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Molecular and morphological observations on *Parasitodiplogaster sycophilon* Poinar, 1979 (Nematoda: Diplogastrina) associated with *Ficus burkei* in Africa

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Summary – A *Parasitodiplogaster* sp. was isolated from syconia of *Ficus burkei* from Pretoria, South Africa, and determined to be conspecific with *P. sycophilon*, originally described by Poinar in 1979 from Harare, Zimbabwe, and also from *F. burkei*. Examination of type material of *P. sycophilon* revealed inaccuracies in the former description necessitating a redescription which is provided herein. Additionally, the original description lacked molecular data, which is also provided. Originally, the stoma of *P. sycophilon* was described as reduced without teeth. However, we observed a large dorsal stegostomatal tooth and an almost equally-sized right subventral tooth which was typologically similar to the stoma of *P. laeviagata* from Florida. In addition, a pore-like phasmid was observed in both males and females just above the tail tip. Most other characters were as formerly described. Based upon molecular inferences from sequences of the D2/D3 expansion segments of the rDNA of the large subunit (LSU), *P. sycophilon* is not clearly defined relative to the neotropical *Parasitodiplogaster* species that have been described and sequenced from figs in the section Urostigma, subsection Americana (*i.e., P. laevigata*, *P. popenema*, *P. citrinema*, and *P. trigonema*), or to *P. australis* from Australia ex *F. virens* (section Urostigma, subsection Urostigma), or to *P. maxinema* from neotropical figs from the section Pharmacosycea, subsection Pharmacosycea. Further work is needed to elucidate the molecular phylogeny of the *Parasitodiplogaster* lineages that may have co-speciated with the African figs of the section Urostigma, subsection Galoglychia.

Keywords - *Ficus* spp., morphology, morphometrics, *Parasitodiplogaster*, redescription, syconia, taxonomy.

Poinar (1979) described *Parasitodiplogaster* Poinar, 1979 as the first parasitic nematode genus from fig wasps and as the first example of adult parasitic diplogastrid nematodes from the body cavity of their living insect hosts. Subsequent studies have shown that *Parasitodiplogaster* is a natural grouping of derived diplogastrid nematodes that penetrate adult female agaonid wasps as infective dauer juveniles as the wasps gather pollen to leave and seek a new fig for pollination and reproduction (Herre, 1989; Bronstein, 1992). Once in the new fig, the fig wasp simultaneously pollinates the fig and oviposits into female florets creating wasp galls. Concordantly, the infective dauer juvenile(s) begin to grow at the expense of their host and ultimately emerge as adults to mate within the sycone and produce the next generation of infective dauer juveniles (Poinar & Herre, 1991; Giblin-Davis *et al*., 1995, 2006; Bartholomaeus *et al*., 2009; Kanzaki *et al*., 2012).

Parasitodiplogaster comprises 15 valid species: *P. sycophilon* Poinar, 1979 and *P. doliostoma* Kanzaki, Giblin-Davis, Davies & Center, 2012 from Africa; *P. australis* Bartholomaeus, Davies, Ye, Kanzaki & Giblin-Davis, 2009 from Australia; and *P. citrinema* Poinar & Herre, 1991, *P. duganema* Poinar & Herre, 1991, *P. laevigata* Giblin-Davis, Ye, Kanzaki, Williams, Morris & Thomas, 2006, *P. maxinema* Poinar & Herre, 1991, *P. nymphanema* Poinar & Herre, 1991, *P. obtusinema* Poinar & Herre, 1991, *P. paranema* Poinar & Herre, 1991, *P. pharmaconema* Kanzaki, Giblin-Davis, Ye, Herre & Center, 2013, *P. pertanema* Poinar & Herre, 1991, *P. popenema* Poinar & Herre, 1991, *P. trigonema* Poinar & Herre, 1991, and *P. yoponema* Poinar & Herre, 1991 from North and Central America. The type species, *P. sycophilon*, was isolated from the fig wasp pollinator, *Elisabethiella stuckenbergi* (Grandi) from *Ficus burkei* (Miq.) in Harare, Zimbabwe (Poinar, 1979). This wasp is reportedly the chief pollinator of *F. burkei* over a range from Zimbabwe into South Africa, whereas *Alfonsiella brongersmai* Wiebes and *A. longiscapa* Joseph have been reported as pollinators of *F. burkei* further north (Berg & Wiebes, 1992).

In this study, *Parasitodiplogaster* sp. was isolated from syconia of *F. burkei* from Pretoria, South Africa, and determined to be conspecific with *P. sycophilon*. Examination of type material of *P. sycophilon* revealed discrepancies in the original description necessitating a redescription which is provided herein. Additionally, the original description lacked molecular data, which is also provided.

Material and methods

We surveyed the area around Hartbeespoort Dam, Northwest Province and Pretoria, Gauteng, for *F. burkei* samples. Five *F. burkei* trees in close proximity were sampled around Hartebeesport Dam and three trees around Pretoria. Repetitive observations and sampling enabled harvesting at suitable fig phases B-D as illustrated in Giblin-Davis *et al.* (2003).

Syconia were dissected with a scalpel and observed under a dissecting microscope for nematode presence. The pollinator wasp *E. stuckenbergi* was observed in *F. burkei* sycones during dissection. Addition of tap water into the syconia improved nematode yields, so a second batch of *F. burkei* syconia were dissected open with a scalpel and placed in tap water for up to 15 min. Live nematodes were hand-picked into 95% ethanol for DNA extraction. A smaller portion of nematodes was collected into water at room temperature, heat-killed, and an equal volume of double strength formalin-glycerin fixative added, and processed into 100% glycerin for permanent mounting (Southey, 1970). Another 15 live nematodes were placed in DESS to conserve morphological features for further observation. The permanent mounts were used for morphological observation. Stomatal morphology was observed on live material and rehydrated DESS conserved material (Yoder *et al.*, 2006). Drawings and measurements of nematodes were done with the aid of a camera lucida and a stage micrometer. Photomicrographs were taken with an Olympus E-410 attached to the phototube of an Olympus BH-2 microscope and edited using Adobe Photoshop Element 2.0 or on a Leica DM5500B equipped with a photomontage image capture system.

The original holotype and allotype specimens of *P. sycophilon* with the slide numbers UCNC 1864 and UCNC1865 were observed with the assistance of Dr Steve Nadler at the UC Davis Nematology type collection.

MOLECULAR STUDIES

Nematode samples were collected into 95% ethanol and stored for a maximum of 5-6 days prior to DNA extraction using a Qiagen microDNA kit. The extracted DNA concentration was measured using Nanodrop spectrophotometry for accurate PCR setup. The D2/D3 segment of ribosomal LSU DNA was amplified and sequenced as described by Nunn (1992).

Table 1. *The* Ficus *and pollinator association of* Parasitodiplogaster *species for which D2/D3 sequence data including the nematode sequence accession codes.*

Ficus species	*Ficus* section	Pollinator species	*Parasitodiplogaster* species	Genbank Acc #
F. maxima Mill.	*Pharmacosycea*	*Tetrapus americanus*	*P. pharmaconema*	AB810254
			P. maxinema	AY840559
F. burkei	*Galoglychia*	*Elisabethiella stuckenbergi*	*P. sycophilon*	KF211402
F. laevigata	*Americana*	*Pegoscapus assuetus*	*P. laevigata*	AY840557
				AY840558
				AY840556
F. citrifolia	*Americana*	*Pegoscapus tonduzi*	*P. citrinema*	AY840555
F. trigonata	*Americana*	*Pegoscapus grandii*	*P. trigonema*	AY840562
F. popenoi	*Americana*	*Pegoscapus gemellus*	*P. popenema*	AY840560
F. virens	*Urostigma*	*Platyscapa coronata*	*P. australis*	EU018051

The molecular sequence determined in the present study was deposited in the GenBank database with accession numbers KF211402 and were compared to those of other *Parasitodiplogaster* and closely related species stored in the database. The GenBank accession numbers for the sequences obtained are presented in Table 1. The relationship of the new species to other *Parasitodiplogaster* species was determined using Bayesian analysis. The compared sequences were aligned using the ClustalW 2.1 (Higgins *et al.*, 1994; Larkin *et al.*, 2007; Bioinformatics and Computational Biology Group, Department of Bioengineering, University of California, San Diego, CA, USA, http://workbench.sdsc.edu). The base substitution model selection was conducted using MODELTEST 3.7 (Posada & Crandall, 1998) and the determined parameters were used in Bayesian analysis, which includes the Akaike-supported Tamura-Nei model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the Akaike information criterion (AIC), which were used to perform and confirm the tree topology using MrBayes 3.2 (Ronquist *et al.*, 2012) by running the chain for 1×10^7 generations and setting the 'burn in' at 10,000 with every 100 trees recorded. We used Markov Chain Monte Carlo methods (Larget & Simon, 1999) within a Bayesian framework to estimate the posterior probabilities of the clade and parameters, and constructed a consensus tree using a 50% majority rule.

A base pair differences and p-distance table was constructed using MEGA 5 distance estimation analysis, including transition and transversion substitutions as well as Gamma distributed rate variation among sites (Tamura *et al*., 2011).

Results

Parasitodiplogaster sycophilon Poinar, 1979

(Figs 1-5)

MEASUREMENTS

See Table 2.

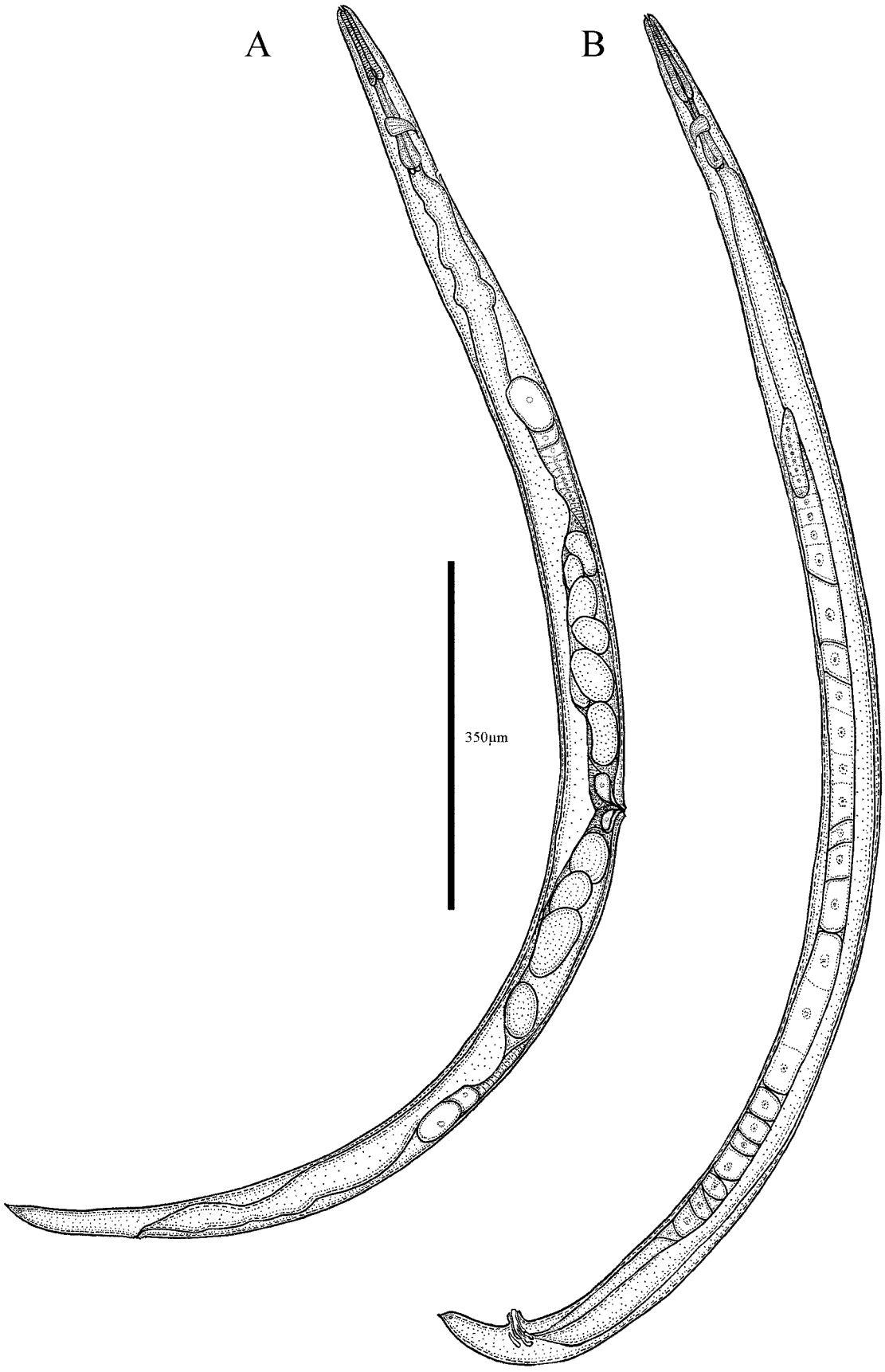
A
B
350µm

Fig. 1. Adults of *Parasitodiplogaster sycophilon*. A: Male; B: Female.

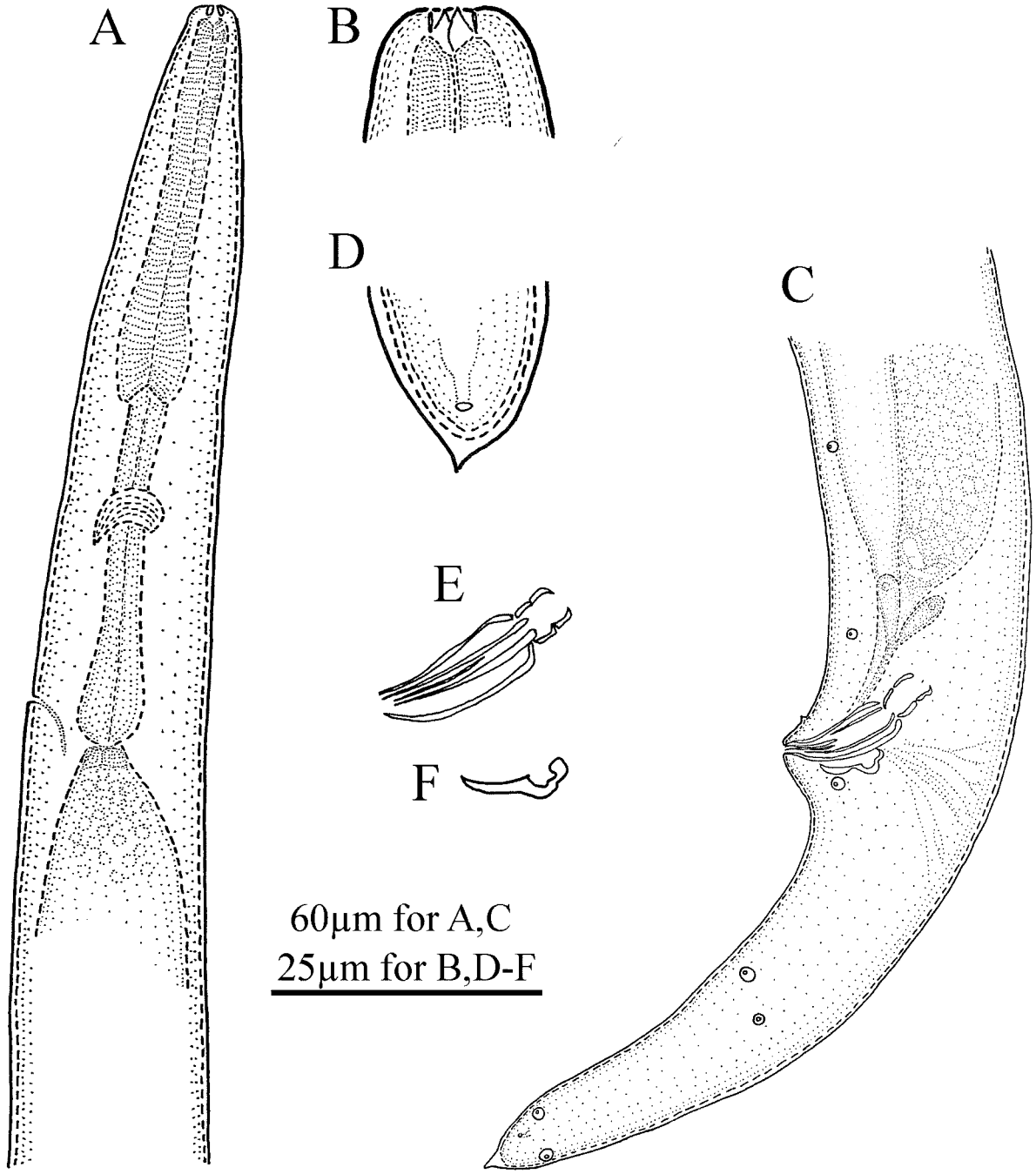

Fig. 2. Adults of *Parasitodiplogaster sycophilon*. A: Anterior part; B: Stoma (ventral view); C: Tail region (male); D: Tail tip (female); E: Spicule (lateral view); F: Gubernaculum (lateral view).

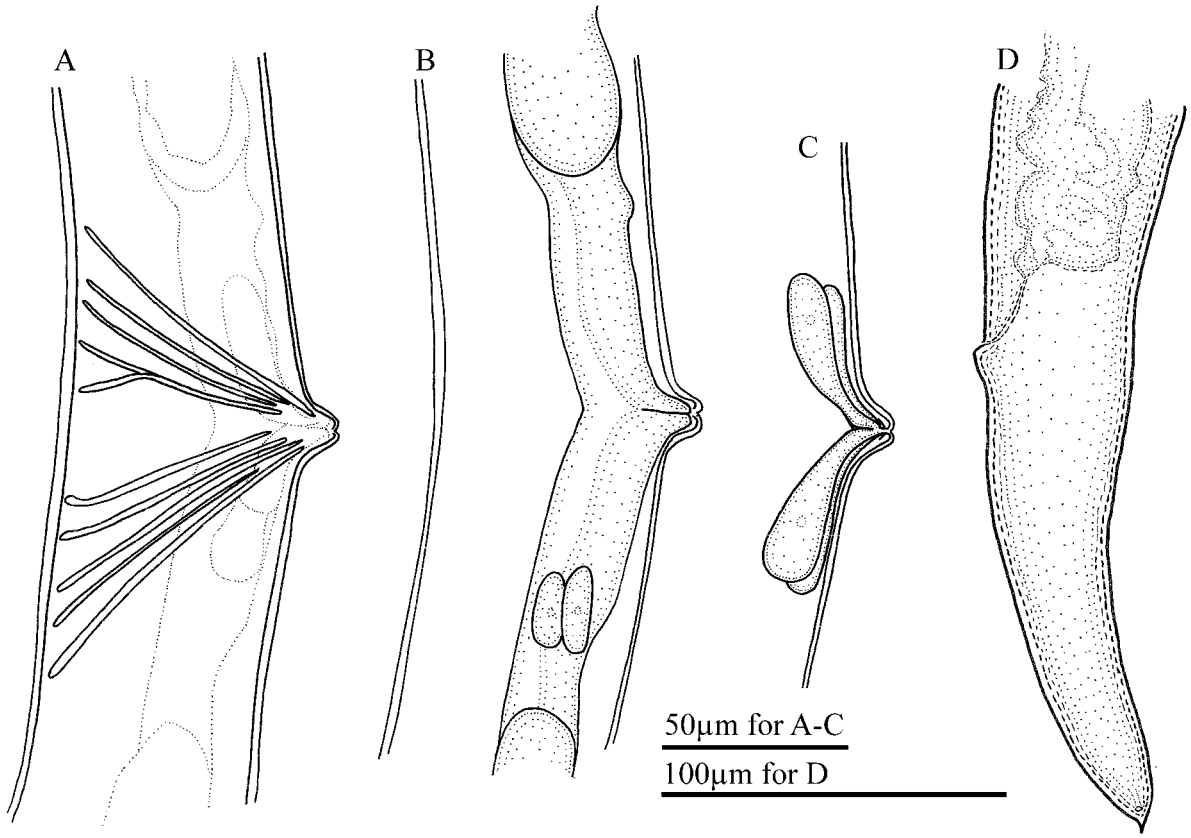

Fig. 3. Adult female of *Parasitodiplogaster sycophilon*. A: Vulval region (overview and muscle); B: Vulval region (oviducts and eggs); C: Vulval region (vaginal glands); D: Tail region (lateral view).

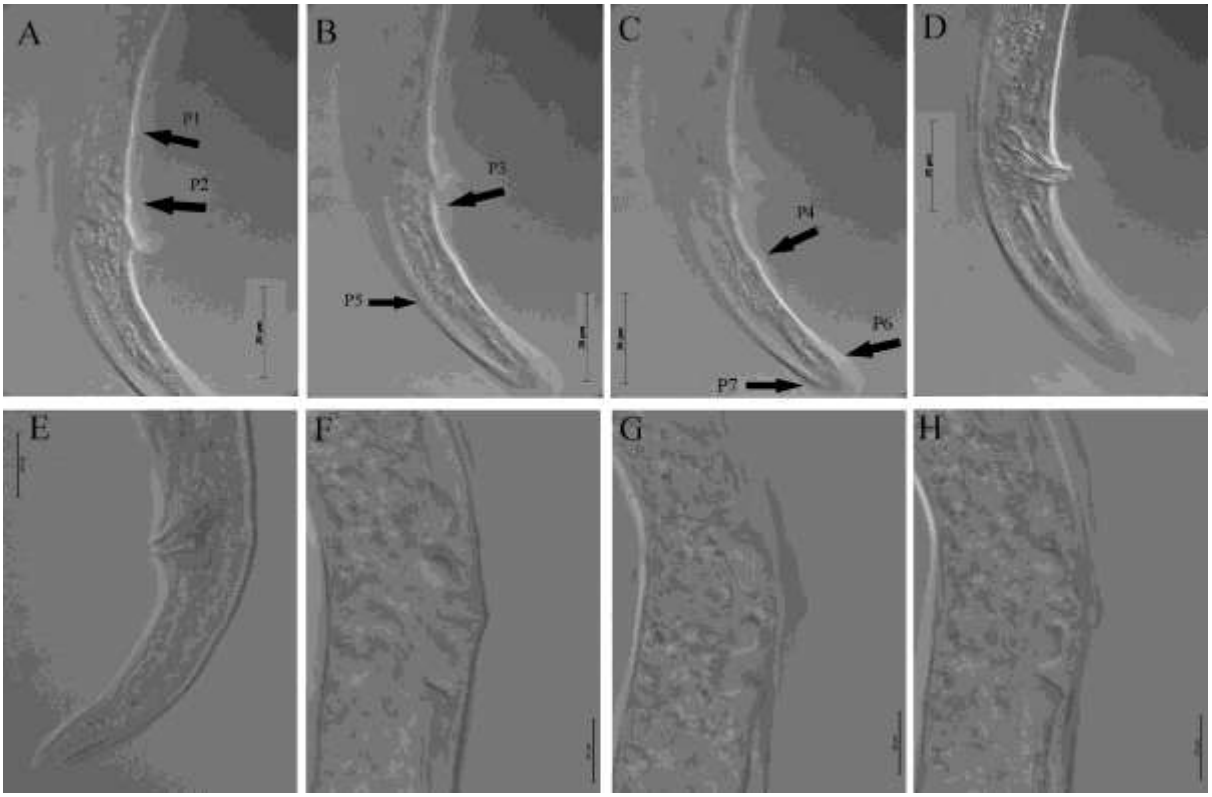

Fig. 4. Adults of *Parasitodiplogaster sycophilon*. A-C: Stomatal morphology observed on live nematodes (right lateral view); D: Systematic representation of the stoma and stomatal elements (ch = cheilostom; gym

=gymnostom; meta = metastegostom; pro/meso = pro/mesostegostom; telo = telostegostom; dpg = dorsal pharyngeal gland).

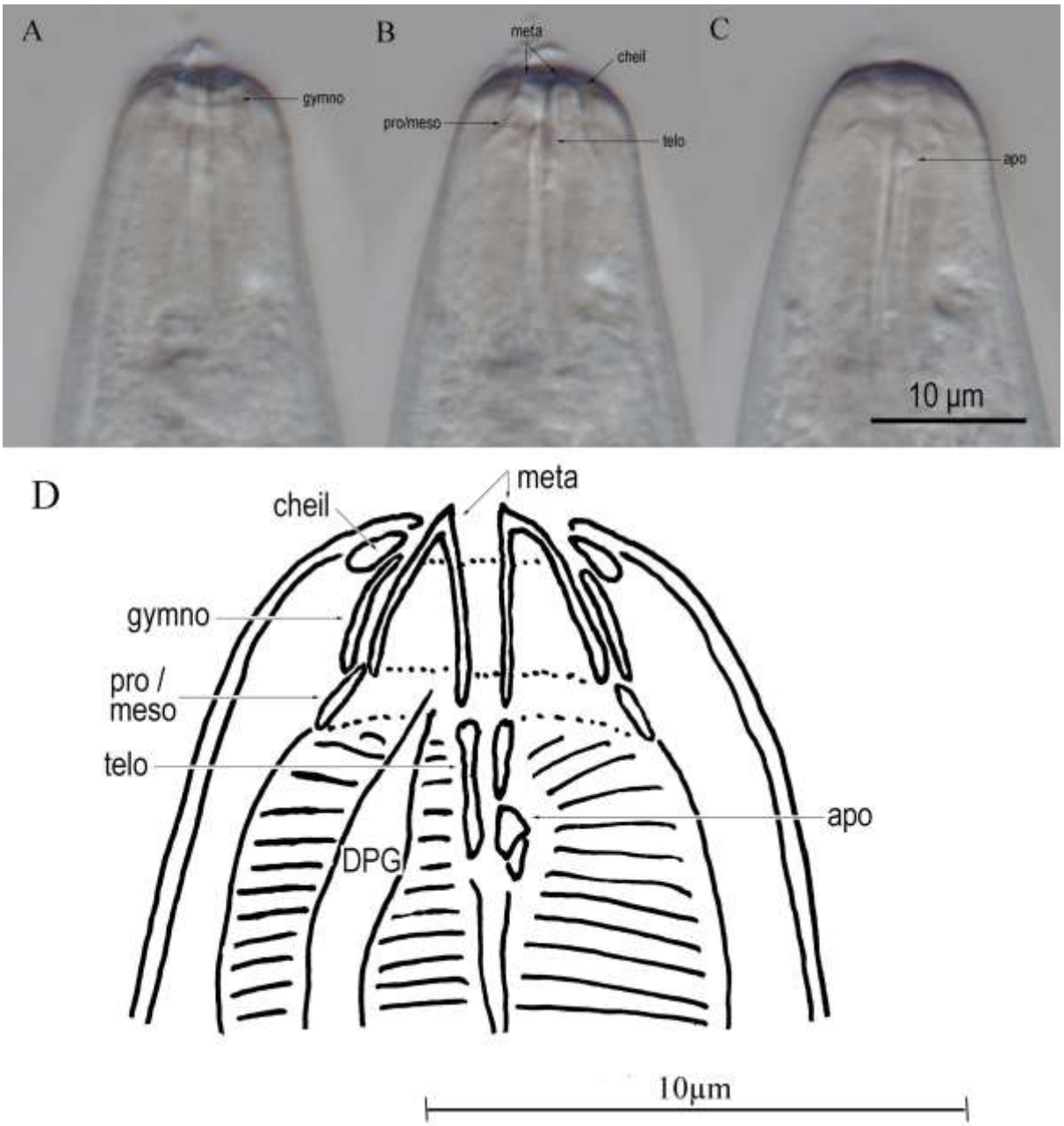

Fig.5. Photomicrographs of adults of *Parasitodiplogaster sycophilon.* A-C: Male tail region with papillae (P1-P7) indicated by arrows; D, E: Spicule and gubernaculum (ventral view); F-H: Female vulval region in different focal planes.

Table 2. *Morphometrics of* Parasitodiplogaster sycophilon *Poinar, 1979. All measurements in µ*m *and in the format: mean* ± SD *(Range).*

	Hartbeespoort Dam, South Africa		Original description	
	Male	Female	Male	Female
n	18	23	10	10
L	1330.4±396.9 (803-2092)	1395.923± 309.5 (1054-1902)	1500 (920-1890)	1500 (1260-1890)
Pharynx length	156.3±17.6 (130-171)	155±51 (144-195)	196 (160-220)	202 (170-223)
Anterior pharynx	84.8±8.9 (72-95)	88.4±6.9 (77-98)	* [2)]	* [2)]
Posterior pharynx	71.4±9.6 (56-81)	80.3±9.7 (67-97)	* [2)]	* [2)]
A/P ratio	0.84±0.06 (0.75-0.92)	0.9±0.27 (0.8-1.01)	* [2)]	* [2)]
a	20.6 ±3.8 (15.1-26.8)	20.6±3.6 (16.4-26.1)	* [2)]	* [2)]
b	8.7± 2.1 (6.1-12.3)	8.4±1.4 (6.7-11.2)	* [2)]	* [2)]
c	15.6 ± 2.5 (13.0-21.3)	12±1 (10.5-13.2)	* [2)]	* [2)]
c’	2.1 ± 0.25 (1.9-2.5)	3.2±0.44 (2.6-3.8)	* [2)]	* [2)]
T or V	73.5 ± 7 (63.5-80.3)	51.6±1.2 (49.2-53.6)	* [2)]	50 (44-56)
Stoma width	* [1)]	* [1)]	1.47 (0.9-2.65)	1.8 (1.1-2.1)
Stoma length	* [1)]	* [1)]	3.3 (3.2-4.8)	3.4 (3.0-4.2)
Maximum body diam.	63.5±7.9 (53-78)	67.4±6.85 (56-77)	55 (38-70)	59 (50-75)
Distance head to nerve ring	* [1)]	* [1)]	150 (124-176)	158 (143-189)
Excretory pore	167±12.2 (154-181)	160.5±24.3 (133-198)	173 (150-202)	171 (147-205)
Testis length	1017.3±320.7 (540-1445)	-	-	-
Spicule (line)	27.75±3.3 (21-33)	-	28 (20-35)	-
Spicule (curve)	30.5±3.5 (23-35)	-	* [2)]	-
Gubernaculum length	12±1.1 (10-13)	-	11 (8-13)	-
Gubernaculum width	5.1±0.75 (4-6)	-	* [2)]	-
Length of mucron	* [1)]	* [1)]	1.06 (0.5-2.60)	1.1 (0.8-1.6)
Cloacal or anal body diam.	38.4±6.7(27-51)	36.5±7.1 (28-52)	33 (25-49)	* [2)]
Tail length	84±14 (61-98)	115.3±18.7 (92-144)	83 (63-110)	126 (109-143)

1) not measured in new description

2) not given in the original description

DESCRIPTION

Male (from figs)

Body large, white translucent, ventrally arcuate when heat-killed. Tail region ventrally curved. Cuticle finely annulated. Lateral field not observed by light microscopy (LM). Lips almost continuous with body and weakly separated into six lip sectors, each sector bearing one small labial sensillum. Two subdorsal and two subventral dome-like cephalic papillae present slightly posterior to level of labial sensilla. Stoma with large claw-like dorsal tooth and slightly smaller right subventral claw-like tooth, both appearing to fill stomatal cavity depending upon level of stegostomatal protraction. Cheilostom and gymnostom not easily observed in fixed material with LM. In live specimens, cheilostomatal elements apparently degenerated rather than solid cuticular rings or plates, short and tapering from a narrow anterior to a wider posterior part. Cheilostom appearing as tear-shaped dots just posterior to stomatal opening. Gymnostom *ca* half diam. of cheilostom and appearing as a tube-like solid ring *ca* three times length of cheilostomatal element and bearing fine annulations. Stegostom consisting of three parts with anterior edge of pro/mesostegostom extends partly into the posterior edge of gymnostom, and posterior edge widening towards anterior pharynx. Metastegostom bearing a pair of claw-like teeth. Telostegostom narrow and almost funnel-shaped, about same length as gymnostom. Live specimens were observed with ventral apodemes at level of posterior end of telostegostom and anterior end of pharynx. Dorsal pharyngeal gland orifice observed penetrating base of dorsal tooth. Anterior pharynx (procorpus + median bulb) muscular, procorpus cylindrical, widening into slightly pyriform median bulb in lateral view. Posterior pharynx (isthmus + terminal bulb) glandular, about equal in length and form to anterior pharynx. Cardia present. Hemizonid not observed. Nerve ring large, surrounding middle of isthmus. Excretory pore opening ventrally, usually at level of terminal bulb, however, in some fixed material it was observed as anterior as median bulb. Deirids observed at level of nerve ring near basal bulb. Testis on right of intestine, reflexed or extended, spermatocytes arranged in multiple rows in anterior part of testis, in mid-testis well-developed spermatocytes arranged in single row, sperm amoeboid, *vas deferens* not clearly separated from male genital tract. Three cloacal glands at intestine/rectum junction. Spicules separate, paired with slight ventral arch and tapering distal end, dorsal and ventral limbs of lamina appearing cuticularly compartmentalised, manubrium rectangular in shape. Gubernaculum slender, *ca* one-third length of spicules in lateral view. Bursa absent. Tail

ventrally arcuate, tapering conoid, *ca* two cloacal body diam. long, with cloacal protuberance extending for about length of gubernaculum, tail tip with short blunt mucron. Five pairs of subventral or ventral genital papillae plus two subdorsal or dorsal pairs, of which one almost in lateral field. First and second pairs of papillae at 1.5 and 0.5 cloacal body diam. anterior to cloacal slit, respectively, third pair just posterior to cloacal slit, at level of gubernaculum, fourth pair one cloacal body diam. posterior to cloacal slit, fifth pair subdorsal *ca* one cloacal body diam. posterior to cloacal slit, sixth pair nearly ventral, *ca* 2.5 cloacal body diam. posterior to cloacal slit, near tail end, and seventh pair dorsally located at level of phasmid *ca* 2.5 cloacal body diam. from cloacal slit. Phasmids near tail tip, pore-like.

Female (from figs)

Body large, weakly curved dorsally when heat-killed. Cuticle finely annulated. Pharynx and stoma morphology similar to male. Ovaries amphidelphic and reflexed, in some cases antidromously (= each ovary reflexed for its entire length). Anterior reproductive tract situated on right of intestine, posterior tract on left. Anterior and posterior reproductive tracts almost identical to each other so only anterior one described here. Ovary extended, usually not reaching vulval region, oocytes arranged in multiple rows in distal third, in single file in posterior part. Oviduct serving as uterus and spermatheca, usually containing two to more than ten eggs at single or two-celled stage. Vulva protuberant, four large vaginal gland cells present. Vagina perpendicular to body surface. Vulval muscle conspicuous, filaments arranged as a quarter sector in lateral view. Rectum *ca* 0.5 anal body diam. long. Intestine-rectum junction constricted by sphincter muscle, three anal glands present. Tail weakly tapering, conoid towards a short rounded tip, small mucron present. Phasmid pore-like, near tail tip.

VOUCHER MATERIAL

Voucher material was deposited with the Nematology Collection at the University of California at Davis. The original type material by Poinar (1979) is also deposited there (slide numbers UCNC 1864/1865).

DIAGNOSIS AND RELATIONSHIPS

The nematodes described as *P. sycophilon* by Poinar (1979) were isolated from *F. burkei*, previously synonymised with *F. thonningii*, and the associated pollinating wasp *E. stuckenbergi* (Burrows & Burrows, 2003). Critical nematode morphological observation, foundress wasp identification and fig tree morphology lead to the conclusion that the *Parasitodiplogaster* species at hand is, in fact, *P. sycophilon*.

Parasitodiplogaster sycophilon is characterised by its wide and cylindrical stoma completely occupied by two large protruding teeth, mucronate tail tip of male and female and slender tail, seven pairs of genital papillae, of which one pair is subdorsally and another pair dorsally oriented, with an arrangement of <P1, P2/P3, P4, P5d, P6, P7d, Ph>, stout and complicated spicule with square manubrium, and gubernaculum thin and bent.

Based upon the stomatal structure, spicule and gubernaculum morphology and the number and arrangement of male genital papillae, *P. sycophilon* is similar to *P. popenema* and *P. citrinema*. These three species share relatively wide and stout spicules, seven pairs of genital papillae and a wide and open stoma (Poinar, 1979; Poinar & Herre, 1991). It is similar to *P. australis* in that it only has two precloacal papillae, whereas most other *Parasitodiplogaster* species have three or more pairs of papillae anterior to the cloacal slit. *Parasitodiplogaster sycophilon* is distinguished from *P. australis* by having didelphic *vs* monodelphic ovaries, number and arrangement of male genital papillae, stomatal structure, and spicule and gubernaculum shape.

Parasitodiplogaster sycophilon is distinguished from *P. citrinema* based upon the absence *vs* presence of three bristle-like setae on each lip sector, the shape of the metastegostomatal teeth, which are smaller and thinner in *P. citrinema*, and the arrangement of male genital papillae <P1, P2,/P3, P4, P5d, P6, P7d, Ph> *vs* <P1, P2, P3 / P4, P5, (P6, P7), Ph>.

Parasitodiplogaster sycophilon is distinguished from *P. popenema* by absence *vs* presence of three bristle-like setae on each lip sector, the shape of the metastegostomatal teeth, which are smaller and thinner in *P. popenema*, the form of the male and female tail tip (mucronate *vs* bluntly pointed or weakly mucronate), the nearly dorsal location of P7 in *P. sycophilon*, spicule morphology (stout spicule blade *vs* a thinner spicule blade) and male tail (slender *vs* broad immediately posterior to the cloacal slit) (Poinar & Herre, 1991; this study).

Parasitodiplogaster pharmaconema and *P. maxinema* form a monophyletic clade and have the *Ficus* section *Pharmacosycea* as host plants. The other monophyletic clade,

including *P. laevigata*, are isolates from trees of the *Ficus* section *Americana*. The singleton branch of *P. australis* is a nematode associated with the *Ficus* section *Urostigma* and the herein redescribed species *P. sycophilon* is isolated from the *Ficus* section *Galoglychia.* The current phylogeny does not resolve the placement of samples from the four subsections with respect to each other.

Discussion

Parasitodiplogaster sycophilon, in the original observation by Poinar, was described from 3% formalin-fixed material that was posted to the author, who then processed the samples into glycerin. The type specimens were observed and found to be in good condition when compared with the newly collected specimens. Morphometric differences are presented in Table 1 between the original 1979 description and the samples re-collected for this study. The mean in some cases is below that originally described, but all values for the material measured here fall within the originally described range. The most significant difference occurred for pharynx length, where the average is much lower than originally described. The range, however, still shows slight overlap. This may be due to the time at which the samples were collected. The originally described *P. sycophilon* was isolated from foundresses in which adult nematodes are generally observed, but in this study, material was isolated from sycones of various ages and, depending on the fig's developmental stage, the nematodes may have ranged from juvenile to dauer to adults. However, no dauers were observed in the material and only nematodes with developed stomas were measured, although these may have still been in the early adult stages of development.

By comparing the recollected specimens to the original descriptions, it was revealed that the stomatal morphology was misinterpreted in the original description of *P. sycophilon*. The stoma was formerly described as reduced, lacking well-defined rhabdions and teeth. In the present study we confirmed the presence of metastegostomatal teeth for *P. sycophilon*. In most of the samples that were observed the teeth were protracted and could easily be misinterpreted as thickenings of the stomatal walls rather than teeth. We observed two large teeth of almost equal size, one dorsal and one right subventral, occupying the stomatal cavity. Redescription of multiple species was necessitated due to stomatal collapse caused by the fixation techniques employed (Kanzaki *et al.*, 2010). However, the fixation method did not seem to affect other morphological features, such as the male genital papillae number which was consistent in the original description and in the redescription (Kanzaki *et al.*, 2010, 2013;

this study).

The present study resolves the question posed by Kanzaki *et al.* (2010), in their redescription of *P. citrinema* and *P. popenema*, regarding the stoma of *P. sycophilon*. The dendrogram originally published by Bartholomaeus *et al.* (2009) and extended by Kanzaki *et al.* (2010) suggests that the stoma was a relative indication of typological and possible phylogenetic similarity. The stoma of *P. sycophilon* in both the original description and in this study revealed similarities between the three species *P. sycophilon, P citrinema* and *P. popenema.* The observation of stegostomatal teeth in *P. sycophilon* should morphologically place *P. sycophilon* as being closely related to these two species, yet the phylogenetic analysis and base pair differences table reveal equal distances between *P. sycophilon* and all other previously described nematodes in *Parasitodiplogaster*.

The molecular data and arrangement of the male genital papillae suggest that *P. sycophilon* is in its own clade, a clade which is distantly related to other *Parasitodiplogaster* species. A greater number of isolates from different tree hosts need to be sequenced and included in the phylogenetic tree to elucidate the relationship of *P. sycophilon* to other species in the genus. More sequence data from other genetic loci could also help clarify the molecular inferences.

Apart from the morphological apomorphies, the molecular data highlights differences among these three species. Kanzaki *et al.* (2010) proposed the presence of four clades in *Parasitodiplogaster* based on stomatal morphology. The first proposed clade consists of *P. laevigata* specimens and the second clade includes *P. citrinema* and *P. popenema*; the third clade contains *P. maxinema* and *P. pharmaconema* whilst the forth clade solely contains *P. australis*. Figure 6 supports these four proposed nematode clades except for the placement *P. sycophilon*. Kanzaki *et al.* placed *P. sycophilon* with the *P.citrinema/P. popenema* clade based on stomatal morphology. However, using the D2/D3 segment of ribosomal LSU DNA to infer molecular phylogeny, *P. sycophilon* appears to form its own clade (Figure 6). Figure 6 also includes the nematode's host plant sections which highlight a correlation between the fig and associated nematodes. This study proposes a fifth clade composed of *P. sycophilon* isolated from *F. burkei* of the section Galoglychia. The base-pair difference table (Table 3) supports a new clade as it shows a relationship of *P. sycophilon* as distant to other Parasitodiplogaster species as *P. australis* is to isolates from the New World.

The lack of clustering of *P. sycophilon* from Africa with other nematodes is consistent with a hypothesis of geographically correlated trajectories, however more samples are required to support this finding.

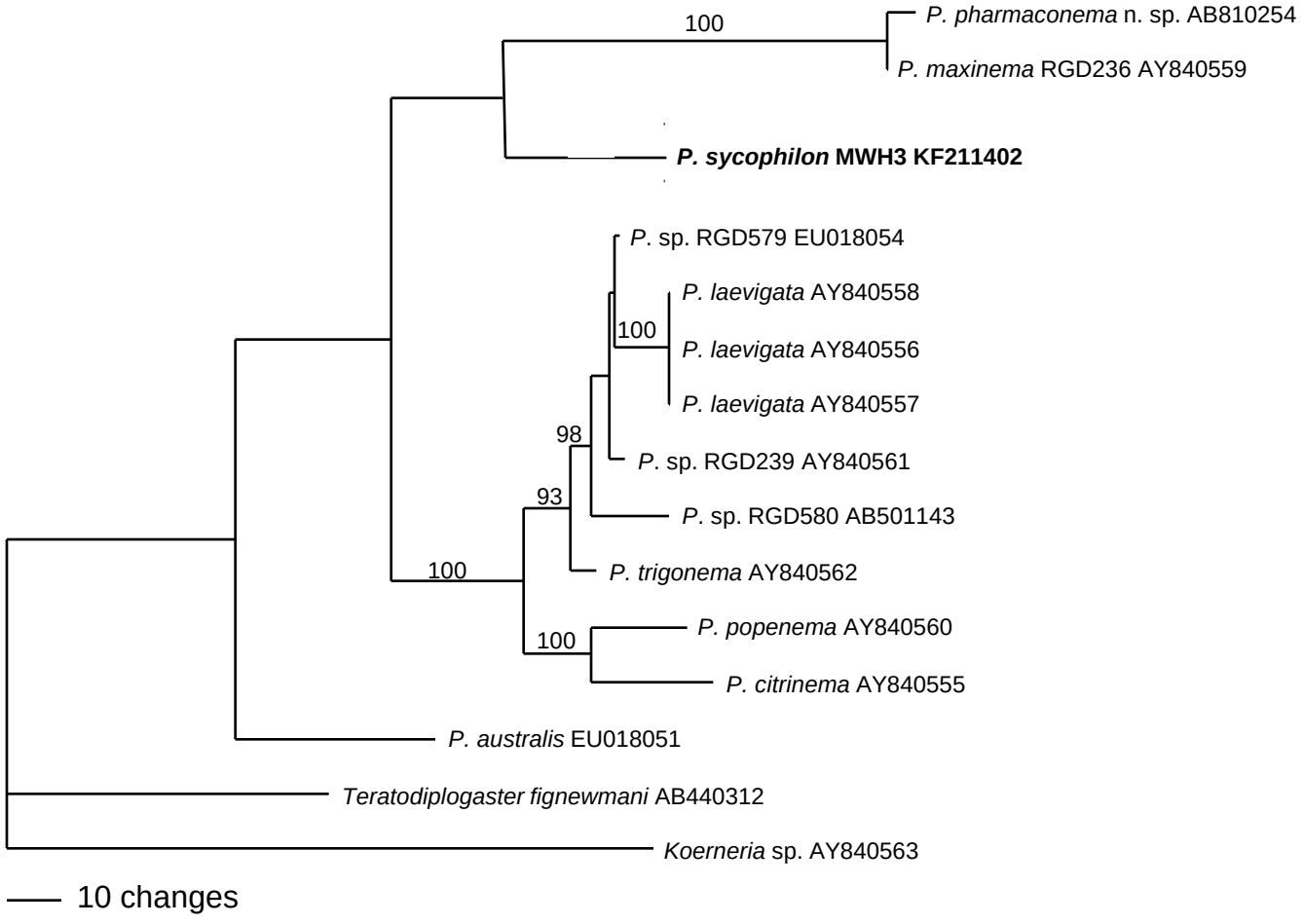

Fig. 6. Molecular phylogeny of Parasitodiplogaster spp. and Koerneria sp. Koerneria sp. is an outgroup for the genus Parasitodiplogaster. Bayesian analysis using the D2/D3 LSU ribosomal subunit including bootstrap values. The 10001st Bayesian tree inferred from D2/D3 under TrN + G model (-lnL = 3276.3745; freqA = 0.1885; freqC = 0.2184; freqG = 0.3343; freqT = 0.2588; R(a) = 1; R(b) = 2.4778; R(c) = 1; R(d) = 1; R(e) = 7.4965; R(f) = 1; Pinva = 0; Shape = 0.3644). Posterior probability values exceeding 50% are given on appropriate clades. The vertical lines to the right of species names unite species isolated from the same plant host with the Ficus section name given next to the vertical line.

Table 3. *Pairwise nucleotide differences among the D2/D3 ribosomal subunit LSU DNA of* Parasitodiplogaster *species and two outgroup samples* Teratodiplogaster *and* Koerneria. *The area below the diagonal line contains the number of nucleotide differences between the species for the 672 bp long fragment. The area above the diagonal contains the calculated p-distance value between species.*

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*P. laevigata* AY840558	-	0.02	0.02	0.04	0.12	0.02	0.06	0.07	0.11	0.16	0.17	0.177	0.232
2	*P.* sp. RGD239 AY840561	14	-	0.01	0.03	0.12	0.02	0.06	0.07	0.12	0.16	0.17	0.177	0.229
3	*P.* sp. RGD579 EU018054	11	5	-	0.03	0.12	0.02	0.06	0.06	0.11	0.16	0.16	0.170	0.222
4	*P.* sp. RGD580 AB501143	26	20	19	-	0.13	0.03	0.07	0.08	0.11	0.17	0.18	0.180	0.232
5	*P. australis* EU018051	82	83	79	85	-	0.12	0.14	0.14	0.14	0.17	0.15	0.168	0.225
6	*P. trigonema* AY840562	16	14	11	22	78	-	0.06	0.06	0.1	0.16	0.16	0.171	0.216
7	*P. popenema* AY840560p	43	40	39	44	95	38	-	0.05	0.13	0.16	0.17	0.177	0.232
8	*P. citrinema* AY840555c	44	45	42	51	93	41	34	-	0.12	0.15	0.16	0.176	0.226
9	*P. sycophilon* MWH3 KF211402	72	78	74	76	92	69	86	77	-	0.13	0.14	0.174	0.201
10	*P. maxinema* RGD236 AY840559	108	109	106	116	114	106	110	101	90	-	0.01	0.180	0.240
11	*P. pharmaconema* n. sp. AB810254	112	113	110	119	118	110	113	105	94	4	-	0.185	0.244
12	*Teratodiplogaster fignewmani* AB440312	119	119	114	121	113	115	119	118	117	121	124	-	0.234
13	*Koerneria* sp. AY840563	156	154	149	156	151	145	156	152	135	161	164	157	-

The morphological complexity of *Parasitodiplogaster*, especially in stomatal morphology, has been emphasised previously. Each new description and re-description of *Parasitodiplogaster* species broadens our understanding of the complexity and relative plasticity of feeding structures in this group. This study includes the first molecular data of an African *Parasitodiplogaster* species. With 25 known native South African *Ficus* species, a plethora of new descriptions and additional morphological manifestations in the associated nematodes can be expected. For example, *P. doliostoma* and *Teratodiplogaster martini* were recently described and give a hint of the morphological variation likely in the African fig nematode world (Kanzaki *et al.*, 2012). Hence, further morphological and molecular investigations into African *Parasitodiplogaster* species and other fig-associated nematodes are justified.

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