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# *Epicharinema keralense* n. gen, n. sp., and comments on Atylenchinae and Ecphyadophorinae (Nemata: Tylenchidae) <sup>(1)</sup>

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## SUMMARY

*Epicharinema keralense* n. gen., n. sp. is described from nematodes collected from soil about the roots of coconut palm in Kerala, India. This genus is related to *Ecphyadophora* and *Ecphyadophoroides* but differs from both by virtue of its greater length; larger stylet, spicules and gubernaculum; and its well-developed metacarpus with a prominent valve. *E. keralense* n. gen., n. sp. also has an elongate, sinuous cleft connected to the amphid which, with the valve in its metacarpus, indicates these belong to the family Tylenchidae. Relationship with *Atylenchus* and *Eutylenchus* is also considered and comments on the subfamilies Atylenchinae and Ecphyadophorinae are provided.

## RÉSUMÉ

*Epicharinema keralense* n. gen., n. sp.  
et commentaires sur les Atylenchinae et les Ecphyadophorinae

Les auteurs décrivent *Epicharinema keralense* n. gen., n. sp., extrait du sol au voisinage des racines de cocotiers, dans l'état de Kerala (Inde). Ce genre est voisin d'*Ecphyadophora* et *Ecphyadophoroides* mais en diffère par la plus grande longueur du corps, du stylet, des spicules et du gubernaculum, ainsi que par un métacarpus bien développé avec une valve proéminente. *E. keralense* n. gen., n. sp., présente également, connectée à l'amphide, une fente allongée et sinueuse, caractère qui, lié à la présence d'une valve dans le métacarpus, indique son appartenance aux Tylenchidae. Les relations de ce nouveau genre avec *Atylenchus* et *Eutylenchus* sont discutées, de même que les caractéristiques des sous-familles Atylenchinae et Ecphyadophorinae.

Soil samples from about the roots of coconut palm, *Cocos nucifera* L., on the Central Plantation Crops Research Institute Regional Station at Kayangulam contained nematodes which closely resemble members of *Ecphyadophora* De Man, 1921 and *Ecphyadophoroides* Corbett, 1964. However, important morphological differences suggest these nematodes represent not only a new species but a new generic taxon as well, a description of which is given below.

## Materials and methods

Nematodes were separated from soil by washing through sieves and then killed by heating in water. Preservation in 2% formalin was followed by storage for several weeks. The specimens then were fixed by transfer into FAA. Dehydration to glycerine followed Cobb's method of 2.5% glycerine in 30% alcohol then transfer to 5% glycerine in 30% alcohol. This

(1) Contribution n° 540 of the Central Plantation Crops Research Institute, Regional Station, Kayangulam.

was allowed to evaporate slowly to glycerine by storage in a Petri dish for several days. Ultimate dehydration was achieved over  $\text{CaCl}_2$  crystals in a desiccation chamber. Mounting was made in dehydrated glycerine. *En face* and transverse sections in glycerine were cut by hand and mounted in glycerine-jelly.

Preparation for scanning electron microscopy was by transfer of nematodes from FAA to alcohol through a graded series from 30% to absolute ethyl alcohol. This was followed by a graded series of amyl acetate in absolute alcohol from 30% finally to absolute amyl acetate. After critical point drying with  $\text{CO}_2$  the specimens were mounted on a stub and coated with 200 Å of gold. Examination and photography was done on an ETEK Autoscan scanning electron microscope at 7,000-8,000 X and 10 KV.

### *Epicharinema* \* n. gen.

#### DIAGNOSIS

Tylenchidae. Body long ( $L = 1.11-1.53$  mm), very slender ( $a = 67-122$ ). Cuticle smooth over most of body, phasmids not seen. Body narrows slightly anteriorly then more abruptly near anterior extremity. Cephalic region flattened dorso-ventrally, with rounded margins, almost truncate anteriorly. Stylet long ( $38-51$   $\mu\text{m}$ ), cone very slender, knobs well-developed. Metacarpus distinctly rounded and set-off, with well-developed valve. Esophageal glands slightly overlapping intestine. Female gonad anteriorly directed; no post-vulval uterine branch observed. Female tail long, slender, narrowing gradually from vulva to acute terminus. Male body narrows abruptly at cloaca, tail almost tubular, very long and slender. Caudal alae prominent, leptoderan, with two sclerotized ribs supporting each ala. Spicules tylenchoid. Gubernaculum  $8-14$   $\mu\text{m}$  long, slender, simple. Testis single, outstretched.

TYPE SPECIES : *E. keralense* n. sp.

\* From the Greek *epicharis* = elegant; *nema* = thread. The species name is from the state in which it was found, Kerala, meaning "land of coconut".

### *Epicharinema keralense* n. sp.

#### DIMENSIONS

*Females* (13) :  $L = 1.30$  (1.11-1.41) mm ;  $a = 82$  (67-106) ;  $b = 9.3$  (8.6-10.9) \*\* ;  $b' = 8.0$  (7.1-8.7) \*\*\* ;  $c = 4.4$  (4.0-5.2) ; stylet = 46 (38-51)  $\mu\text{m}$  ; cone = 30 (26-36)  $\mu\text{m}$  ;  $V = 27(19-40)72(68-79)$  -----.

*Males* (13) :  $L = 1.42$  (1.26-1.53) mm ;  $a = 99$  (78-122) ;  $b = 9.6$  (8.2-10.3) ;  $b' = 8.8$  (7.9-9.8) ;  $c = 4.2$  (3.5-5.4) ; stylet = 46 (41-52)  $\mu\text{m}$  ; cone = 30 (27-34)  $\mu\text{m}$  ; spicules = 41 (39-43)  $\mu\text{m}$  ; gubernaculum = 11 (8-14)  $\mu\text{m}$  ;  $T = 32$  (20-40).

*Holotype* (female) :  $L = 1.41$  mm ;  $a = 94$  ;  $b = 9.5$  ;  $b' = 8.7$  ;  $c = 4.1$  ; stylet = 51  $\mu\text{m}$  ; cone = 30  $\mu\text{m}$  ;  $V = 1972$  --.

*Allotype* (male) :  $L = 1.46$  mm ;  $a = 108$  ;  $b = 10.2$  ;  $b' = 8.4$  ;  $c = 4.2$  ; stylet = 52  $\mu\text{m}$  ; cone = 31  $\mu\text{m}$  ; spicules = 43  $\mu\text{m}$  ; gubernaculum = 11  $\mu\text{m}$  ;  $T = 33$ .

#### Juveniles

*Second-stage* ? (1) :  $L = 0.69$  mm ;  $a = 69$  ;  $b = ?$  ;  $b' = ?$  ;  $c = ?$  ; stylet = 28  $\mu\text{m}$  ; cone = 17  $\mu\text{m}$ .

*Third-stage* ? (1) :  $L = 0.94$  mm ;  $a = 99$  ;  $b = 7.2$  ;  $b' = 6.3$  ;  $c = 3.6$  ; stylet = 35  $\mu\text{m}$  ; cone = 22  $\mu\text{m}$ .

*Fourth-stage* (7) :  $L = 1.17$  (1.09-1.30) mm ;  $a = 89$  (84-93) ;  $b = 8.4$  (7.5-8.9) ;  $b' = 7.6$  (7.0-7.8) ;  $c = 3.5$  ; stylet = 41 (39-42)  $\mu\text{m}$  ; cone = 26 (24-27)  $\mu\text{m}$ .

#### DESCRIPTION

*Female* : Body almost straight when killed by gentle heat, very slender, of uniform diameter except at cephalic region and tail. Cephalic region flattened dorso-ventrally ; outline bottle-shaped in lateral view, narrow (about 10  $\mu\text{m}$  wide at anterior extremity) ; about 10  $\mu\text{m}$  posteriad it begins to widen to general body diameter. Outline in dorso-ventral view almost

\*\*  $b$  = body length divided by esophagus measured to esophago-intestinal valve.

\*\*\*  $b'$  = body length divided by esophagus measured to end of esophageal glands.

cylindrical up to bluntly rounded anterior extremity. *En face*: a flat labial plate, seen in scanning electron microscope (SEM) photographs (Figs 1 & 2), appears to be hexagonal in outline set off by obscure line connecting amphids. Amphids obscure under light microscope; in SEM photographs they appear pore-like, connected to sinuous, deep-cleft infoldings which course posteriad. No papillae or setae evident on SEM photographs. Under light microscope internal innervations to four papillae

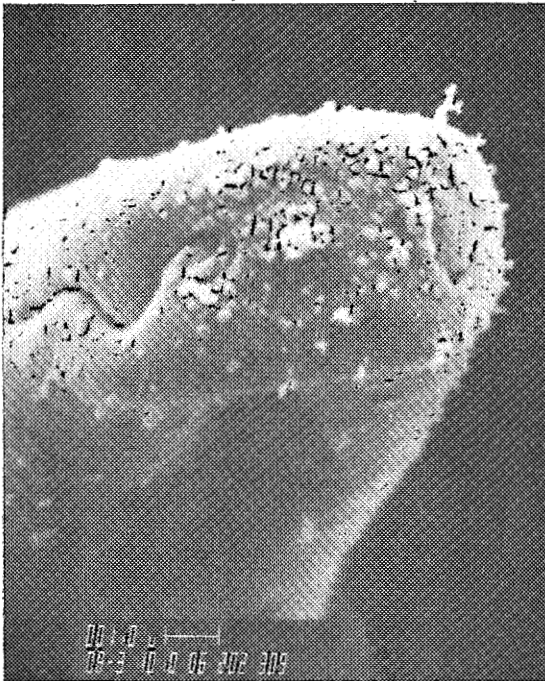


Fig. 1. : *En face* view of female *Epicharinema keralense* n. gen., n. sp., 8,000 X.

evident in submedian sectors. Stylet 51  $\mu\text{m}$  long, base and knobs robust, heavily sclerotized; cone robust at base but narrows to extremely fine shape anteriorly, anterior tip difficult to determine. (Stylet protruding in one paratype confirmed total length of stylet). Knobs rounded, about 4  $\mu\text{m}$  wide, without forward directed processes. Dorsal gland orifice adjacent to knobs. Metacarpus well-developed, set off by slight constrictions anteriorly and posteriorly. Valve 3  $\mu\text{m}$  long, strongly developed. Excretory canal and pore very faint, 147  $\mu\text{m}$  from anterior extremity (not seen in all paratypes), about

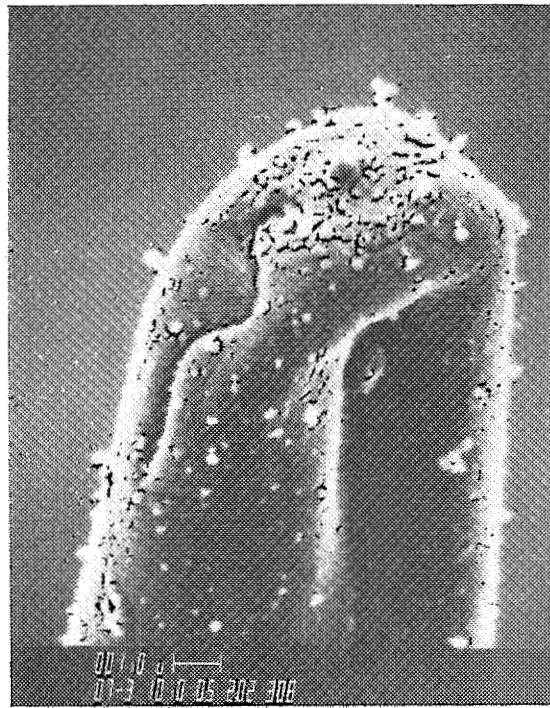


Fig. 2. : Anterior end of female *Epicharinema keralense* n. gen., n. sp. 7,000 X.

one body width anterior to end of oesophageal glands. Connection of esophagus with intestine provided with small sclerotized valve. Isthmus elongate, nerve ring about one body width posterior to median bulb. Gonad anteriorly directed, monodelphic, spermatheca not certainly identified. Vulva marked by slight swelling, opening obscure, apparently bears a short delicate, cuticular flap extending posteriorly from anterior lip; fan-shaped muscles attach vagina to subdorsal wall. Anus obscure; tail long, filiform, delicate, ending in acute terminus. Body smooth except for four faint cross-annulations detectable only by SEM photographs, located posteriad to smooth cephalic region delimited by extent of amphidial grooves. No other cross-annulations or evidence of lateral fields noted in whole mounts or transverse sections. Phasmids not seen.

*Males*: Body and cephalic shape similar to female. General body diameter similar throughout up to attachment of caudal alae then narrows abruptly to cloaca, more abruptly again posterior to cloaca with a small indent on dorsal

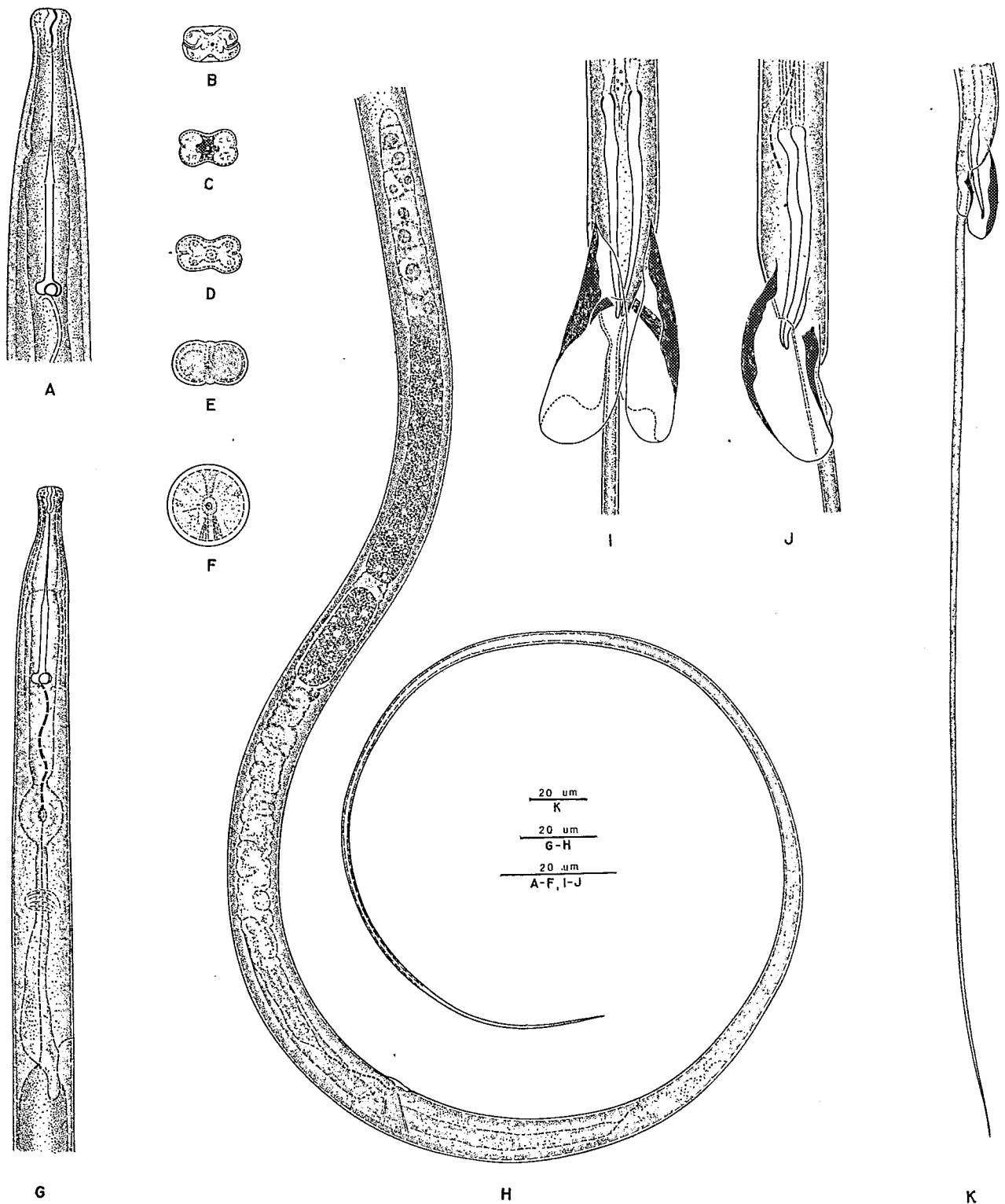


Fig. 3 : *Epicharinema keralense* n. gen., n. sp. Female, A-H. A : Anterior end ; B-F : Transverse sections from anterior surface (B) each one successively posterior up to (F) about 30  $\mu$ m from anterior end ; G : Esophageal region of anterior end ; H : Posterior and tail region. Male, I-K. Spicules and caudal alae, I : Ventral view ; J : Lateral view ; K : Tail region.

side; then continues posteriorly narrowing very gradually resulting in an extremely fine, long, almost tubular tail shape with acute terminus. Caudal alae begin 11  $\mu$ m anterior to cloaca, extend 28  $\mu$ m posterior to cloaca; elongate-oval, flap-like in outline. Two refractive, apparently sclerotized ribs support caudal alae. First pair slightly laterad to ventral margin, somewhat irregular in outline, presenting strongly marked ventral margin when seen in lateral view. Second pair more dorsad, narrows to fine point at margin of caudal alae. Testis single, outstretched. Spicules slender, slightly curved ventrally; distally, recurve dorsally near tip, terminus rounded. Gubernaculum 11  $\mu$ m long, slender, simple.

**Juveniles:** Similar to adult female in general shape. Second-stage (?) juvenile has weakly developed, backward-sloping knobs on stylet. Female and male fourth-stage juveniles appear to be distinguishable by shape at developing vulva vs. cloaca. Some (males ?) slightly rounded on dorsal side, others (females ?) smooth outline.

**TYPE HOST:** Sandy loam soil about roots of coconut palm, *Cocos nucifera* L.

**TYPE LOCALITY:** Palm No. 65, Block No. 1 of Central Plantation Crops Research Institute Regional Station, Kayangulam, Kerala State, India.

**HOLOTYPE:** Female, collected June 16, 1979 by V.K. Sosamma, slide number 1598, University of California Nematode Collection (UCNC), Davis, California.

**ALLOTYPE:** Male, same data as holotype, slide number 1598, UCNC.

**PARATYPES:** 29 females, 24 males, 41 juveniles, same data as holotype deposited as follows: 23 females, 18 males, 35 juveniles, UCNC; 1 female, 1 male, 1 juvenile, each to the following: National Nematode Collection, Indian Agricultural Research Institute, New Delhi, India; Nematology Laboratory, CPCRI Regional Station, Kayangulam, Kerala State, India; USDA Nematode Collection, Beltsville, Maryland; Nematology Department, Rothamsted Experimental Station, Harpenden, England; Plantenziektenkundige Dienst, Wageningen, Netherlands; Commonwealth Institute of Helminthology, St. Albans, Herts., England.

## DIAGNOSIS

As the monotypic species of *Epicharinema*, *E. keralense* has the characters that distinguish the genus. *E. keralense* clearly is closely related to *Ecphyadophora* and to *Ecphyadophoroides* by its cylindrical but very slender shape throughout most of the body length; slender overlapping esophageal glands; fine anterior part of the stylet; dorsal gland orifice very close to stylet base; single anteriorly directed gonad; general position of vulva; flapshaped, leptoderan, caudal alae; abrupt narrowing of male tail; and elongate, fine tail of both sexes. It is further similar to *Ecphyadophora* by its smooth cuticle (finely annulated in four species, smooth in two species of *Ecphyadophora*). It is similar to *Ecphyadophoroides* by the dorsal-ventral flattening of the cephalic region. It is distinctive from all species of both genera by the greater length (range = 0.51-0.75 mm except for 0.84-0.94 for *E. tarjani* and 0.65-1.02 mm for *E. tenuissima* as reported by Tarjan (1957); large stylet: 38-52  $\mu$ m (6-12  $\mu$ m range for all species of the other two genera); distinct median bulb with prominent valve; large spicules 39-43  $\mu$ m (9-20  $\mu$ m range for all species of other two genera) and gubernaculum 8-14  $\mu$ m (2-7  $\mu$ m range for all species of other two genera).

## Relations of Atylenchinae and Ecphyadophorinae

The presence of a well-developed, valvated median bulb in *E. keralense* raises important questions regarding the relationship of all three genera to other groups. Skarbilovich (1959) proposed the family Ecphyadophoridae with a subfamily Ecphyadophorinae for the single species, *E. tenuissima*. More generally accepted is the action of Goodey (1963) recognizing Ecphyadophorinae Skarbilovich, 1959 but placing it in the family Neotylenchidae. However, the valvated metacarpus of *E. keralense* puts into question the validity of that relationship and suggests these belong in the family Tylenchidae. In fact there is doubt whether absence of a valvated metacarpus as a valid character by itself should constitute the basis for a separate family as originally proposed by

Thorne (1949). Many disparate species are thus brought together. Given a valve a large percentage of these "neotylenchs" would be considered tylenchs. Indeed Paramonov (1968) transferred *Nothanguina* to the Anguininae (Tylenchidae). Under the proposals of Maggenti (in press) these and the herein described species would be members of Tylenchidae.

It is also significant that the sinuous amphidial grooves of *E. keralense* are very similar to those of several tylenchs and "neotylenchs" reported by Andr ssy (1963), Geraert (1965, 1974), Kheiri (1972) and Siddiqi (1979). They describe a number of species as having relatively large amphidial apertures including longitudinal radial clefts. Furthermore, Siddiqi proposed two new genera in a new subfamily Duosulciinae (Tylenchidae) which possess a non-muscular metacarpus (postcorpus of Siddiqi) without inner valve plates. The other genera of that subfamily have well-developed metacarpus with valve. This is further evidence of a closer relationship of Ecphyadophorinae with Tylenchidae.

The only character not consistent with Tylenchidae is the nature of overlapping esophageal glands of Ecphyadophorinae. However, *E. keralense* does have a relatively short overlap which could be considered intermediate between the long, slender and extended overlap of the other two genera and the enclosed glands of most Tylenchidae. Furthermore, there are examples of varying degrees of overlapping glands in some genera of Tylenchidae such as *Anguina* and *Ditylenchus*.

As members of Tylenchidae it becomes logical to note the relationship of these species to members of the *Atylenchus* and *Eutylenchus*. Skarbilovich (1959) was the first to propose a separate family Atylenchidae and subfamily Atylenchinae for *Atylenchus* and *Eutylenchus*. Hirschmann (1954) reported *A. decalineatus* from Germany but did not discuss its taxonomic position. Chitwood and Tarjan (1957) later redescribed that species but also did not refer to its familial relationship. Thorne (1961) and Goodey (1963) disagreed with Skarbilovich but Sher, Corbett and Colbran (1966) accepted Atylenchidae when they revised the family. Paramonov (1972) believed *Atylenchus* and *Eutylenchus* represented a subfamily Atylenchinae

in the Tylenchidae. Golden (1971) proposed a superfamily Atylenchoidea with one family Atylenchidae and a subfamily Atylenchinae but that has not gained acceptance. Szczygiel (1969) proposed another genus *Pleurotylenchus* for *P. sachsi* which was described as *Tylenchus sachsi* by Hirschmann (1952). This was transferred to *Tylenchus* (*Aglenchus*) *sachsi* by Andr ssy (1954) and later to *Aglenchus sachsi* by Meyl (1961). Most recent was the action of Andr ssy (1976) in which he proposed two subfamilies, Atylenchinae and Pleurotylenchinae, in a family Atylenchidae.

There is an obvious and close similarity linking all these species of *Atylenchus*, *Eutylenchus*, *Pleurotylenchus*, *Ecphyadophora*, *Ecphyadophoroides* and *Epicharinema* by virtue of their long, slender shape, delicate stylet, mostly with leptoderan, flap-like caudal alae, and long slender tails. Presence of seta-like structures on the lip region and longitudinal ridges on the body as found in *Atylenchus* and *Eutylenchus* do not necessarily represent separate family status for those forms. The inclusion by Andr ssy (1976) of *Pleurotylenchus sachsi*, which bears no cephalic setae, in Atylenchidae further weakens the case for a separate family. On the other hand the characters listed above are strong indicators of relationship with *Ecphyadophora* and its relatives. Acceptance of these relationships leads to the following placement of genera in the family Tylenchidae which follows the proposals of Maggenti (in press) :

SUBFAMILY Atylenchinae Skarbilovich, 1959

Genus *Atylenchus* Cobb, 1913

Species : *A. decalineatus* Cobb, 1913

Genus *Eutylenchus* Cobb, 1913

Species : *E. setiferus* (Cobb, 1893) Cobb, 1913

*E. africanus* Sher, Corbett & Colbran, 1966, syn. *E. orientalis* Husain & Khan, 1968

*E. vitiensis* Orton Williams, 1979

Genus *Pleurotylenchus* Szczygiel, 1969

Species : *P. sachsi* (Hirschmann, 1952) Szczygiel, 1969

SUBFAMILY Ecphyadophorinae Skarbilovich, 1959

Genus *Ecphyadophora* De Man, 1921



Species : *E. tenuissima* De Man, 1921  
*E. quadralata* Corbett, 1964  
*E. goodeyi* Husain & Khan, 1965  
*E. tarjani* Husain & Khan, 1965  
*E. acuta* Husain & Khan, 1968  
*E. vallipuriensis* Husain & Khan, 1968  
*E. basiri* Verma, 1972  
*E. tritici* Verma, 1972

Genus *Ecphyadophoroides* Corbett, 1964

Species : *E. annulatus* Corbett, 1964  
*E. tenuis* Corbett, 1964  
*E. graminis* Husain & Khan, 1968  
*E. indicus* Verma, 1972

Genus *Epicharinema* n. gen.

Species : *E. keralense* n. sp.

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