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# ***Teratodiplogaster variegatae* n. sp. (Nematoda: Diplogastridae) isolated from the syconia of *Ficus variegata* Blume on Ishigaki Island, Okinawa, Japan**

Natsumi KANZAKI<sup>1,\*</sup>, Gavin C. WOODRUFF<sup>1</sup> and Ryusei TANAKA<sup>2,3</sup>

<sup>1</sup>Forest Pathology Laboratory, Forestry and Forest Products Research Institute,

1 Matsunoso, Tsukuba, Ibaraki 305-0035, Japan

<sup>2</sup>Division of Parasitology, Faculty of Medicine, University of Miyazaki, Miyazaki, Miyazaki 889-1692, Japan

<sup>3</sup>Max Planck Institute for Developmental Biology, Department of Evolutionary Biology,  
Spemannstraße 37, Tübingen, Germany

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**Summary** – *Teratodiplogaster variegatae* n. sp. is described and illustrated from *Ficus variegata* on Ishigaki Island, Okinawa, Japan. *Teratodiplogaster variegatae* n. sp. is characterised by its scoop-like lip, the presence of large sac-like *receptaculum seminis* in the female gonads, and eight pairs of male genital papillae with an arrangement of (P1, P2, P3, vs, C, (P4, P5d), (P6, P7), P8d, Ph). It is distinguished from its close relatives *T. fignewmani* and *T. martini* by its stomatal morphology, spicule and gubernaculum morphology, structure of female reproductive organs, and the characteristic star-shaped appendage on the tail tip of males and females. Molecular phylogenetic analyses based upon near-full-length SSU and D2-D3 expansion segments of LSU corroborate a monophyletic origin of the genus *Teratodiplogaster*.

**Keywords** – description, fig tree, molecular, morphology, morphometrics, new species, phylogeny, taxonomy.

Fig-associated nematode species reveal multiple patterns of divergence and convergence (Herre, 1993; Giblin-Davis *et al.*, 2004; Davies *et al.*, 2010). However, these patterns have led to taxonomic difficulties. For instance, the aphelenchid genus *Schistonchus* Cobb, 1927 (Fuchs, 1937) was described based on morphological characteristics. However, molecular phylogenetic analyses separate this group into three (or more) clearly different clades, and this case is regarded as a good example of morphological convergence in plant-parasitic nematodes evolved from fungal feeders (*e.g.*, Davies *et al.*, 2010; Kanzaki *et al.*, 2014a). On the other hand, two diplogastrid genera, *Parasitodiplogaster* Poinar, 1979 and *Teratodiplogaster* Kanzaki, Giblin-Davis, Davies, Ye, Center & Thomas, 2009, which are hypothesised to be insect parasites evolved from bacteria feeder/nematode predator species, are easily distinguished by morphological characters. However, regardless of clear morphological differ-

ences, these genera are not clearly resolved by molecular phylogenetic analysis, *i.e.*, *Teratodiplogaster* is nested within the *Parasitodiplogaster* clade as a sister of the bacteria feeder/nematode predator genus, *Allodiplogaster* (Giblin-Davis *et al.*, 2006; Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2009, 2010, 2012a, 2014a). Thus, this clade can also be used to address questions regarding morphological divergence occurring in sympatric speciation.

In previous studies, Kanzaki *et al.* (2012a, 2014a) reported undescribed *Teratodiplogaster* species from *Ficus variegata* Blume from Japan and Northern Australia, although further information was not provided because of insufficient material for morphological and molecular characterisation. In the present study, a *Teratodiplogaster* species, based upon the materials obtained from Okinawa, Japan, is described and illustrated as *T. variegatae* n. sp. and its molecular profile presented.

\* Corresponding author, e-mail: nkanzaki@affrc.go.jp

## Materials and methods

### NEMATODE ISOLATION AND MORPHOLOGICAL OBSERVATION

The materials were collected from syconia of *F. variegata* on May 28, 2014 from Ishigaki Island, Okinawa, Japan (GPS: 24°37'57"N, 124°15'58"E, 78.0 m a.s.l.). The collected syconia were dissected such that the surface tissue of each syconium was peeled with a scalpel to remove and reduce fig latex, and the peeled syconium was then cut into small pieces in sterilised water in a plastic Petri dish. Nematodes that crawled out from dissected syconia were hand-picked and collected in sterilised water. Some of the collected nematodes were placed into nematode digestion solution (NDS: Kikuchi *et al.*, 2009; Tanaka *et al.*, 2012) or DESS (Yoder *et al.*, 2006) for molecular profiling and phylogenetic analyses, and the others were killed by heat (60°C, 1 min), fixed in TAF (triethanolamine:formalin:distilled water = 2:7:91), processed through a glycerin-ethanol series using a modified Seinhorst's method (Minagawa & Mizukubo, 1994) and mounted in glycerin using the Maeseneer & d'Herde method (Hooper, 1986). These glycerin-mounted materials were used as the type specimens, and morphometric values were taken from them.

Drawings and measurements of nematodes were done with the aid of a camera lucida and a stage micrometer. Morphological terminologies employed for the previously described *Teratodiplogaster* species (see Kanzaki *et al.*, 2009, 2012a) were used to describe general morphology.

### MOLECULAR CHARACTERISATION AND PHYLOGENY

Nematode DNA materials prepared with NDS were used for molecular profiles and phylogenetic analyses. The DNA base sequences of partial ribosomal DNA (= *ca* 1.7 kb of SSU and *ca* 0.7 kb of D2-D3 extension segments of LSU) were determined following the methods of Kanzaki & Futai (2002) and Ye *et al.* (2007) and deposited in the GenBank database with accession numbers LC004467 and LC004468. The molecular phylogenetic status of *T. variegatae* n. sp. was inferred based on the SSU and D2-D3 LSU ribosomal RNA genes. The sequences obtained were compared to those of other diplogastrid species belonging to the *Allodiplogaster* Paramonov & Sobolev in Skrjabin, Shikobalova, Sobolev, Paramonov & Sudarikov, 1954 + *Teratodiplogaster* + *Parasitodiplogaster* clade and outgroup taxa, *Koerneria luziae* (Körner, 1954) Meyl, 1960 deposited in the Gen-

Bank database, as *Teratodiplogaster* and *Parasitodiplogaster* are known to form a monophyletic clade with *Allodiplogaster*, where *Allodiplogaster* is always inferred as a sister of *Teratodiplogaster* + *Parasitodiplogaster* (Kanzaki *et al.*, 2014b, c, d; Wöhr *et al.*, 2014).

The molecular phylogenetic analyses were conducted based on SSU and D2-D3 LSU using Bayesian analyses. The compared sequences were aligned separately using MAFFT (Katoh *et al.*, 2002; available online at <http://align.bmrb.kyushu-u.ac.jp/mafft/software/>) and the base substitution model was determined for each gene locus using MODELTEST version 3.7 (Posada & Crandall, 1998) under the AIC model selection criterion, and GTR + I + G and TrN + G were selected for SSU and D2-D3 LSU, respectively. The Akaike-supported model, log likelihood ( $\ln L$ ), Akaike information criterion values, proportion of invariable sites, gamma distribution shape parameters and substitution rates were applied to the phylogenetic analysis. Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001); four chains were run for  $4 \times 10^6$  generations. Markov chains were sampled at intervals of 100 generations (Larget & Simon, 1999). Two independent runs were performed and after confirming the convergence of runs and discarding the first  $2 \times 10^6$  generations as burn in, the remaining topologies were used to generate a 50% majority-rule consensus tree.

## Results

### *Teratodiplogaster variegatae*\* n. sp. (Figs 1-7)

#### MEASUREMENTS

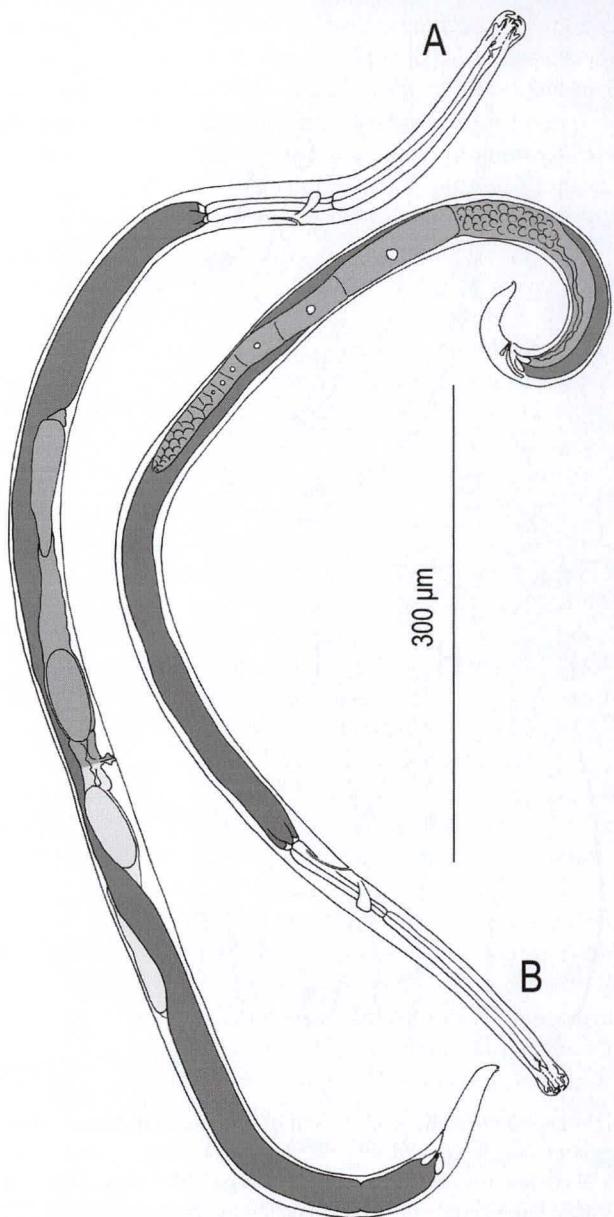
See Table 1.

#### DESCRIPTION

##### Adults

Medium-sized (0.8-1.3 mm) species of diplogastrid. Body cylindrical, slender. Cuticle thick, strongly annulated except for lip region. Lateral field present, narrow, number of the lines (incisures) not confirmed. Deirid present on lateral field at level of basal bulb, but not clearly observed probably because of material condition.

\* The new species is named after the species epithet of its host tree, *Ficus variegata*.



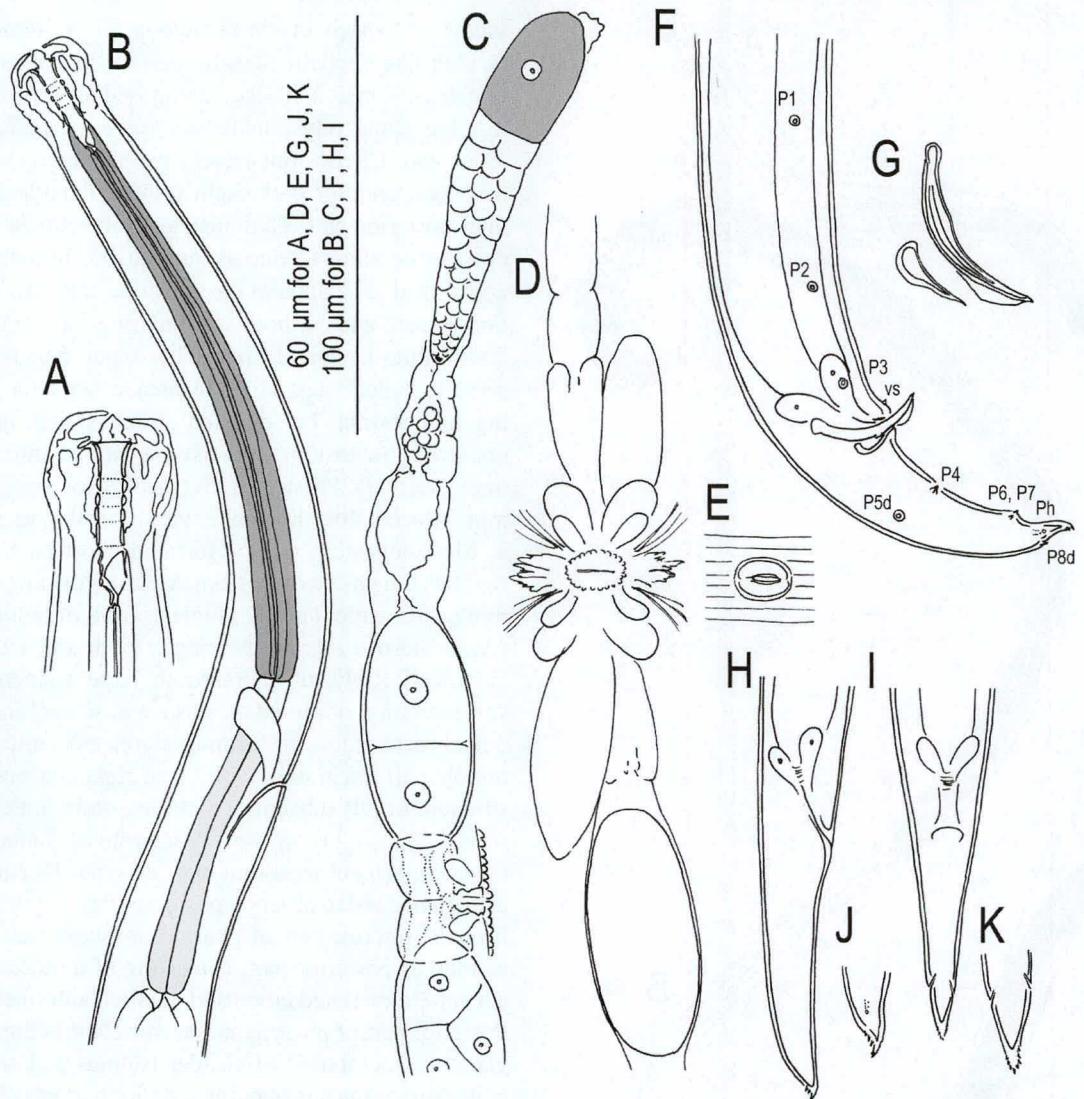
**Fig. 1.** *Teratodiplogaster variegatae* n. sp. A: Entire female, right lateral view; B: Entire male, left lateral view.

Lip expanded to form a large scoop-like structure. Both right and left lateral lips thin, membranous, shaped as a half-circle, with small oval-shaped extension. Two (right and left) subventral lips fused to form a large scoop-like membrane consisting of a large spoon-like central region and two small fin-like triangular flaps on either side. Dorsal lips forming a structure mirroring ventral lips. Labial sensilla not observed, probably embedded into each lip

sector or located inside the scoop. Four small setiform or club-like cephalic sensilla present in both males and females. Stoma tube-like, composed of seven sections that are tentatively labelled as sect1 to sect7 from anterior end. Cheilostom (sect1) visibly sclerotised, short, tube-like, anterior part slightly more flexible and wider than posterior part. Gymnostom short, cuticle tube-like, dorsal side almost same as ventral side in length. Gymnostomat tube apparently separated into two clear sections (sect2 and 3), both sections ring-like, appearing as fractal dots in lateral view. This separation is hypothesised to reflect separation of arcade syncytia surrounding gymnostom, but detailed structure was not clearly observed. Stegostom tube-like, separated into four sections (sect4-7). Prostegostom (sect4) short, ring-like, forming fractal dots in lateral view, similar to sect2 and 3. Mesostegostom (sect5) forming short cuticular tube, twice as long as prostegostom. Metastegostom (sect6) forming short cuticular tube, same as, or a little longer than, pro + mesostegostom, bearing a tooth and a thick cuticular wall. Right subventral tooth, large, triangular, transverse striation observed on surface in several individuals; dorsal metastegostom forming a concave cuticular plate, middle part integrating with tip of right subventral tooth, structure of left subventral metastegostom not clearly observed. Telostegostom (sect7) sclerotised, funnel-shaped, connecting metastegostom and pharynx. Dorsal pharyngeal gland hard to observe, position of its opening not confirmed. Anterior part of pharynx muscular, ca 1.8 times as long as posterior part, consisting of a muscular cylinder (procorpus) and elongated median bulb (metacorpus). Posterior part of pharynx not as muscular as anterior part, glandular, composed of slender isthmus and small basal bulb. Nerve ring surrounding anterior part of isthmus. Excretory pore at level of middle to posterior end of isthmus. Cardia present, probably open, but not well developed. Deirid not confirmed probably because of material condition.

#### Male

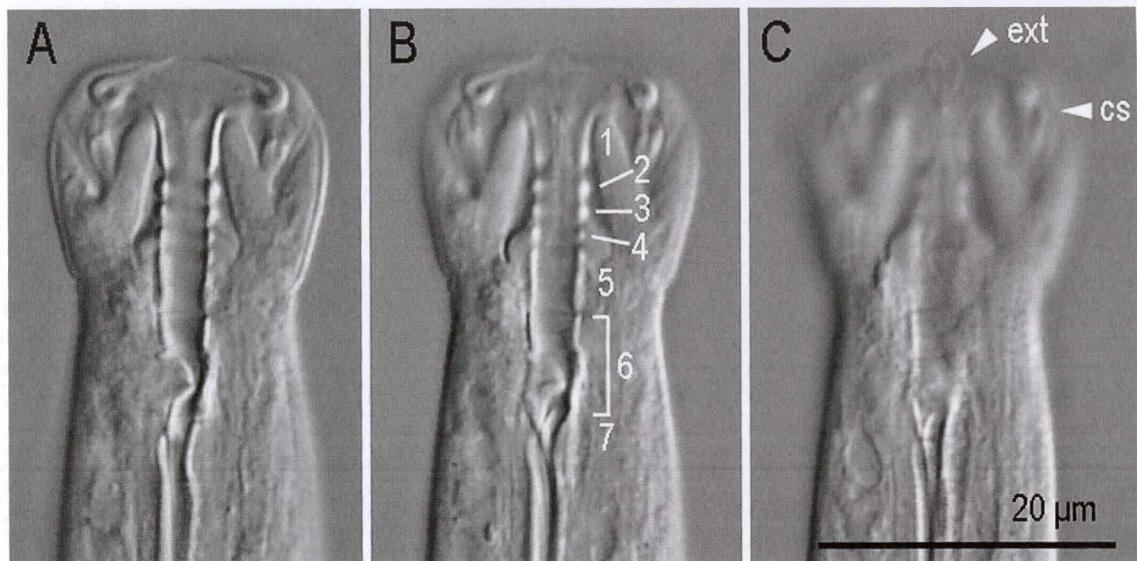
Body straight except for tail region, which is strongly arcuate in fixed specimens. Testis single, usually outstretched. Spermatocytes small, arranged irregularly as single or double rows around anterior end, then becoming larger and consistently arranged in double rows, and finally in a single row. Posterior part of testis, ca four cloacal body diam. (CBD) long, forming *vas deferens*, many mature sperm packed between well developed large spermatocyte and *vas deferens*. Spicule weakly arcuate, consisting of small and rounded manubrium with arcuate



**Fig. 2.** *Teratodiplogaster variegatae* n. sp. A: Lip region of female, right lateral view; B: Neck region of female, right lateral view; C: Anterior gonad branch of female, right lateral view; D: Vulval region of female, ventral view; E: Vulval opening, ventral view; F: Male tail, right lateral view (d = dorsal; P + number = papilla; Ph = phasmid; vs = single mid-ventral papilla); G: Spicule and gubernaculum, right lateral view; H: Female tail, right lateral view; I: Female tail, ventral view; J: Female tail tip, right lateral view; K: Female tail tip, ventral view.

dorsal and ventral limbs, a low membranous keel-like extension present on distal third of dorsal limb. Gubernaculum short with rounded anterior part and pointed posterior (distal) end in lateral view. Eight pairs of genital papillae present, with an arrangement of (P1, P2, P3, vs, C, (P4, P5d), (P6, P7), P8d, Ph), where 'vs' denotes the ventral single papilla, 'C' denotes the cloacal aperture, and 'd' refers to the dorsally or laterally located and closely associated papillae which are enclosed in parentheses. First

pair (P1) far anterior to cloacal aperture, i.e., more than cloaca-tail tip length, or three CBD, second pair (P2) at midpoint between P1 and cloacal aperture (= ca 1.5 CBD anterior to cloacal aperture); third pair (P3) ca one CBD posterior to P2 (= ca 0.5 CBD anterior to cloacal aperture); fourth pair (P4) and fifth pair (subdorsally directed P5d) almost same level, slightly less than 1.0 CBD posterior to cloacal slit; sixth and seventh pair (P6, P7), obviously smaller than other six pairs, close



**Fig. 3.** Lip region of *Teratodiplogaster variegatae* n. sp. A-C: Female in different focal planes. Stomatal sections (1-7), oval-shaped extension (ext) and cephalic sensilla (cs) are indicated. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

to each other, *ca* 2.5 CBD posterior to cloacal aperture (or 0.5 CBD anterior to tail tip); eighth pair (subdorsally located P8d), slightly posterior to P6/P7. Phasmids lateral, small, difficult to observe, midway between P6/P7 and tail tip. Three large anal gland cells (two ventral, one dorsal) observed in lateral view. Tail broad, strongly ventrally arcuate, smoothly tapering to tail tip. Tail terminus with a small star-shaped appendage.

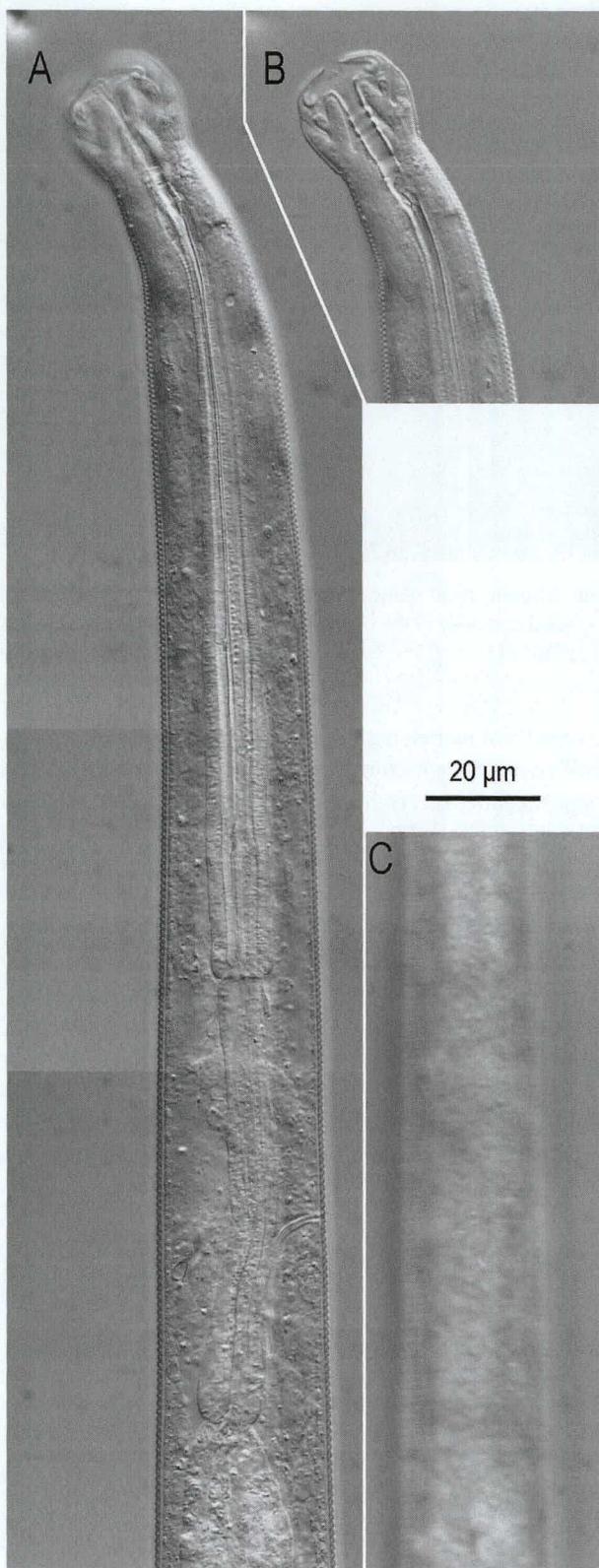
#### Female

Gonads paired. Anterior ovary to right of intestine, posterior ovary to left. Anterior and posterior gonads with similar morphology, thus anterior gonad is described here from anterior end. Ovary reflexed its total length (= antitidromous), oocytes arranged in multiple rows in distal half of ovary and then in single file. Largest (most developed) oocyte showing granular texture, darker than other oocytes in colour. Oviduct a slender tube, posterior part clearly expanded and serving as spermatheca, containing rounded (amoeboid) sperm. Posterior part of oviduct (spermatheca) formed by large cells, connected to crustaformeria-like tissue formed by large cells. Crustaformeria-like tissue connecting spermatheca and uterus. Uterus with thick and dense wall, sometime containing one or two well developed egg(s). Short and rounded sac-like structure, possibly a *receptaculum seminis*, observed in dorsal and ventral view on left side of

oviduct and uterus, but structure difficult to confirm in lateral view. This structure also present in posterior gonad on opposite side, *i.e.*, right of oviduct and uterus. Four small vaginal glands and four large rounded cells present. Large cells assumed to function as valve separating uterus and vagina. Vagina perpendicular to body surface. Vulva protuberant, sclerotised, circular in ventral view, without any flap apparatus at vulval opening. Rectum *ca* one anal body diam. long, sphincter muscle present but not conspicuous. Three large anal gland cells (two ventral, one dorsal) observed. Prerectum-like constriction observed at distal part of intestine, but could be an artefact as it was observed in only one individual out of ten observed, and was not clear in other individuals. Anus dome-shaped slit, not protuberant. Tail broad, smoothly tapering to short and conical distal end. Tail terminus with star-shaped appendage visible in ventral view, appendage appearing as three dorsally orientated hairs in lateral view. Phasmids small, close to tail terminus.

#### TYPE HOST AND LOCALITY

Isolated from the figs (syconia) of *Ficus variegata* collected at Ishigaki Island, Okinawa, Japan (GPS: 24°37'57"N, 124°15'58"E, 78.0 m a.s.l.) on May 28, 2014. Besides, the type materials (type locality).



#### OTHER LOCALITIES

Also isolated from Ishigaki and Iriomote Islands during a field survey conducted during 2013 (Kanzaki *et al.*, 2014a).

#### TYPE MATERIAL

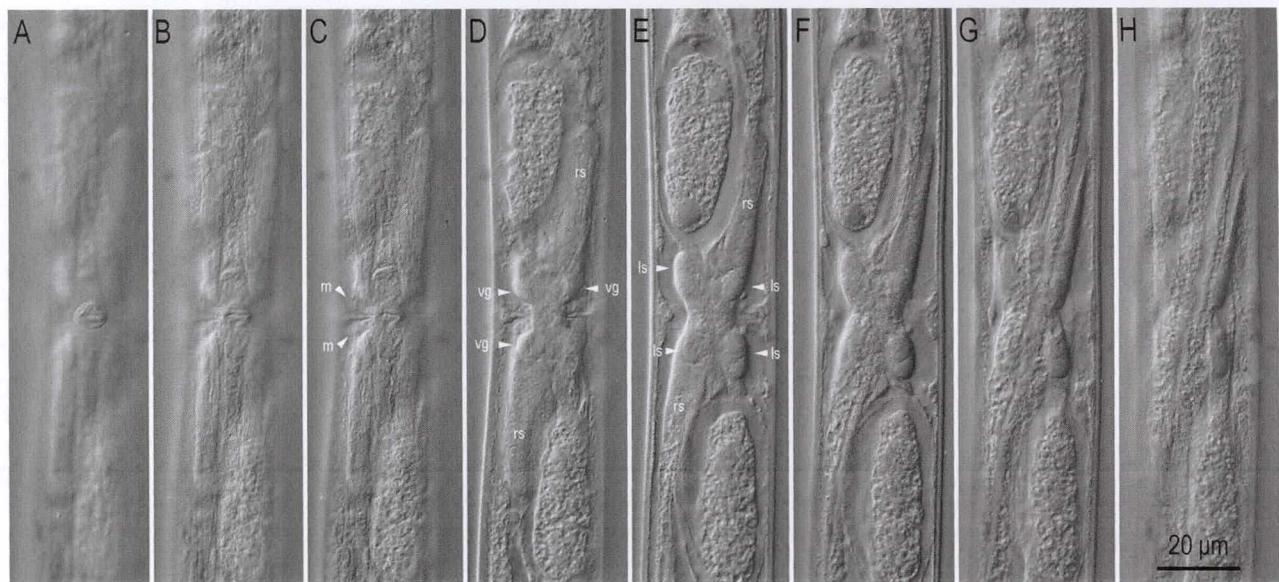
Holotype male, four paratype males and five paratype females deposited in the USDA Nematode Collection, Beltsville, MD, USA, and five paratype males and five paratype females deposited in the Forest Pathology Laboratory Collection, Forestry and Forest Products Research Institute, Tsukuba, Japan. Besides the type materials, several voucher specimens from the type locality are deposited at the Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL, USA.

#### DIAGNOSIS AND RELATIONSHIPS

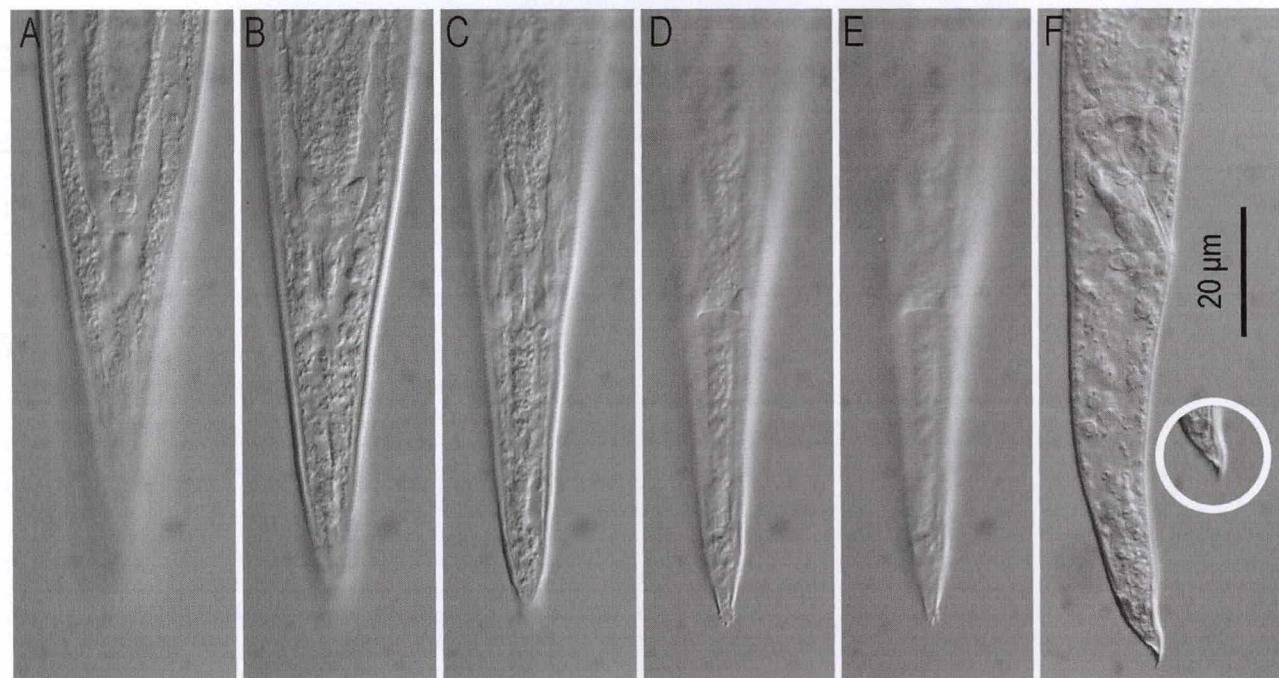
Besides its generic characters (*i.e.*, scoop-like lip morphology, long stoma with large triangular right subventral tooth) the new species is characterised by the female gonad composition (*i.e.*, possessing a sac-like *receptaculum seminis*), male spicules and gubernaculum (*i.e.*, weakly arcuate spicules with small and rounded manubrium and membranous dorsal extension or keel), gubernaculum with rounded anterior part and pointed distal end, eight pairs of male genital papillae with an arrangement of (P1, P2, P3, vs, C, (P4, P5d), (P6, P7), P8d, Ph), and star-shaped appendage at the tail tip of males and females.

*Teratodiplogaster* currently contains only two species, *T. fignewmani* Kanzaki, Giblin-Davis, Davies, Ye, Center & Thomas, 2009 and *T. martini* Kanzaki, Giblin-Davis, Davies & Center, 2012a. *Teratodiplogaster variegatae* n. sp. is distinguished from *T. fignewmani* by the shorter body length of both male (854–1283 vs 1850–2700 μm) and female (805–1173 vs 2160–3895 μm), body proportion of both male ( $a = 29.9\text{--}47.7$  vs  $52.9\text{--}75.9$ ) and female ( $a = 26.1\text{--}45.6$  vs  $59.0\text{--}92.0$ ), stomatal morphology (anteriorly widened tube-like cheilostom, four-sectioned gymnostom and stegostom without subventral denticle vs anteriorly narrowed tube-like cheilostom, gymnostom with-

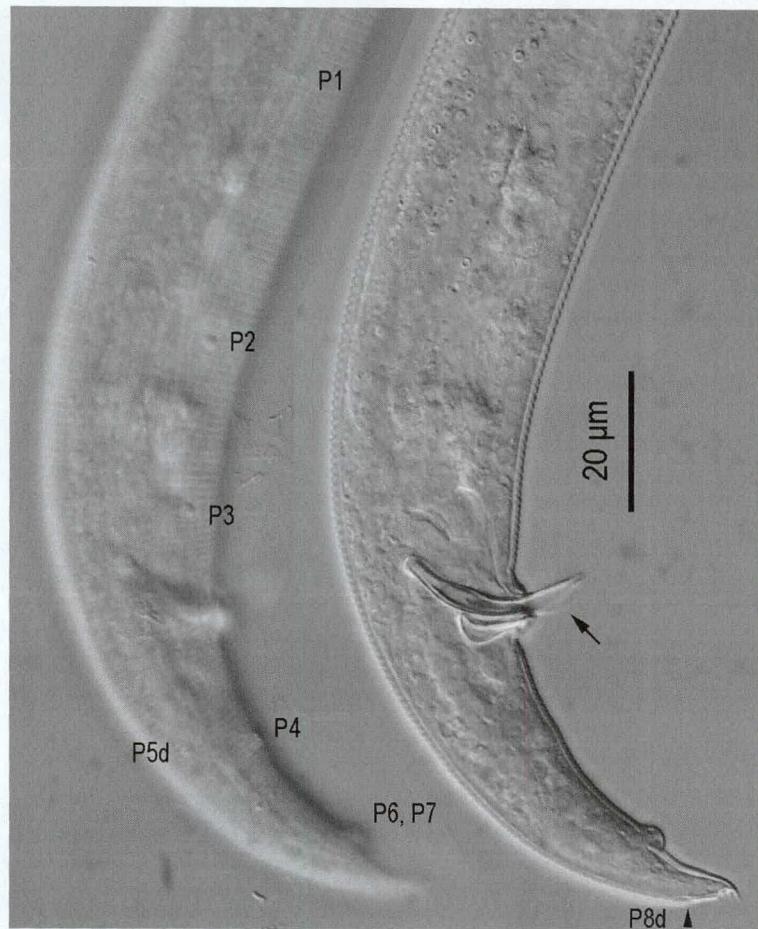
**Fig. 4.** Neck region of female *Teratodiplogaster variegatae* n. sp. A: Entire neck region; B: Anterior end in different focal plane; C: Body surface. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.



**Fig. 5.** Vulval region of *Teratodiplogaster variegatae* n. sp. in different focal planes. A-H: Different focal planes, ventral view. A: Vulval opening; B, C: Vagina sphincter muscle (m); D-H: Vaginal glands (vg), *receptaculum seminis* (rs) and large-celled structure (ls). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.



**Fig. 6.** Female tail of *Teratodiplogaster variegatae* n. sp. A-E: Ventral view in different focal plane; F: Right lateral view of entire tail and tip (circled). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.



**Fig. 7.** Male tail region of *Teratodiplogaster variegatae* n. sp. in two different focal planes. Genital papillae are marked as 'P + number'; the spicule keel is indicated by an arrow. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

**Table 1.** Morphometrics of *Teratodiplogaster variegatae* n. sp. and other *Teratodiplogaster* spp. All measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  s.d. (range).

| Character | <i>Teratodiplogaster variegatae</i> n. sp. |                               |                               | <i>T. fignewmani</i>          |                                | <i>T. martini</i>             |                               |
|-----------|--|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
|           | Male                                       |                               | Female                        | Male                          | Female                         | Male                          | Female                        |
|           | Holotype                                   | Paratypes                     | Paratypes                     | Paratypes                     | Paratypes                      | Paratypes                     | Paratypes                     |
| n         | —  | 9                             | 10                            | 10                            | 11                             | 10                            | 13                            |
| L         | 1093                                       | 1019 $\pm$ 117<br>(854-1283)  | 965 $\pm$ 120<br>(805-1173)   | 2119 $\pm$ 311<br>(1850-2700) | 2705 $\pm$ 636<br>(2160-3895)  | 2171 $\pm$ 267<br>(1825-2650) | 2253 $\pm$ 325<br>(1750-3100) |
| a         | 40.9                                       | 40.9 $\pm$ 4.6<br>(29.9-47.4) | 30.7 $\pm$ 5.8<br>(26.1-45.6) | 67.8 $\pm$ 6.6<br>(52.9-75.9) | 79.9 $\pm$ 10.6<br>(59.0-92.0) | 62.6 $\pm$ 4.6<br>(52.6-70.0) | 67.1 $\pm$ 6.9<br>(54.5-79.5) |
| b         | 4.6  | 4.4 $\pm$ 0.4<br>(3.8-5.4)    | 4.2 $\pm$ 0.3<br>(3.8-4.9)    | 9.1 $\pm$ 1.3<br>(7.8-11.7)   | 11.0 $\pm$ 1.5<br>(9.5-13.5)   | 8.2 $\pm$ 0.5<br>(7.7-9.3)    | 8.1 $\pm$ 0.7<br>(6.7-9.3)    |
| c         | 17.0                                       | 16.9 $\pm$ 1.4<br>(15.0-19.7) | 18.2 $\pm$ 1.2<br>(17.0-19.9) | 19.4 $\pm$ 2.6<br>(16.9-26.0) | 13.0 $\pm$ 1.9<br>(10.2-16.4)  | 21.6 $\pm$ 2.4<br>(18.1-25.0) | 19.4 $\pm$ 2.9<br>(15.9-26.3) |

**Table 1.** (Continued.)

| Character                                       | <i>Teratodiplogaster variegatae</i> n. sp. |                           |                           | <i>T. fignewmani</i>      |                           | <i>T. martini</i>         |                           |
|---|--|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|   | Male                                       |                           | Female                    | Male                      | Female                    | Male                      | Female                    |
|   | Holotype                                   | Paratypes                 | Paratypes                 | Paratypes                 | Paratypes                 | Paratypes                 | Paratypes                 |
| c'  | 3.1  | 3.1 ± 0.1<br>(3.0-3.4)    | 3.5 ± 0.2<br>(3.2-3.8)    | 5.1 ± 0.6<br>(3.4-5.7)    | 9.3 ± 1.8<br>(6.8-11.9)   | 4.2 ± 0.4<br>(3.5-4.9)    | 5.8 ± 0.6<br>(4.8-7.3)    |
| T or V  | 51.3                                       | 40.2 ± 6.2<br>(29.8-51.3) | 59.4 ± 1.0<br>(58.3-61.1) | 61.9 ± 9.2<br>(51.9-83.7) | 50.0 ± 1.5<br>(46.2-52.2) | 69.1 ± 6.8<br>(58.5-80.6) | 50.5 ± 1.7<br>(47.8-53.3) |
| Max. body diam.                                 | 25   | 25 ± 4.1<br>(21-35)       | 32 ± 3.4<br>(24-35)       | 32 ± 8.2<br>(26-51)       | 35 ± 13.0<br>(25-66)      | 35 ± 5.5<br>(29-45)       | 34 ± 5<br>(25-44)         |
| Stoma diam.                                     | 4.8  | 4.4 ± 0.6<br>(3.4-5.3)    | 4.2 ± 0.6<br>(3.4-4.8)    | n/a                       | n/a                       | 5.0 ± 0.8<br>(4.0-6.0)    | 5.0 ± 0.9<br>(4.0-7.0)    |
| Stoma length                                    | 21   | 22 ± 1.2<br>(20-23)       | 22 ± 1.1<br>(20-24)       | 23 ± 1.4<br>(20-25)       | 23 ± 2.4<br>(20-28)       | 26 ± 3.4<br>(21-30)       | 27 ± 5.7<br>(15-34)       |
| Anterior pharynx length                         | 135  | 130 ± 6.8<br>(120-142)    | 125 ± 7.3<br>(115-138)    | 126 ± 8.0<br>(112-134)    | 139 ± 24<br>(102-178)     | 168 ± 28<br>(140-240)     | 174 ± 17<br>(150-200)     |
| Posterior pharynx length                        | 75   | 74 ± 4.8<br>(63-80)       | 76 ± 5.1<br>(70-85)       | 81 ± 7.3<br>(68-92)       | 82 ± 14<br>(43-98)        | 73 ± 10<br>(50-85)        | 75 ± 17<br>(50-110)       |
| Ratio of anterior and posterior pharynx         | 1.8  | 1.8 ± 0.1<br>(1.6-2.0)    | 1.7 ± 0.1<br>(1.5-1.7)    | 1.6 ± 0.1<br>(1.4-1.7)    | 1.7 ± 0.3<br>(1.4-2.4)    | n/a                       | n/a                       |
| Nerve ring from anterior end                    | 185  | 177 ± 10<br>(162-189)     | 172 ± 10<br>(156-188)     | 188 ± 13<br>(171-214)     | 202 ± 33<br>(143-252)     | n/a                       | n/a                       |
| Nerve ring from posterior end of metacorpus     | 22   | 19 ± 5.1<br>(13-28)       | 20 ± 4.9<br>(11-27)       | n/a                       | n/a                       | n/a                       | n/a                       |
| Excretory pore from anterior end                | 210  | 190 ± 12<br>(167-210)     | 186 ± 13<br>(170-206)     | 217 ± 19<br>(202-261)     | 235 ± 49<br>(166-321)     | n/a                       | 228 ± 17<br>(206-252)     |
| Excretory pore from posterior end of metacorpus | 47   | 32 ± 7.5<br>(18-47)       | 34 ± 7.9<br>(17-46)       | n/a                       | n/a                       | n/a                       | n/a                       |
| Testis length                                   | 408  | 408 ± 71<br>(327-561)     | —                         | 1334 ± 409<br>(1050-2260) | —                         | 1493 ± 168<br>(1225-1750) | —                         |
| Anterior ovary length                           | —  | —                         | 76 ± 11<br>(58-93)        | —                         | 294 ± 174<br>(150-630)    | —                         | 233 ± 44<br>(170-325)     |
| Posterior ovary length                          | —  | —                         | 85 ± 17<br>(65-115)       | —                         | 268 ± 141<br>(160-560)    | —                         | 230 ± 54<br>(130-350)     |
| Anal or cloacal body diam.                      | 21   | 19 ± 1.8<br>(16-21)       | 15 ± 1.3<br>(13.5-17.5)   | 22 ± 3.3<br>(19-30)       | 23 ± 6.4<br>(17-35)       | 24 ± 2.8<br>(21-29)       | 20 ± 2.9<br>(17-28)       |
| Tail length                                     | 64   | 60 ± 4.5<br>(51-65)       | 53 ± 4.7<br>(46-59)       | 110 ± 11<br>(98-136)      | 207 ± 27<br>(163-256)     | 101 ± 8.7<br>(88-116)     | 118 ± 13<br>(92-138)      |
| Spicule length <sup>1)</sup>                    | 30   | 27 ± 2.4<br>(24-30)       | —                         | 27 ± 2.1<br>(25-32)       | —                         | 25 ± 3.6<br>(18-28)       | —                         |
| Spicule length <sup>2)</sup>                    | 24   | 23 ± 2.0<br>(20-26)       | —                         | 24 ± 2.0<br>(23-29)       | —                         | 24 ± 3.8<br>(17-30)       | —                         |
| Gubernaculum length                             | 11.5                                       | 11 ± 0.7<br>(10-12)       | —                         | 15.0 ± 1.0<br>(13-16)     | —                         | n/a                       | —                         |

<sup>1)</sup> Curved along arc from the tip of manubrium to distal end.<sup>2)</sup> Manubrium tip to distal end measured in a straight line.

n/a – data not given in original description.

out clear section and stegostom with subventral denticles), male sperm amoeboid vs lemon or almond-shaped, spicule shape arcuate with rounded manubrium and low dorsal keel *vs* with small manubrium and half-circle-shaped spicule, gubernaculum with rounded anterior end and pointed distal end *vs* narrow and flattened in lateral view, arrangement of genital papillae (P1, P2, P3, *vs*, C, P4, P5d, P6d, Ph, (P7, P8)) *vs* (P1, P2, P3, *vs*, C, (P4, P5d), (P6, P7), P8d, Ph), female gonad morphology (presence of sac-like *receptaculum seminis* and absence of clear sphincter structure between uterus and spermatheca *vs* absence of sac-like *receptaculum seminis* and presence of clear sphincter structure between uterus and spermatheca), tail shape of both male (short and conical *vs* long and slender; length = 51-65 *vs* 98-136  $\mu\text{m}$ ;  $c' = 3.0\text{-}3.4$  *vs* 3.4-5.7) and female (short and conical *vs* long and slender; length = 46-59 *vs* 163-256  $\mu\text{m}$ ;  $c' = 3.2\text{-}3.8$  *vs* 6.8-11.9), and tail tip appendage of both male and female being star-shaped *vs* with a blunt mucron (Kanzaki *et al.*, 2009). In addition to morphological (typological) characters, host association (habitat) (*F. variegata* *vs* *F. racemosa* L.) and some other morphometric values (Table 1) can be used to distinguish these two species (Kanzaki *et al.*, 2009).

*Teratodiplogaster variegatae* n. sp. seems close to *T. martini* as both have a *receptaculum seminis*, a feature not reported in *T. fignewmani*. However, the new species is distinguished from *T. martini* by the shorter body length of both male (854-1283 *vs* 1825-2650  $\mu\text{m}$ ) and female (805-1173 *vs* 1750-3100  $\mu\text{m}$ ), body shape of both male ( $a = 29.9\text{-}47.7$  *vs* 52.6-70.0) and female ( $a = 26.1\text{-}45.6$  *vs* 54.5-79.5), the morphology of the stegostom (with *vs* without clear sections in pro + mesostegostom), spicule shape arcuate with rounded manubrium and low dorsal keel *vs* short and arcuate without keel-like extension, gubernaculum with rounded anterior end and pointed distal end *vs* L-shaped in lateral view, the arrangement of the genital papillae (P1, P2, P3, *vs*, C, P4, P5d, P6d, Ph, (P7, P8)) *vs* (P1, P2, P3, *vs*, C, P4d, P5, (P6, P7), P8d, Ph), female gonad morphology (presence *vs* absence of sac-like large *receptaculum seminis*), the tail shape of both male (short and conical *vs* long and conical; length = 51-65 *vs* 88-116  $\mu\text{m}$ ;  $c' = 3.0\text{-}3.4$  *vs* 3.5-4.9) and female (short and conical *vs* long and slender; length = 46-59 *vs* 92-138  $\mu\text{m}$ ;  $c' = 3.2\text{-}3.8$  *vs* 4.8-7.3), and tail tip appendage of both male and female (star-shaped *vs* with blunt mucron) (Kanzaki *et al.*, 2012a). In addition to morphological (typological) characters, the host association of *F. variegata* *vs* *Ficus* sp. (the African species is probably *F. sur* Forssk.) and some other

morphometric values (Table 1) also distinguish these two species (Kanzaki *et al.*, 2012a).

## MOLECULAR PROFILES AND PHYLOGENY

*Teratodiplogaster variegatae* n. sp. forms a well-supported clade (posterior probability for Bayesian Inference is 100% for D2-D3 LSU and 96, 91, 88% for near-full-length SSU) with *T. fignewmani*, i.e., the monophyly of the genus was supported (Fig. 8). The genus *Teratodiplogaster* was phylogenetically nested in the *Parasitodiplogaster* + *Teratodiplogaster* clade.

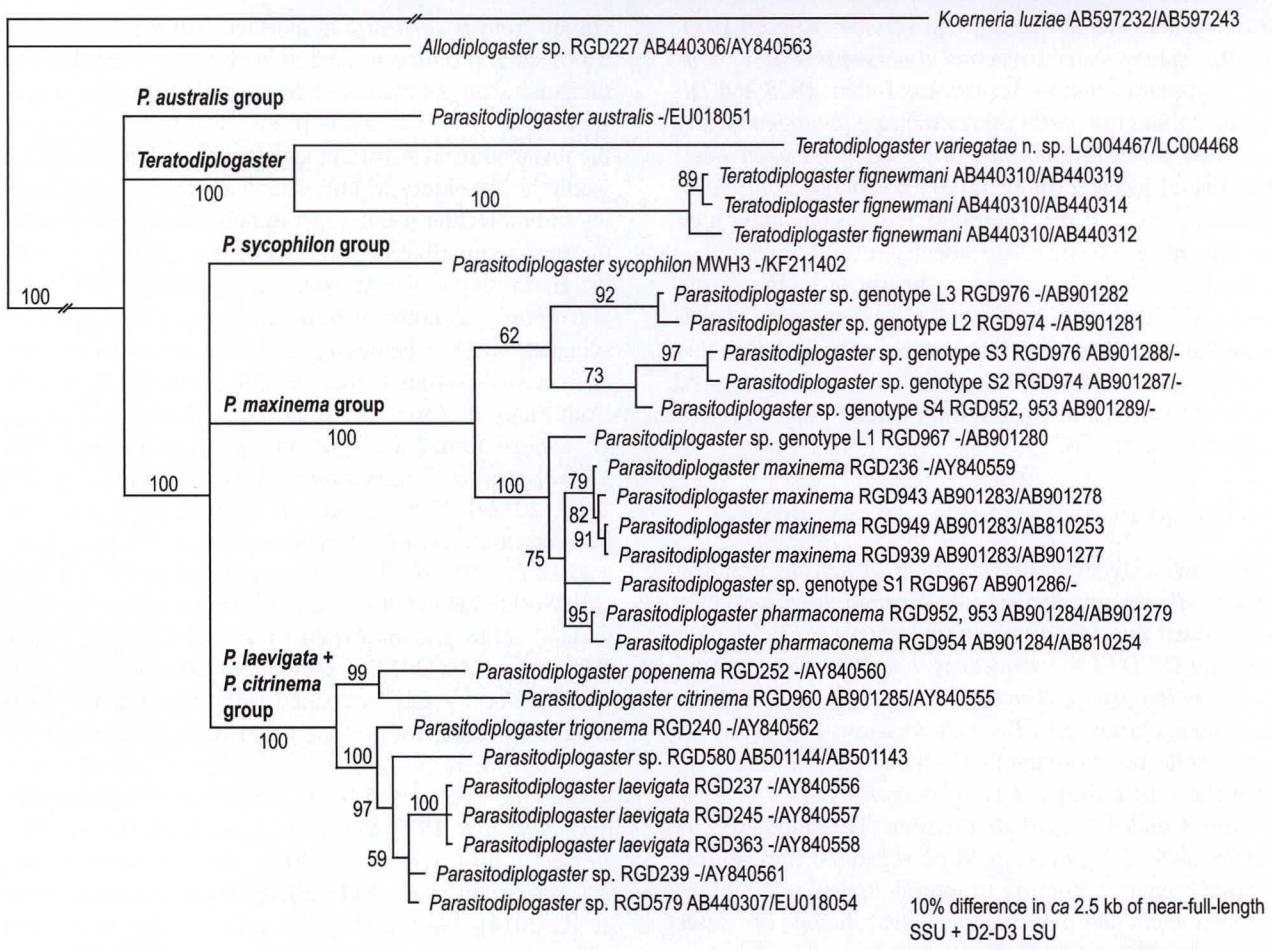
## Discussion

### MORPHOLOGICAL CHARACTERS

In this study, we describe only the third *Teratodiplogaster* species. Although the three species share some unique diagnostic traits, especially the highly derived morphology of the lips, there are some interesting morphological differences.

First, the separation of the stomatal elements differs among the three species. *Teratodiplogaster fignewmani* has a simple tube-like stoma without conspicuous separation, whereas *T. martini* and *T. variegatae* n. sp. have two-sectioned gymnostoms. Furthermore, *T. variegatae* n. sp. has three more sections in the stegostom (Figs 2, 3). As suggested previously (e.g., Baldwin *et al.*, 1997; Fürst von Lieven & Sudhaus, 2000; Sudhaus & Fürst von Lieven, 2003; Giblin-Davis *et al.*, 2006), the gymnostom of diplogastrid nematodes is associated with two-layered arcade syncytia cells. Thus, it is inferred that, in *T. martini*, each section of the two-sectioned gymnostom is associated with one syncytial ring, and the pro- and mesostegostom are fused to form a single and often less conspicuous element. However, the pro- and mesostegostom of *T. variegatae* n. sp. show a clear separation which is not common in the other diplogastrids, including its congeners and sister genera. Ultrastructural observations and comparison with its close relative, e.g., the omnivore genus *Allodiplogaster*, the sister of *Teratodiplogaster*, will be interesting in assisting our understanding of the formation of the characteristic stomatal morphology of *Teratodiplogaster*.

Second, the structure of the female reproductive tract, i.e., composition and the shape of each organ, is highly variable among species. *Teratodiplogaster fignewmani* does not have a *receptaculum seminis*, whereas the other two species do, albeit of different types. The four-celled



**Fig. 8.** Molecular phylogenetic relationship in *Allodiplogaster*/the *Parasitodiplogaster*/*Teratodiplogaster* clade. The consensus Bayesian tree inferred from near-full-length of SSU under GTR + I + G model ( $\ln L = 4732.9414$ ; freqA = 0.2538; freqC = 0.2107; freqG = 0.2685; freqT = 0.2671; R(a) = 1.2936; R(b) = 3.3318; R(c) = 2.9398; R(d) = 0.7444; R(e) = 8.3423; R(f) = 1; Pinvar = 0.4486; Shape = 0.7052) and D2-D3 LSU under TrN + G model ( $\ln L = 4684.6245$ ; freqA = 0.1896; freqC = 0.2185; freqG = 0.3376; freqT = 0.2543; R(a) = 1; R(b) = 2.1338; R(c) = 1; R(d) = 1; R(e) = 6.5843; R(f) = 1; Shape = 0.3946). The accession numbers of the compared sequences are indicated in the form: SSU/D2-D3 LSU. Posterior probability values exceeding 50% are given on appropriate clades.

structure in *T. martini* may be a homologue of the four large and dark cells in *T. variegatae* n. sp. (Figs 2, 5). The large, sac-like structure of the *receptaculum seminis* in *T. variegatae* n. sp. is very conspicuous in ventral/dorsal view (Figs 2, 5) and it would be difficult to overlook if such a conspicuous *receptaculum seminis* was present in *T. fignewmani*. On the other hand, the characteristic sphincter organ reported from *T. fignewmani* is not found in the other two species (Kanzaki *et al.*, 2009, 2012a). This organ consists of very well developed muscle and is also easily seen (Kanzaki *et al.*, 2009).

*Teratodiplogaster* belongs to a clade including *Allodiplogaster*, *Parasitodiplogaster*, and *Teratodiplogaster* (e.g., Kanzaki *et al.*, 2011, 2013a, 2014b, d). The *receptaculum seminis* found in *T. martini* and *T. variegatae* n. sp. and sphincter reported in *T. fignewmani* have not been reported for any other groups within this clade. Thus, these structures are considered as species- or species group-specific apomorphies.

Furthermore, the sac-like shape of the *receptaculum seminis* of *T. variegatae* n. sp. (Figs 2, 5) seems somewhat similar to those of *Diplogasteroides* de Man, 1912 (an-

*drassyi* clade) and *Pseudodiplogasteroides* Körner, 1954, and the kidney-shaped *receptaculum seminis* of *T. martini* is similar to that of *Acrostichus* Rahm, 1928 and *Diplogasteroides* (*magnus* clade) which are phylogenetically separated from *Teratodiplogaster* and from each other (Fürst von Lieven & Sudhaus, 2000; Kiontke *et al.*, 2003; Kanzaki *et al.*, 2013b). Therefore, the *receptaculum seminis* may have evolved independently several times.

No *Teratodiplogaster* species has been cultured successfully and thus it is difficult to examine the developmental process of their characteristic organs. Detailed morphological analyses, *e.g.*, TEM observation may be important for examining the ultrastructure and development of these organs.

#### PHYLOGENETIC RELATIONSHIP AND TAXONOMY

As previously suggested (Wöhr *et al.*, 2014), a monophyletic *Parasitodiplogaster* + *Teratodiplogaster* clade is separated into five groups based upon near-full-length SSU and D2-D3 LSU sequences, *i.e.*, *P. australis* group, *P. sycophilon* group, *P. maxinema* group, *P. laevigata* + *P. citrinema* group, and *Teratodiplogaster* (Fig. 8). Thus, to reconcile the morphological characters with the phylogenetic relationships, *Teratodiplogaster* could be synonymised under *Parasitodiplogaster*. The alternative is that *Parasitodiplogaster* could be separated into several different genera according to morphological and biological characters and their phylogenetic relationship. After considering the morphological characters, *i.e.*, no clear synapomorphy can be found for these two genera, the separation of *Parasitodiplogaster* seems reasonable. Several characteristic stomatal morphologies and other typological characters have been reported recently (Giblin-Davis *et al.*, 2006; Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2009, 2010, 2012, 2014b, e; Wöhr *et al.*, 2014), and these characterise the species groups of *Parasitodiplogaster*. Detailed morphological and phylogenetic comparisons followed by generic-level taxonomic analysis will be necessary to resolve these issues.

#### PLANT HOST (HABITAT) AND DISTRIBUTION

*Ficus variegata*, the host (habitat) tree of *T. variegatae* n. sp., is widely distributed from Japan to Northern Australia (Harrison *et al.*, 2012). In previous field surveys of fig-associated nematodes in Australia (Lloyd & Davies, 1997; Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2009, 2012a; Davies *et al.*, 2010, 2013a, b) and Indonesia (Sriwati *et al.*, 2013), *Teratodiplogaster* sp. has been re-

corded from *F. variegata* in Northern Australia, although a molecular profile was not obtained. It is conceivable that the Australian population of *Teratodiplogaster* sp. is conspecific with *T. variegatae* n. sp. However, considering the distance from Australia and Japan, such a conclusion would be premature without detailed morphological studies and molecular profiles. More field surveys may reveal the precise distribution range of *T. variegatae* n. sp. and its relationship to the Australian populations.

Currently, all nominal *Teratodiplogaster* species are associated with figs belonging to the subgenus *Sycomorus*: *T. fignewmani* from *F. racemosa* in Australia; *T. martini* from *Ficus* sp. (probably *F. sur*) in Zimbabwe; *T. variegatae* n. sp. from *F. variegata* in Japan, and a *Teratodiplogaster* sp. from *F. variegata* in Australia (Kanzaki *et al.*, 2009, 2012a). Furthermore, an unidentified species has been reported from *F. auriculata* Lour in Vietnam (Kanzaki *et al.*, 2012b). The subgenus *Sycomorus* is a typical Old World clade of figs and is distributed from the tropics and subtropics of Africa to the West Pacific region (Harrison *et al.*, 2012). *Teratodiplogaster* appears to be an Old World genus associated with the subgenus *Sycomorus*, since the former has never been found from the other subgenera of the *Ficus* regardless of intensive surveys in the Central and North American tropics and subtropics (Poinar, 1979; Poinar & Herre, 1991; Herre, 1993, 1995; Giblin-Davis *et al.*, 2006; Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2009, 2010, 2012a, 2013a, b; Wöhr *et al.*, 2014). More intensive surveys of *Sycomorus* figs will doubtless reveal the distribution, evolutionary history and host/carrier relationships of *Teratodiplogaster* spp.

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