Pakira orae Yeates, 1967 from Transkei (Nematoda: Leptolaimidae)

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Pakira orae Yeates, 1967 which was described from dune sands in New Zealand, was recently rediscovered in coastal areas in the Cebe Nature Reserve in Transkei. A more complete description is given of the morphology of this rare nematode, based on light as well as scanning electron microscopy.

Pakira orae Yeates, 1967 wat beskryf was van duinesand in Nieu-Seeland, is onlangs langs die kus van die Cebe Natuurreservaat in Transkei herontdek. 'n Meer volledige studie van die morfologie van hierdie seldsame nematood is met behulp van lig- en aftaselektronmikroskopie gedoen.

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The monotypic genus *Pakira* has never again been reported in the literature since its description from dune sands in New Zealand by Yeates in 1967. During a nematode survey in the Cebe Nature Reserve, a coastal reserve at the mouth of the Cebe River in Transkei, the authors found several specimens which they regard as conspecific with *Pakira orae* Yeates. This afforded the opportunity to study the morphology of this species in more detail. The present specimens were obtained from four different samples. The sample which yielded most specimens came from a high stabilized dune covered with indigenous forest with hardwood trees as well as *Strelitzia nicolai* Regel & Koern, close to the beach and bordering on a lagoon. Other specimens came from samples under grasses and trees, on very sandy soil but somewhat further inland, the furthest about 1 km from the coast. Specimens used for light microscopy (measurements and drawings) were processed according to standard procedures as described in nematological literature. Preparation for SEM was done according to the technique described by Luc, Coomans & Sarr (1987).

Pakira orae Yeates, 1967 (Figures 1–5 & Table 1)

Live specimens very agile, the long body often curving zigzag-like. Body posture of heat-relaxed specimens strongly ventrally curved into a closed C shape or loose spiral in both female and male. Cuticle about the same width over the entire body, in both female and male, 2–2,5 μm thick. Prominent transverse striae about 2 μm apart over greater part of body, but narrower towards head and tail, about 1,5 μm in both female and male. However, annules sometimes seem to be double because of fainter striae between the main striae. When the body bends, the annules on the outside are stretched and the intermediate striae may disappear, while those on the inside bend are accentuated, so that annules here may appear half as wide as normal. Lip region with six equal lips, well separated, appearing petal-shaped when seen *en face* (Figure 4B). Inner labial papillae not seen under the light microscope or SEM. Six outer labial papillae conpicuous, consisting of a large (2,5–3 μm diameter) circular elevated structure with a smaller central slightly elevated nipple-like structure carrying a small round pore (Figure 4A–C); the nipple-like part can retract and then only a larger pore is visible on top of the circular basis. Four cephalic papillae, about 2 μm in diameter, appearing as a rounded or bluntly conoid plug-like protrusion in a large round or oval pore under SEM (Figure 4A, C, D). Amphids with slit-like to narrow oval openings (Figures 1C & 4C, D), fovea stirrup-shaped, fusus large, close behind fovea (Figure 1C, F).

Numerous conspicuous body pores connected with large, unicellular epidermal glands arranged in eight rows, two subdorsal, two subventral and two on each side, sublateral (Figure 1A, C–F, I). These pores do not all have the same appearance owing to protrusion or retraction of an internal element (receptor or secretion?). Apart from pores, papillae sporadically occur near the anterior end and subventrally as well as laterally in the caudal region of the male (Figures 4C, G & 5B, C).

Epidermal nuclei all very clear, roundish to polygonal when compressed. Nuclei of epidermal glands usually obvious, but sometimes obscure. Nuclei of muscle cells elongate (Figure 1G, H). Excretory pore located mid-ventrally opposite basal swelling of pharynx, leading to a long, relatively wide, cuticularized duct. The duct is flattened and biconcave in cross section; it can be followed for a distance of about 2–3 body widths behind the base of the pharynx, where it ends in a short or longer loop; the latter is probably connected with a ventral gland cell, but this has not been identified with certainty. Close to, but anterior to the loop, a transparent (hyaline) cell with a clear nucleus containing a prominent nucleolus is constantly present (Figure 1A, B). This cell may represent the ventral gland; it is accompanied by one or two coelomocytes. The duct and the loop may be situated at the same or at different body sides.

Numerous large coelomocytes occur in groups of two

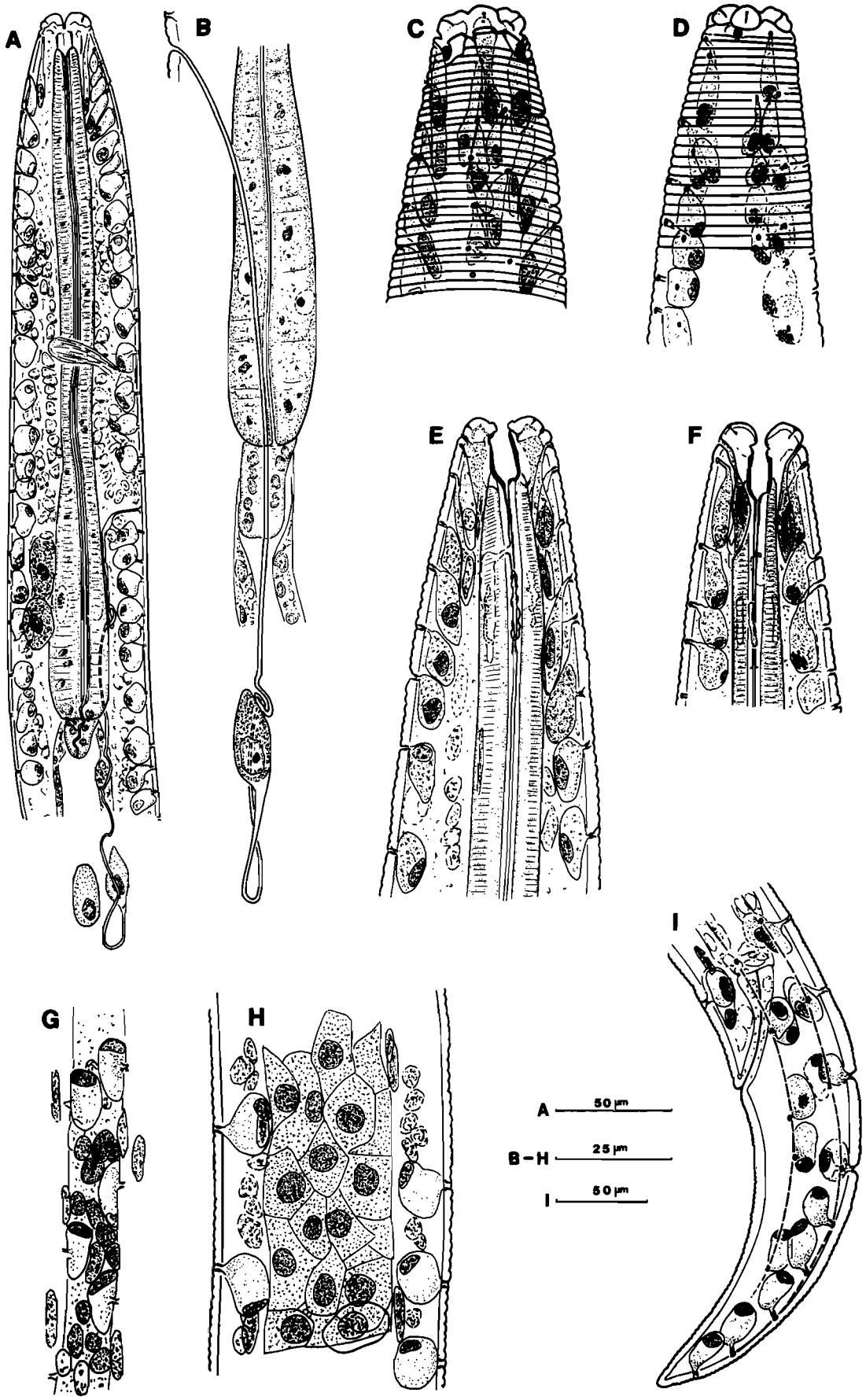

Figure 1 *Pakira orae*. A: Anterior part of female, showing excretory duct. B: Excretory system and basal part of pharynx of juvenile specimen. C & D: Surface view of head end in lateral and subdorsal view respectively. E & F: Optical section through head end in lateral and dorso-ventral view respectively. G: Lateral chord, also showing some elongate nuclei of somatic muscle cells. H: Cells of intestinal wall, also showing epidermal glands and several elongate nuclei of somatic muscle cells. I: Female tail.

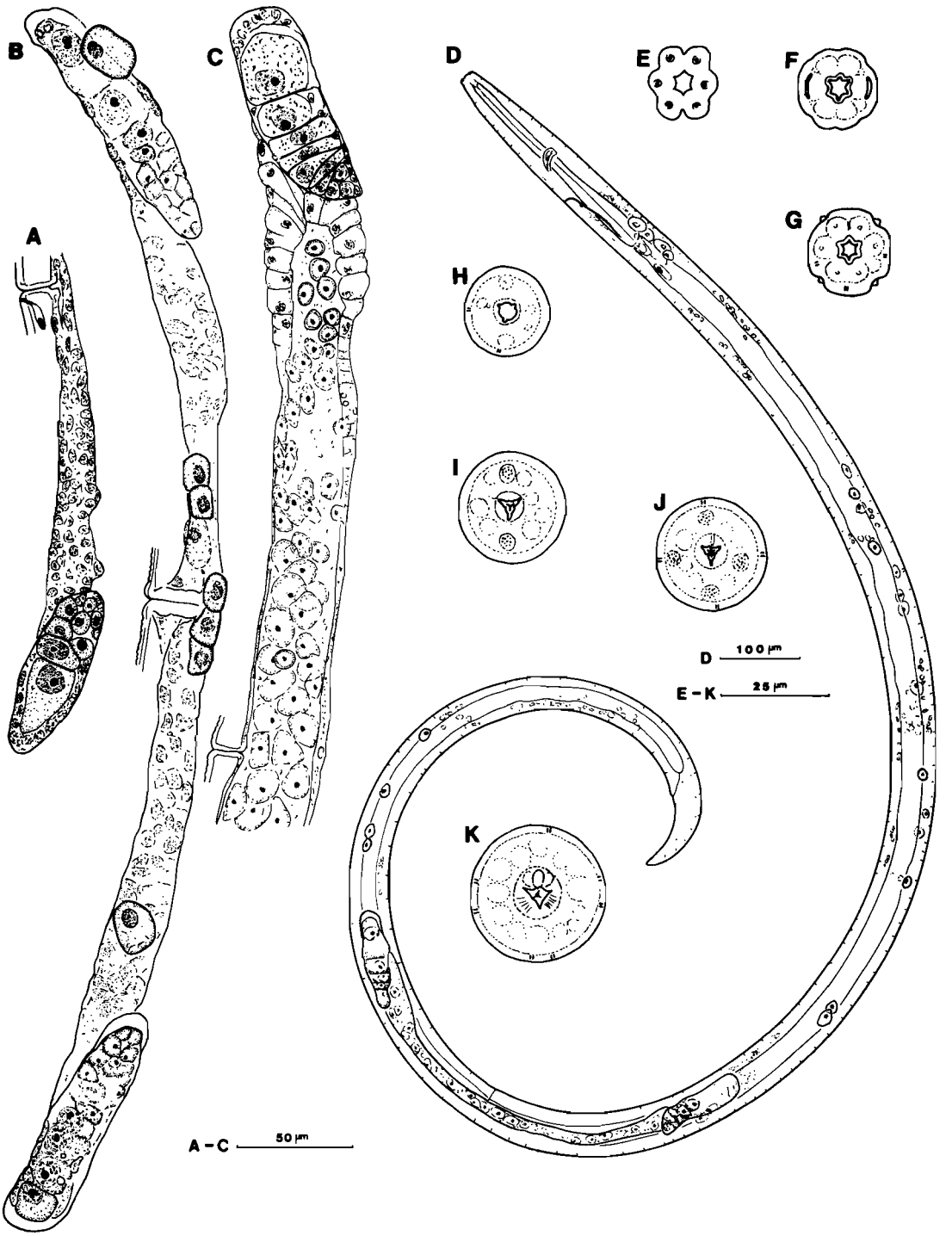

Figure 2 *Pakira orae*. A: One branch of female reproductive system, not containing spermatozoa, and showing numerous nuclei in the gonoduct wall. B: Complete reproductive system, with accompanying coelomocytes. C: One branch of female reproductive system, containing spermatozoa (small, near ovary) and possibly spermatids (larger, near vagina). D: Entire female. E–K: Face view and cross sections at different levels of stoma and pharynx (juvenile specimen). E: Face view. F: At level of amphid apertures. G: At level of cephalic papillae. H: Slightly more posterior, still in the area of the stoma. I: At level of anterior differentiated part of pharynx. J: Slightly more posterior, showing ducts of four body pores. K: Still more posterior, showing ducts of seven body pores, and ampulla of dorsal gland in pharynx.

or three irregularly distributed along base of pharynx, always with at least one coelomocyte ventrally somewhat posterior to base of pharynx, and usually dorsally or subdorsally along the entire intestine (Figures 1A, 2B, D & 3A–D).

Stoma barrel-shaped, well sclerotized, 6 × 10 µm in female, 5 × 9,5 µm in male; followed by a differentiated tubular anterior part of the pharynx, apparently 36,4 (33–44) µm in female, 34,2 (32–36) µm in male. The pharynx is sub-cylindroid, with a slight, gradual basal swelling. (Although Yeates described the pharynx as dorylaimoid, his Figure 2A does not show a typical dorylaimoid pharynx). Pharynx with weak radial muscles over its entire length and with secretory granules mainly

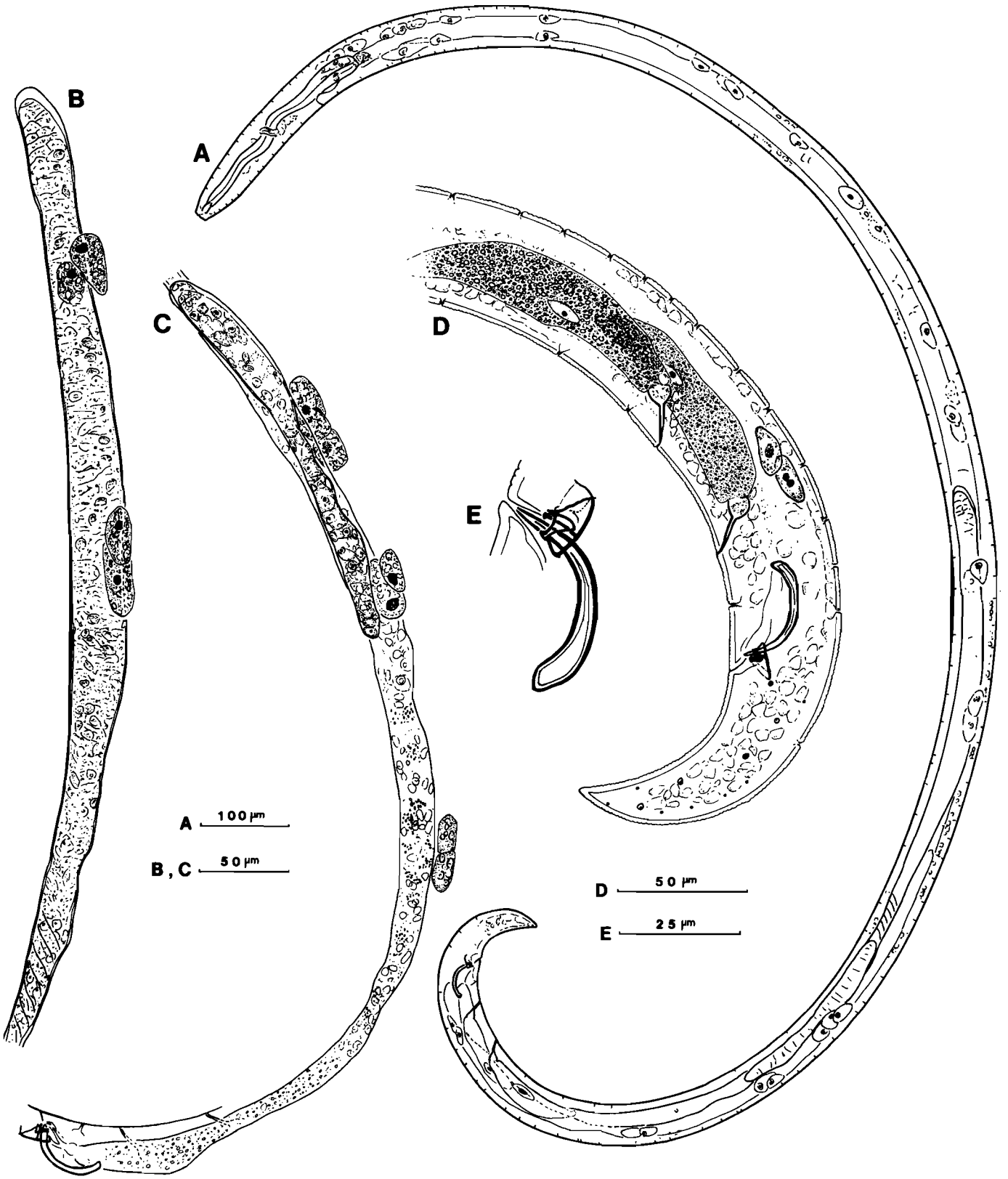

Figure 3 *Pakira orae*. A: Entire male. B: Anterior testis with accompanying coelomocytes. C: Posterior testis, gonoduct and spicules, plus coelomocytes. D: Male posterior part, showing large glands of supplements. E: Spicule and gubernaculum.

confined to the posterior part. DO slightly more than one stoma length behind stoma; SO closely behind nerve ring; gland nuclei obscure. Cardia elongate-conoid, apparently consisting of about eight loosely associated cells, with the transition from pharynx to cardia sometimes indistinct, the first two cells of the cardia apparently situated in the transition. Intestine with few cells in circumference, these intestinal cells and their nuclei a conspicuous feature, their arrangement and appearance sometimes almost like fish-scales (Figure

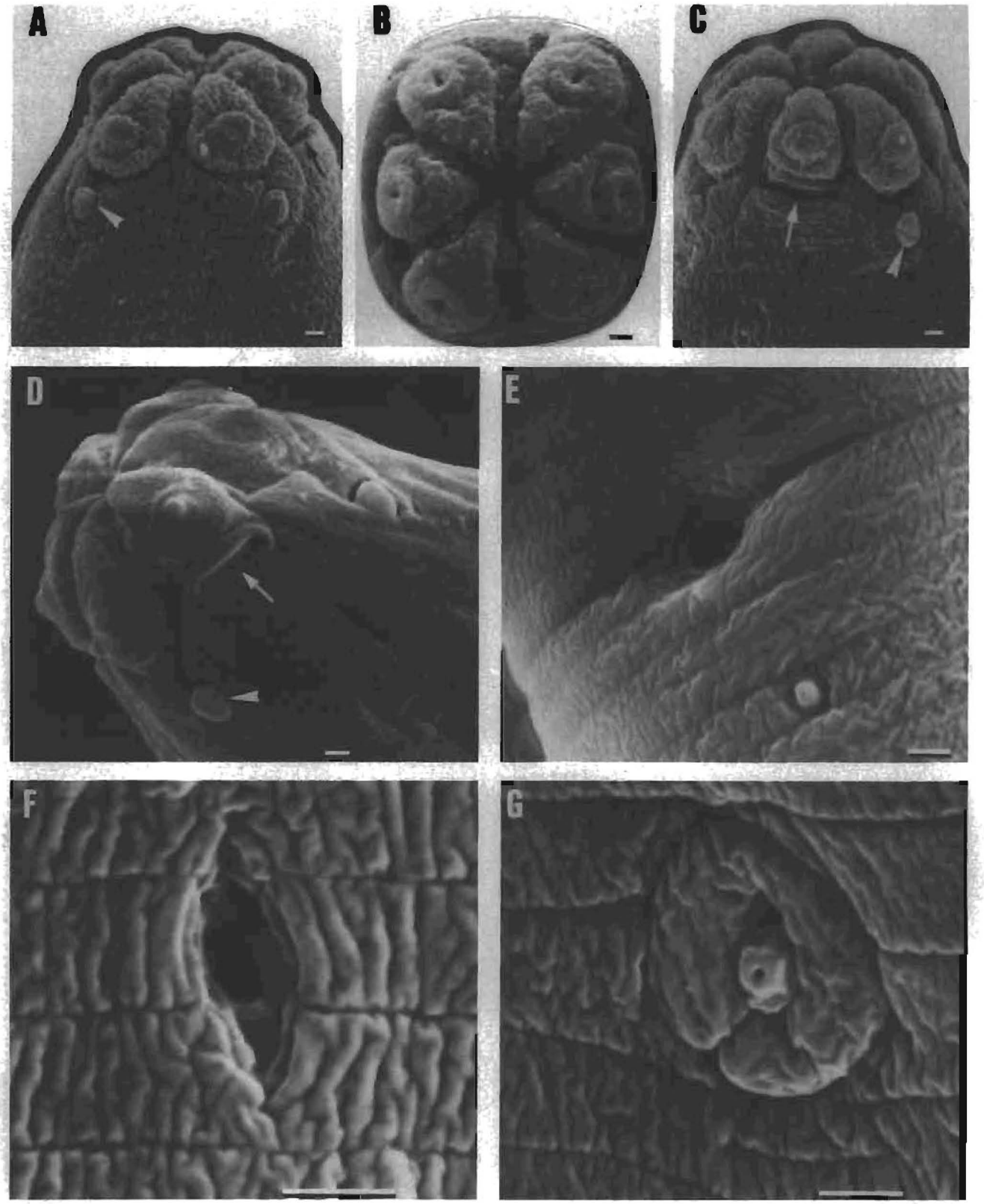

Figure 4 SEM photomicrographs of *Pakira orae*. A: Male head end in dorso-ventral view. B: Juvenile, *en face*. C: Male head end in lateral view. D: Juvenile head end in lateral view. Arrowheads in A, C and D point towards cephalic sensilla, arrows point towards amphid apertures. E: Excretory pore and ventral body pore, male. F: Body pore, juvenile. G: Postcloacal subventral papilla, male. Bar = 1 µm.

5E, F), and the cells in lateral view bulging beyond the contour of the intestine into the pseudocoel. Rectum about one anal body diameter in length. Tail conoid, ventrally curved, the terminus rounded. No ventral pores present on tail.

Female didelphic amphidelphic, with ovaries reflexed,

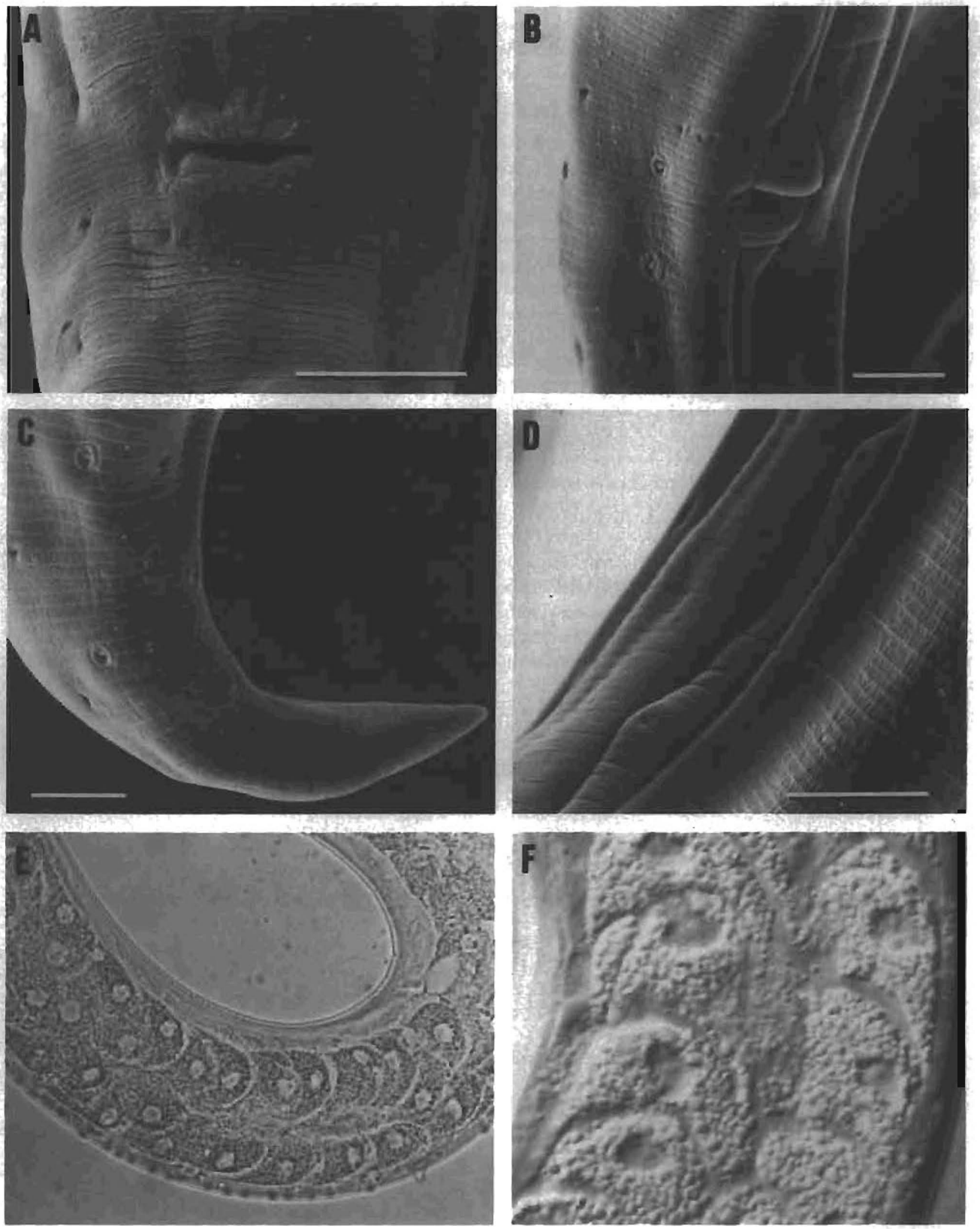

Figure 5 *Pakira orae*. SEM and LM photomicrographs. A: Anus, juvenile. B: Cloacal opening, male. C: Lateral view of male tail. D: Supplements, male. E & F: Intestinal cells as seen under light microscope, bright field and Nomarski, respectively. Bar (A–D) = 10 µm.

anterior ovary to the right, posterior ovary to the left side of the intestine. Vulva a transverse slit. Vagina perpendicular to body axis, leading to broad, undifferentiated gonoducts.

Male diorchic, with both testes opposed and at different body sides; anterior testes to the right, posterior one to the left of the intestine. Spicules slender, gently curved. Gubernaculum with complicated

structure, somewhat variable in different specimens, consisting of a rod-like part with knobbed distal end, carrying a conspicuous ventral apophyse lying medially between the spicules, a large dorsal median apophyse, and more delicate lateral structures apparently attached to the median apophyse (Figure 3E).

Two ventromedian supplements, each with a sclerotized duct leading to a large elongate gland (Figure 3D). The openings of these ducts are situated on two elevations lying between two subventral longitudinal grooves, which extend on either side of the cloacal opening (Figure 5D). The cloacal opening is a transverse slit, with the anterior and posterior lips slightly elevated, the posterior lip showing a series of internal grooves (Figure 5B).

One fully developed female with normal gonads was found which had two supplements in the same position as in the male. The measurements of this female are listed separately in Table 1.

Juveniles basically similar to adult females in appearance, except for size and lack of reproductive system.

Discussion

Although somewhat larger, the specimens from Transkei agree fairly well morphologically with the type population described from New Zealand (Yeates 1967). The most obvious differences are in the greater overall length, the length of the spicules and the location of the supplements (see Table 1).

There are other apparent differences between our specimens and the description of Yeates, such as the shape and position of amphid openings, the structure of the pharynx (= oesophagus of Yeates) which was described as dorylaimoid with a more glandular basal bulb (figured by Yeates, in his Figure 2A as having a cylindrical, muscular anterior two thirds, and a broader glandular basal third). The anterior differentiated part of the pharynx (called posterior narrow part of the stoma by Yeates) was reported as about 20 µm long (25 µm calculated from Yeates, Figure 2A), while we report it as about 32–44 µm in our specimens. These discrepancies we ascribe to differences in interpretation and in population, and we find no reason to describe our population as a new species, but regard it as a geographical variant of *Pakira orae*.

Yeates (1967) assigned *Pakira* to Plectidae Örley, 1880, but Andrássy (1973) transferred the genus to Leptolaimidae Örley, 1880 (subfamily Leptolaiminae). Lorenzen (1981) agreed with this action on the following arguments (see Lorenzen l.c.pp. 203–204): cuticularized excretory duct not coiled, pharynx with only a glandular enlargement at its base and absence of deirids.

The genus *Pakira* fits the diagnosis of Leptolaimidae as given by Lorenzen (1981: 195–197) but our findings differ from what Lorenzen (see above) mentions about the excretory duct. His statement was undoubtedly based on the original description of the genus. Since we found a terminally coiled excretory duct (similar to that in Plectidae, but with the coil **behind** the pharyngo-intestinal junction) the first argument to transfer *Pakira*

Table 1 Biometrical data of *Pakira orae* from New Zealand and Transkei

	New Zealand (From Yeates 1967)		Transkei		
	♀	♂	♀	♀ (with supplements)	♂
n	12	12	11	1	6
L(mm)	2,44 (2,03–2,86)	2,03 (1,90–2,34)	2,94 (2,20–3,97)	2,23	2,52 (2,32–3,20)
a	44 (37–52)	44 (36–53)	52 (41–67)	38	55 (41–74)
b	8,8 (7,5–10,2)	7,7 (6,2–9,0)	9,6 (7,6–13,1)	8,2	9,7 (8,6–11,9)
c	24 (20–27)	22 (18–26)	28 (21–36,5)	25	25 (21–28,5)
Tail length (µm)	102*	102*	105 (95–112)	90	100 (87–114)
c′	2,9 (2,4–3,6)	2,8 (2,1–3,6)	2,7 (2,3–3,3)	2,3	2,6 (2,1–2,8)
V	62 (58–67)	–	64 (59–66)	62	–
Stoma length (µm)	8–9		10 (8,5–11)	9	9,5 (9–10)
Lip region width (µm)	21*		21 (19–23)	18,5	18 (16,5–19,5)
Nerve ring from front end (µm)	–		146 (128–173)	126	128 (123–134)
Excretory pore from front end (µm)	188*		212 (184–260)	181	186 (172–205)
Pharynx length (µm)	290*		301 (273–355)	273	261 (243–274)
Spicule length along median line (µm)	–	38,5*	–	–	50,5 (47–52,5)
Cloaca to supplement 1 (µm)	–	36,5*	–	48	56,5 (46–65)
Cloaca to supplement 2 (µm)	–	56 (48–65)	–	99	101 (86–110)

*Calculated from Yeates (1967) Figure 2.

from Plectidae to Leptolaimidae is no longer valid. The other two still stand. Furthermore, a coiled excretory duct occurs in at least two other genera of the Leptolaimidae (*Anonchus* Cobb, 1913 and *Paraplectonema* Strand, 1934) (see Lorenzen l.c.: 196), so that the presence of this character is not an argument for removing *Pakira* from Leptolaimidae. The shape of the amphid is rather unusual for Leptolaimidae, but again similar types are found in other genera (e.g. *Anomonema, Stephanolaimus*) while such a variation in amphid shape is also found in other families, e.g. Plectidae.

In conclusion, we agree with a position of *Pakira* in the Leptolaimidae, subfamily Leptolaiminae and consider the special characters (extensive epidermal gland system, absence of caudal glands, short cephalic sensilla, etc.) as related with the terrestrial habitat.

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References

ANDRÁSSY, I. 1973. Nematoden aus Strand- und Höhlenbiotopen von Kuba. *Acta Zool. Acad. Sci. Hungaricae* 19: 233–270.

LORENZEN, S. 1981. Entwurf eines phylogenetischen Systems der freilebenden Nematoden. *Veröff. Inst. Meeresforsch. Bremerh.* Suppl. 7: 1–472.

LUC, M., COOMANS, A. & SARR, E. 1987. Redescription of *Neodolichodorus rostrulatus* (Siddiqi, 1976); Siddiqi, 1977 (Nematoda: Tylenchina). *Revue de Nématologie* 10: 29–37.

YEATES, G.W. 1967. Studies on nematodes from dune sands 2. Araeolaimida. *N. Zeal. J. Sci.* 10: 287–298.