; B : *G. rostochiensis*, lateral view; C : *G. solanacearum*, end view; D : *G. solanacearum*, lateral view; E : *G. virginiae*, end view; F : *G. virginiae*, lateral view; G : *G. tabacum*, end view; H : *G. tabacum*, lateral view; I : *G. pallida*, end view; J : *G. pallida*, lateral view. Scale in end view (A-G, J) as shown for A; scale in lateral view (B-F, I, K, M) as shown for B.

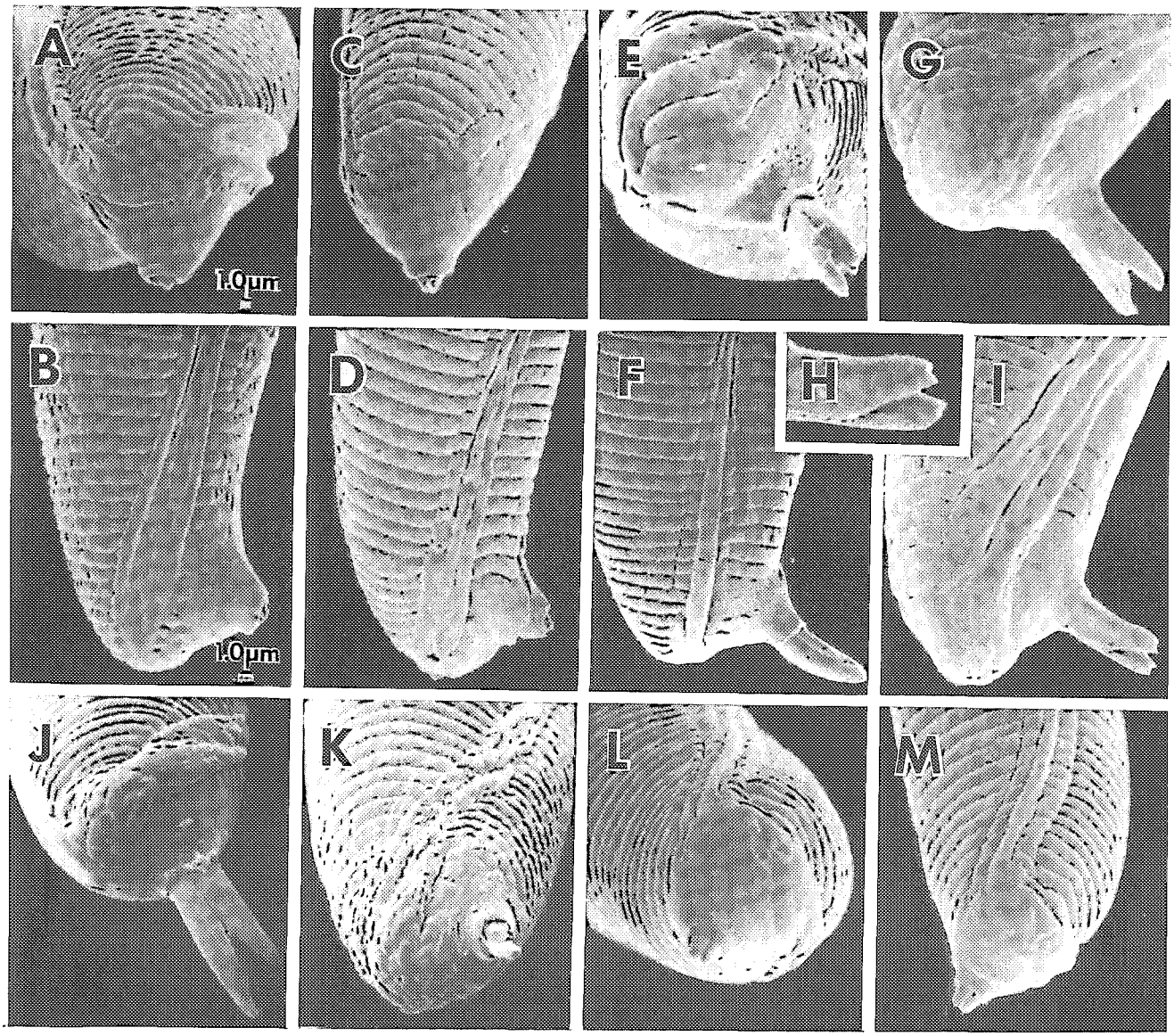

Fig. 5. Tail region of males of *Cactodera* and *Punctodera*. A : *C. cacti*, end view; B : *C. cacti*, lateral view; C : *C. eremica*, end view; D : *C. eremica*, lateral view; E : *C. weissi*, end view; F : *C. weissi*, lateral view; G : *C. betulae*, end view; H : *C. betulae*, enlargement of bifid spicule tip from I; I : *C. betulae*, lateral view; J : *P. punctata*, end view; K : *P. punctata*, lateral view; L : *P. chalcoensis*, end view; M : *P. chalcoensis*, lateral view. Scale in 1-G, J, L as shown for A; B-F, I, K, M as shown for B.

among cyst-forming genera this feature is only shared with *Heterodera* spp. (Othman, 1985). These findings may indicate the *en face* pattern of J2 of *C. betulae* is intermediate in a transformation series between *Cactodera* or *Punctodera*, and *Heterodera*. Bifid spicule tips are shared by *Cactodera* including *C. betulae* and *Heterodera* but do not occur in *Punctodera* or other cyst nematodes, perhaps, further supporting a link between *Cactodera* and *Heterodera*.

Monophyly of *Globodera* may be supported by the presence of protuberances on the head region, but it is not known whether or not these are homologous with the fewer protuberances of *Sarisodera*, *Rhizonema*, or *Hylonema* (Luc, Taylor & Cadet, 1978; Othman & Baldwin, 1985).

Lip patterns of J2 of *Punctodera* are distinctive from *Globodera* and *Cactodera* (excluding *C. betulae*) by partial fusion of the adjacent submedial lips with the labial disc, supporting monophyly of this genus. In addition, the characters of fused adjacent submedial lips and fusion of the large lateral lips (*versus* small lateral lips in *Cactodera*) with the labial disc are shared with most *Globodera* and could support a hypothesis that these two genera are sister groups. The hypothesis of *Globodera* and *Punctodera* as sister group is also supported by the shared characters of a small vulva and circular area lacking the body surface pattern around the vulva, reduction or absence of a cone in females, and details of the development of fenestrae which apparently differ from *Cactodera* (Othman, 1985). This is in partial contrast to Stone's view (1975, 1979) that *Cactodera* evolved from *Globodera*, and *Punctodera* developed from an independent line; it also slightly varies from the phylogeny suggested by Wouts (1985) which considers *Cactodera* and *Punctodera* sister groups which collectively share a common ancestor with *Globodera*.

Stone (1975) reported SEM of en face patterns of J2 of *Globodera*, *Cactodera*, and *Punctodera**; however previous SEM techniques resulted in collapsed specimens and results were inadequate to interpret many details. Misinterpretation of *en face* patterns may have influenced phylogenetic schemes proposed by Stone and subsequent investigators (Ferris, 1979; Wouts, 1985). *Cactodera* was considered by Stone (1975) as having the type 2 pattern in which submedial lips are fused with adjacent head annules. This character occurs only in *C. betulae*, the three other species examined typically have six separate lips. Stone designates a type 6 pattern as applying to *Punctodera*. This pattern is characterized by fused submedial lips, one which could not have been distinctive from many *Globodera*. We have noted that another feature of *Punctodera*, fusion of the labial disc with submedial lips, is apparently distinctive for the genus. Stone recognized variation in *Globodera* spp. with respect to fusion or lack of fusion between submedial lips. However, six separate lips were illustrated and designated the type 1 pattern to which *Globodera* was ascribed; our observations indicate that, with the exception of *G. rostochiensis*, the typical *Globodera* pattern has fused submedial lips, as has been also demonstrated for *G. zelandica* (Wouts, 1984).

The position of phasmids in J2 and posterior termination of lateral lines in J2 and males indicate interesting differences, but these characters have only limited promise for defining genera and are apparently too mosiac in distribution to be useful in phylogenetic analysis of the family. Phasmids are probably absent in males of most species of cyst-forming genera; this contrasts with their presence in *Meloidodera* (Othman & Baldwin, 1985). The presence or absence of phasmids in males has been confirmed by TEM in several heteroderids (Carta & Baldwin, unpubl. observ.), and may be useful characters in interpreting phylogeny of Heteroderidae.

Our investigation indicates characters which can be useful in identification and phylogenetic analysis of Heteroderidae, but such hypotheses of phylogeny are subject to further testing through use of larger samples as well as additional populations and species. For example, we noted that lip patterns of J2 and males are often highly variable, even within a population. Variation of some structures including certain features of overall cuticular and perineal patterns of females and cysts apparently vary with age (Othman, 1985); while these show promise as characters for further testing phylogenies, their most effective use must follow thorough comparative morphological-developmental investigations.

* At the time of Stone's description these genera were not recognized as distinct from *Heterodera*.

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