

1 NemXXXX

2
3 ***Sachsia putridicola* n. sp. (Rhabditida: Diplogastridae), isolated from**
4 **an *Onthophagus dung* beetle (Coleoptera: Scarabaeidae) from**
5 **Tsukuba, Japan**

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20 **Summary** – An undescribed *Sachsia* species (Diplogastridae) was isolated from a dung
21 beetle, *Onthophagus* sp. cf. *atripennis*, and the species is described here as *Sachsia*
22 *putridicola* n. sp. The new species is characterised by its cheilostom; anterior part
23 forming a cuticular ring; posterior part in the form of a thin-walled tube; anisotopic
24 gymnostom; metastegostom with dorsal small tooth and no subventral armature; and
25 setiform male genital papillae with the arrangement <v1d, v2, v3/v4, ad, ph, v5-7, pd>.
26 *Sachsia putridicola* n. sp. is typologically similar to two previously described
27 congeners, *Sachsia zurstrasseni* and *Sachsia postpapillata*, but can be readily
28 distinguished by its anisotopic gymnostom (*vs* isotopic gymnostom in the two nominal
29 species) and the direction of the anterior three pairs of genital papillae, which are
30 directed sublaterally in the new species (*vs* second or third pair directed sublaterally in
31 *S. zurstrasseni* and *S. postpapillata*, respectively). Phylogenetically, the new species is
32 close to *Eudiplogasterium evidentum*, not *S. zurstrasseni*. Based on the typological and
33 phylogenetic characters, the status of *S. zurstrasseni* is discussed.

34

35 **Keywords** – dung beetle, morphology, morphometrics, new species, phylogeny,
36 taxonomy.

37

38 Nematodes are associated with many different groups of insects as parasites,
39 parasitoids, pathogens and phoretic associates. Among these host/carrier species, dung
40 and carcass-associated beetles have many characteristic nematodes, which are possibly
41 adapted to nutrient-rich and quickly decomposed substrates (environments), such as an
42 aphelenchoidid endoparasite, *Peraphelenchus* Wacheck, 1955 (Wacheck, 1955;
43 Kanzaki *et al.*, 2013), three-gendered rhabditids, *Auanema* Kanzaki, Kiontke, Tanaka,
44 Hirooka, Schwarz, Müller-Reichert, Chaudhuri & Pires da Silva, 2017, three-gendered
45 viviparous rhabditids, *Tokorhabditis* Kanzaki, Yamashita, Lee, Shih, Ragsdale &
46 Shinya, 2021 (Kanzaki *et al.*, 2017a, 2021; Ragsdale *et al.*, 2022), viviparous
47 diplogastrid, *Sudhausia* Herrmann, Ragsdale, Kanzaki & Sommer, 2013 (Herrmann *et*
48 *al.*, 2013; Kanzaki *et al.*, 2017b), and several predators, including *Mononchoides* Rahm,
49 1928 and *Fictor* Paramonov, 1952 (Mahboob & Tahseen, 2022; Mahboob *et al.*, 2022)
50 and a characteristic omnivore, *Onthodiplogaster* Kanzaki, Ikeda & Shinya, 2023
51 (Kanzaki *et al.*, 2023). In addition, mutualistic relations between the dung beetle
52 *Onthophagus taurus* (Schreber, 1759) and its associated nematodes have been
53 recognised (Ledón-Rettig *et al.*, 2018).

54 During a field survey of dung- and carcass-associated nematodes, an
55 undescribed species of *Sachsia* Meyl, 1960 was isolated from *Onthophagus* sp. *cf.*
56 *atripennis* (Coleoptera: Scarabaeidae).

57 Here, the newly isolated *Sachsia* species is described and illustrated as *Sachsia*
58 *putridicola* n. sp. based on its typological characters and molecular sequence profile. In
59 addition, the taxonomic status of the genus is discussed.

60

61 **Materials and methods**

62

63 NEMATODE COLLECTION AND CULTURE

64

65 Six adult *Onthophagus* spp. (not identified at the species level, but seemingly
66 *Onthophagus atripennis* Waterhouse) were collected from rotten mushrooms (*Boletus*
67 *reticulatus* Schaeff.) from the campus of the University of Tsukuba in Ibaraki, Japan, on
68 7 July 2021. The beetles were dissected individually on water agar (2.0% agar without
69 nutrients). The dissected beetles were kept in the laboratory (approximately 25°C) and
70 observed occasionally for 1 month. Propagated nematodes were observed under a
71 dissecting microscope (S8 Apo; Leica Microsystems, Wetzlar, Germany) to determine
72 their feeding habits and transferred to appropriate medium (NGM seeded with
73 *Escherichia coli* strain OP50). Successfully propagated nematodes were subcultured
74 and further identified based on typological characters using a compound microscope
75 (Eclipse Ni; Nikon, Tokyo, Japan) with differential interference contrast optics.

76

77 LIGHT MICROSCOPIC OBSERVATION AND PREPARATION OF TYPE SPECIMENS

78

79 Adult nematodes were collected from two-week-old cultures, after which they
80 were heat-killed and fixed in TAF (triethanolamine:formalin:distilled water = 2:7:91)
81 for one week. Fixed material was processed to glycerin using a modified Seinhorst's
82 method (Minagawa & Mizukubo, 1994) and mounted in glycerin according to the
83 methods of de Maeseneer & d'Herde (1963). Mounted specimens were used for
84 morphometrics and kept as type material. In addition, live adults from two-week-old
85 cultures were used for detailed morphological observations following the methods of

86 Kanzaki (2013). All micrographs were obtained using a digital camera system (MC170
87 HD; Leica, Wetzlar, Germany) and morphological drawings were made using a drawing
88 tube connected to the microscope.

89

90 MOLECULAR PROFILES AND PHYLOGENETIC STATUS

91

92 Several adult individuals were hand-picked from a culture (see above), and
93 transferred to nematode lysis solution (Kikuchi *et al.*, 2009; Tanaka *et al.*, 2012)
94 individually for DNA extraction. These nematodes were digested at 55°C for 20 min.,
95 and the lysates served as PCR template DNA. First, the materials were individually
96 amplified and sequenced for their D2-D3 expansion segments of the large subunit of
97 ribosomal RNA (D2-D3 LSU) according to Ye *et al.* (2007) to confirm their species
98 identity. Thereafter, *ca* 4 kb of ribosomal RNA region including near-full-length small
99 subunit (SSU: *ca* 1.7 kb), internal transcribed spacer region (ITS: *ca* 0.9 kb) and D1-D4
100 expansion segments of the large subunit (D1-D4 LSU: *ca* 1.4 kb) were determined
101 following the methodology by Ekino *et al.* (2017) and Kanzaki *et al.* (2019). In
102 addition, partial code of mitochondrial cytochrome oxidase subunit I (mtCOI: 660 bps)
103 was determined following the methodology by Kanzaki & Futai (2002). The sequences
104 of the new species were deposited in the GenBank database with accession numbers
105 **LC773616** (rDNA) and **LC773617** (mtCOI).

106 The SSU and D2-D3 LSU were employed for combined molecular phylogenetic
107 analysis. First, both sequences were compared with those deposited in the database
108 using BLAST homology search program
109 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch

110 h&LINK_LOC=blasthome). According to the BLAST results and previous publications
111 (Gonzalez *et al.*, 2021; Kanzaki *et al.*, 2023), sequences to be used in phylogenetic
112 analyses were retrieved from the database. The selected sequences were summarized in
113 Suppl. Table S1.

114 The sequences of each dataset were aligned using MAFFT (Kato *et al.*, 2002;
115 Kuraku *et al.*, 2013: available online at <http://align.bmr.kyushu-u.ac.jp/mafft/software/>).
116 The substitution model and parameters were determined by MEGA 7 (Kumar *et al.*,
117 2016) for each locus, and the Bayesian phylogenetic analysis was conducted with
118 MrBayes 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012); four chains were
119 run for 4×10^6 generations. Markov chains were sampled at intervals of 100 generations
120 (Larget & Simon, 1999). Two independent runs were performed, and, after confirming
121 the convergence of runs and discarding the first 2×10^6 generations as burn-in, the
122 remaining topologies were used to generate a 50% majority-rule consensus tree.

123

124 **Results**

125

126 NEMATODE ISOLATION

127

128 *Tokorhabditis atripennis* Ragsdale, Kanzaki, Yamashita & Shinya, 2022 was
129 isolated from all dissected individuals, while *S. putridicola* n. sp. was found from one
130 individual beetle.

131

132 PHYLOGENETIC STATUS

133

134 Phylogenetic relationship among diplogastrid nematodes including the new
135 species is shown in Fig. 1 and Suppl. Figs S1 and S2. *Sachsia putridicola* n. sp.
136 belonged to a subclade of Diplogastridae with *Cutidiplogaster* Fürst von Lieven, Uni,
137 Ueda, Barbuto & Bain, 2011, *Eudiplogasterium* Meyl, 1960, *Mononchoides* Rahm,
138 1928, *Neodiplogaster* Cobb, 1924, *Onthodiplogaster* Kanzaki, Ikeda & Shinya, 2023,
139 *Paroigolaimella* Paramonov, 1952, *Sachsia*, and *Tylopharynx* de Man, 1876, and close
140 to *Eudiplogasterium* and *Paroigolaimella*. However, the new species did not form a
141 clade with its congener, *S. zurstrasseni* (Sachs, 1950) Meyl, 1960, *i.e.*, the new species
142 formed a well-supported (100% posterior probability) clade with *E. levidentum*
143 (Weingärtner, 1955) Meyl, 1960, with *S. zurstrasseni* being closer to *Paroigolaimella*
144 spp.

145

146 ***Sachsia putridicola* n. sp.**

147 (Figs 2-6)

148

149 MEASUREMENTS

150

151 See Table 1.

152

153 DESCRIPTION

154

155 *Adults*

156

157 Medium to small species in the family, *i.e.*, 486-607 and 575-746 μm in male
158 and female, respectively. Body cylindrical, relatively stout. Cuticle thick, with fine
159 annulation and clear longitudinal striations where dot-like and chain-like patterns can be
160 observed in different focal planes. Lateral field weakly developed, sometimes difficult
161 to distinguish from striations, but can be distinguished with lack of annulation,
162 seemingly composed by two separate bands, *i.e.*, four lines were observed. Six equal-
163 sized lip sectors not clearly separated from each other, forming a dome-shape, without
164 clear constriction, *i.e.*, lip part continuous with body contour. Six short, setiform labial
165 sensilla present in male and female, and four long, setiform cephalic papillae present in
166 male. Amphid large, inverted triangular, located at the level of the posterior end of
167 cheilostom. Stomatal dimorphism not observed. Stoma separated into three sections,
168 cheilo-, gymno- and stegostom. Cheilostom forming a short tube; anterior part forming
169 a ring at the stomatal opening which is seemingly separated into six or 12 sections, but
170 the separation was weakly observed only in live materials, and not clearly observed in
171 fixed or glycerin material; posterior part short tube with thin and rather flexible wall.
172 Gymnostom forming thick-walled short tube, internally overlapping with cheilostom;
173 anisotropic, *i.e.*, dorsal side is about half of ventral side in length (depth); separated into
174 two (anterior and posterior) subsections each derived from different arcade syncytia, but
175 the margin was observed weakly in live materials. Stegostom separated into three
176 subsections, pro-meso-, meta- and telostegostom. Pro-mesostegostom not developed,
177 not clearly cuticularized, internally overlapping with gymnostom. Metastegostom with a
178 small dorsal tooth on a mound; no tooth, ridge or denticule was observed on subventral
179 sectors, but the sectors forming (surrounded by) slightly sclerotized ring. Stegostom not
180 well-developed; funnel-shaped, connecting metastegostom and pharynx. Although the

181 dorsal pharyngeal gland was observed, the gland orifice was not clearly observed.
182 Anterior part of pharynx (= pro- and metacarpus) and posterior pharynx (isthmus and
183 basal bulb) almost same in length. Procorpus muscular tube without conspicuous
184 internal lining, occupying about 1/3 of corresponding body diam. Metacarpus muscular,
185 forming weakly-developed and somewhat roundish rectangular median bulb. Isthmus
186 narrow, not muscular. Basal bulb glandular. Pharyngo-intestinal junction clearly
187 observed, well developed. Nerve ring usually surrounding posterior part of isthmus.
188 Secretory-excretory pore visible, but not conspicuous, ventrally located at 1/2 body
189 diam. posterior to basal bulb. Deirids observed laterally, at the level of one body diam.
190 posterior to secretory-excretory pore. Postdeirid present, on latero-dorsal side of the
191 body, and the position will be described for male and female separately. Lateral glands
192 not observed.

193

194 *Male*

195

196 Body weakly ventrally arcuate, strongly ventrally curved at tail region when
197 killed by heat. Testis single, on the right ventral of intestine, anterior part reflexed to
198 right side. Spermatogonia arranged in multiple (three to five) rows in reflexed part, then
199 well-developed spermatocytes arranged as multiple (one to three) rows in anterior two-
200 thirds of main branch, then mature amoeboid spermatids arranged in multiple rows in
201 remaining, proximal part of gonad. *Vas deferens* occupying about 1/3 of total gonad
202 length. Posterior end of gonad (= posterior end of *vas deferens*) and intestine fused to
203 form a cloacal tube. Postdeirid around the anterior end of *vas deferens*. Spicules paired,
204 separate. Spicules straight to smoothly curved in ventral view, adjacent to each other in

205 distal end, *i.e.*, arranged in ‘V’-shape. Spicule in lateral view smoothly ventrally
206 arcuate, rounded to roundish squared manubrium present at anterior end; anterior 2/5 of
207 lamina/calomus complex with smooth and obvious ventral expansion, then smoothly
208 tapering to bluntly pointed distal end. Gubernaculum conspicuous, about half of spicule
209 in length, bow to low keel-shaped with a manubrium at the anterior end; posterior end
210 dorsally covering spicules. Dorsal side of gubernaculum well sclerotised. Tail conical,
211 with a sharply pointed spike occupying about half of whole tail length. Nine pairs of
212 setiform genital papillae and a papilliform ventral single papilla present. Ventral single
213 papilla (vs) on anterior cloacal lip. Paired genital papillae and a pair of phasmids
214 arranged as <v1d, v2, v3 / v4, ph, ad, v5-v7, pd> in the terminology of Sudhaus and
215 Fürst von Lieven (2003), where sublateral v1d *ca* 1 cloacal body diam. (CBD) anterior
216 to cloacal opening (CO); subventrally located v2 and v3 close to each other, and at the
217 half way between CO and v1d; subventral v4 about 1/3 CBD posterior to CO; phasmid
218 (ph) laterally located at 1/2 CBD posterior to CO; sublaterally located *ad ca* 1 CBD
219 posterior to CO; v5-v7 forming triplet, and the central one (v6) slightly more ventrally
220 located, and longer than the other two; subdorsally directed pd located at level of or
221 slightly posterior to v7. Anterior five pairs (v1-ad) almost equal in size, rather long and
222 conspicuous; v5 and v7 very small; and v6 and pd small but larger than v5 and v7, *i.e.*,
223 intermediate between anterior pairs and v5/v7. Bursa or bursal flap absent.

224

225 *Female*

226

227 Slightly and smoothly arcuate ventrally or straight when killed by heat. Gonad
228 didelphic, amphidelphic. Each genital system arranged from vulva/vagina as uterus,

229 spermatheca, oviduct, connection tissue and ovary; where uterus to ovary form a single
230 tube. Because anterior and posterior gonads are structurally identical, and symmetry to
231 each other, *i.e.*, anterior and posterior gonads extend on the right and left of intestine,
232 only anterior gonad is described here from Vagina/vulva to ovary. Vagina pore-like in
233 ventral view, without any flap apparatus. Vagina perpendicular to body wall,
234 surrounded by sclerotised tissue. Vulval muscle forming X-shape in ventral view,
235 relatively conspicuous. Four vaginal glands visible overlapping with vulval muscle.
236 Uterus to connection tissue extending ventrally and anteriorly on right of intestine and
237 with a totally reflexed (= antidiromous reflexion) ovary extending dorsally. Uterus thick-
238 walled. Middle part of main gonad branch functions as spermatheca where well-
239 developed sperm often present; composed by large rounded-flattened cells. Oviduct
240 simple tube between spermatheca and connection tissue. Connection tissue consisting of
241 oval-shaped cells, somewhat crustaformeria-like, connecting ovary and oviduct.
242 Oocytes mostly arranged in multiple (two to five) rows in distal half, and well-
243 developed oocytes arranged in single row, where the most developed (anterior) oocyte
244 appears darker and more glandular than the other oocytes. Postdeirid at the level of the
245 reflection of posterior gonad. Rectum *ca* 1 anal body diam. (ABD) long, intestinal-rectal
246 junction surrounded by well-developed sphincter muscle. Three rectal glands, two
247 ventral and one dorsal, present. Anus in form of dome-shaped slit, posterior anal lip
248 slightly protuberant. Phasmid conspicuous, located about half (1/3-2/3) ABD posterior
249 to anus. Tail smoothly tapering or slightly elongate conical, with pointed and elongate
250 terminus.

251

252 TYPE HABITAT AND LOCALITY

253

254 The new species was isolated from an adult of *Onthophagus* sp. cf. *atripennis*
255 collected on 7 July, 2021 from a rotten *Boletus reticulatus* in the campus of the
256 University of Tsukuba (36° 06' 26" N, 140° 06' 10" E, 25 m a.s.l.).

257

258 OTHER HABITATS AND LOCALITIES

259

260 The new species was previously isolated from a dissected body of *Creophilus*
261 *maxillosus* (L.) (Staphylinidae) collected with a rotten meat trap in the Chiyoda
262 Experimental Nursery of Forestry and Forest Products Research Institute (36° 11' 00"
263 N, 140° 13' 04" E, 37 m a.s.l.) in June, 2015, but the culture was lost before the
264 collection of taxonomic materials (Kanzaki, Unpubl. Obs.)

265

266 ETYMOLOGY

267

268 The species epithet *putridicola* is derived from the Latin words *putridium* (=
269 rotten) + *cola* (= dwelling) because of the habitat of the new species, *i.e.*, rotten
270 substrates (rotten mushroom and rotten meat).

271

272 TYPE MATERIAL

273

274 Type specimens include a holotype male, nine paratype males, and 10 paratype
275 females were deposited as follows: the holotype male (Collection ID: T-797t), four
276 paratype males (T-7893p to T-7896p), and five paratype females (T-7897p to T-7901)

277 in the USDA Nematode Collection (USDANC), Beltsville, Maryland; five paratype
278 males (*Sachsia putridicola* M01-05) and five paratype females (*Sachsia putridicola*
279 F01-05) in the Forest Pathology laboratory collection in FFPRI, Tsukuba, Japan. In
280 addition, several mounted and unmounted specimens of males and females were
281 deposited in the Kansai Research Center, FFPRI.

282

283 DIAGNOSIS AND RELATIONSHIPS

284

285 *Sachsia* currently contains two species, *S. zurstrasseni* and *S. postpapillata*
286 Mumtaz & Ahmad, 2019. The new species is distinguished from *S. postpapillata* by its
287 stouter body reflected to a values of males reflected to a values, 15.1 (12.7-19.0) vs 23.4
288 (22.6-24.9) and females 13.2 (11.8-14.0) vs 20.8 (19.9-21.6); stomatal structure, *i.e.*,
289 although the detailed structure of cheilostom is not described, the shape of gymnostom
290 seems different as anisotropic vs isotropic; arrangement of male genital papillae, <v1d,
291 v2, v3 / v4, ph, ad, v5-v7, pd> vs <v1, v2, v3d / v4, ph, ad, v5-v7, pd>, *i.e.*, the
292 sublaterally located pair comes to first (v1d) vs third (v3d); and somewhat shorter (not
293 clearly filiform) female tail which is reflected to c' values, 6.2 (4.8-7.7) vs 10.2 (9.3-
294 11.0) (Mumtaz & Ahmad, 2019). In addition, several other morphometric values could
295 be applied to distinguish the new species from *S. postpapillata* (Mumtaz & Ahmad,
296 2019). However, the values for *S. postpapillata* had been calculated from relatively
297 small number of materials (four males and five females), the values are expected to
298 have wider range, and thus, are not mentioned in detail herein.

299 Based upon stomatal structure, *S. putridicola* n. sp. is seemingly closer to *S.*
300 *zurstrasseni*, *i.e.*, although the detailed structure is not described in the text, Sachs

301 (1950) drew a ring-like anterior part and thin-walled posterior part of cheilostom for *S.*
302 *zurstrasseni*. However, *S. putridicola* n. sp. can be distinguished from *S. zurstrasseni* its
303 gymnostomatal shape, anisotopic *vs* somewhat isotopic; arrangement of male genital
304 papillae, <v1d, v2, v3 / v4, ph, ad, v5-v7, pd> *vs* <v1, v2d, v3 / v4, ad, v5-v7, pd>
305 (phasmid is not drawn in Sachs (1950)), *i.e.*, the sublaterally located pair comes to first
306 (v1d) *vs* second (v2d); and position of female phasmid, approximately half ABD *vs*
307 more than 1 ABD posterior to anus (Sachs, 1950).

308 Phylogenetically, the new species is clearly separate from *S. zurstrasseni* as
309 reported in Susoy *et al.* (2015) (Fig. 1, Suppl. Figs, S1, S2). However, the species could
310 belong to different undescribed genus, and further comments are given below.

311

312 ADDITIONAL REMARKS

313

314 Both in the present and previous isolations, *S. putridicola* n. sp. could be
315 cultured using common methods on NGM seeded with *E. coli* OP50. However, in both
316 cases, the nematode did not propagate well, and the culture lasted for less than 1 year in
317 our laboratory. Therefore, although the culture could last longer with careful subculture,
318 the habitat (humidity and/or pH conditions) and food microbe preferences of the
319 nematode are thought to be specific to their natural habitats. Similar difficulty in
320 culturing has been reported in the rhabditid *Onthophagus* associate, *Tokorhabditis tauri*
321 (Ragsdale *et al.*, 2022). This suggests that the new species could be useful for studies of
322 the adaptation of nematodes to environments and food sources, and further efforts to
323 reisolate and establish a stable culture of this species are therefore necessary.

324

325 **Discussion**

326

327 GENERIC CHARACTERS OF *SACHSIA*

328

329 The genus *Sachsia* was originally established by Meyl (1960) with *Sachsia*

330 *zurstrasseni* as the type species. Thereafter, Sudhaus & Fürst von Lieven (2003)

331 organised the diplogastrid genera and retained *Sachsia* as a monotypic genus.

332 Diplogastrid genera are usually characterised mainly by the stomatal structure and male

333 and female tail characters, and several genera (or clade)-specific apomorphies have been

334 discussed, such as the presence of kidney-shaped *receptaculum seminis* in *Acrostichus*

335 Rahm, 1928 (Sudhaus & Fürst von Lieven, 2003). Sudhaus & Fürst von Lieven (2003)

336 typologically characterised *Sachsia* as follows: conspicuous cuticular ornamentation;

337 bipartite buccal cavity (stoma); stegostom with dorsal thorn-like tooth; female gonad

338 amphidelphic; gubernaculum resembles a narrow slightly bent strut; and tail short in

339 both sexes.

340 Comparison of these characters in three *Sachsia* spp. indicated that the generic

341 characters can be emended as follows (Sachs, 1950; Sudhaus & Fürst von Lieven, 2003;

342 Mumtaz & Ahmad, 2019):

343 1) conspicuous cuticular ornamentation

344 2) bipartite buccal cavity (stoma)

345 3) stegostom with dorsal thorn-like tooth

346 4) female gonad amphidelphic

347 5) gubernaculum resembles a narrow slightly bent strut

348 The tail is relatively short in *S. zurstrasseni* and *S. putridicola* n. sp., but *S.*
349 *postpapillata* has a filiform female tail and long spike (appendage) in males. Therefore,
350 the tail shape is omitted from the characters. In addition, the arrangement of genital
351 papillae is often identical among close relatives or within the genus. However, in the
352 present study, the arrangement was confirmed to be different among the three species
353 and it was not included as a generic character.

354 Among these characters, 1), 2) and 4) are often found in the other diplogastrids
355 (Sudhaus & Fürst von Lieven, 2003); therefore 3) and 5), could be the most important
356 characters.

357 In the present study, stomatal composition was used as a diagnostic character,
358 *i.e.*, the gymnostom is anisotopic in the new species but somewhat isotopic in the other
359 two species. In addition, cheilostomatal structure was somewhat different among these
360 three species. Although the detailed structure has not been described for *S.*
361 *postpapillata*, the ring-like anterior part found in the other two species was not
362 confirmed in *S. postpapillata* (Sachs, 1950; Mumtaz & Ahmad, 2019), and the posterior
363 part of the cheilostom was thinner in *S. putridicola* n. sp. This type of variation has been
364 found in *Pristionchus* Kreis, 1932, *i.e.*, *P. elegans* Kanzaki, Ragsdale, Herrmann &
365 Sommer, 2012 and its close relatives have membrane-like thin cheilostomatal plates,
366 while others have thick plates (Kanzaki *et al.*, 2012). On the other hand, variation in the
367 gymnostom is not commonly seen within a genus. Compared with these variabilities,
368 the structure in the stegostom seems common among the three species considered here,
369 *i.e.*, a small dorsal tooth on the dorsal metastegostomatal mound and no tooth, denticle
370 or ridge present on the subventral side. The nematodes in this clade usually have
371 sclerotised cuticular structure(s) on the subventral sides (Sudhaus & Fürst von Lieven,

372 2003; Susoy *et al.*, 2015), and secondary loss of the subventral armature could be an
373 important apomorphy of the genus.

374 The other important character, the somewhat flattened shape of the
375 gubernaculum, is also not commonly known in diplogastrids. However, the
376 gubernaculum morphology is sometimes difficult to evaluate, except in some
377 characteristic cases such as the highly complex and/or massive gubernaculum reported
378 in *Acrostichus* (Kanzaki *et al.*, 2020). Therefore, further studies of the characters based
379 on good specimens of more species are required.

380 In addition to the abovementioned characters, the three currently known *Sachsia*
381 spp. commonly have setiform sensillae and papillae, and large amphids (Sachs, 1950;
382 Mumtaz & Ahmad, 2019). These characters are often found in aquatic species such as
383 the *Striata* group of *Allodiplogaster* Paramonov & Sobolev in Skrjabin, Shikobalova,
384 Sobolev, Paramonov & Sudarikov, 1954 (Kanzaki *et al.*, 2014), and those inhabiting
385 humid and nutrition-rich environments including *Butlerius* Goodey, 1929 and
386 *Onthodiplogaster* (Susoy *et al.*, 2015; Girgan *et al.*, 2021; Kanzaki *et al.*, 2023).
387 Therefore, these characters may represent adaptations to their habitat, *i.e.*, may be
388 specific to the intrageneric group sharing the same habitat, and could be added to the
389 generic characters as follows:

390 6) Long and setiform labial and cephalic sensillae

391 7) Long and setiform genital papillae

392

393 REMARKS ON GENERIC AND SPECIES STATUS OF *SACHSIA ZURSTRASSENI*

394

395 In a previous study, a species isolated from manure/humus environment was
396 identified as *S. zurstrasseni* (by N. Kanzaki, the author of the present study) because of
397 its typological similarity and isolation source, *i.e.*, *S. zurstrasseni* was originally isolated
398 from manure (cow dung) in southern Germany (Sachs, 1950), and molecular sequence
399 profiles were given for the species (Susoy *et al.*, 2015). In the present study, however, *S.*
400 *putridicola* n. sp. did not form a clade with *S. zurstrasseni*, and the morphological
401 characters of the previous (Sachs, 1950) and new (Susoy *et al.*, 2015) isolates were
402 therefore compared based on available information, *i.e.*, only morphological drawings
403 and a micrograph of the stomatal part are available for the new isolate (Ragsdale, 2015;
404 Susoy *et al.*, 2015). The stomatal compositions of these two isolates are similar to each
405 other; typically, in metastegostom, both have only a dorsal tooth and no armature is
406 found in the subventral sectors. However, the forms of labial and cephalic sensillae are
407 different, *i.e.*, setiform and papilliform in the previous and new isolate, respectively
408 (Sachs, 1950; Ragsdale, 2015; Susoy *et al.*, 2015). The cheilostomatal shape is slightly
409 different, clearly narrowing anteriorly in the new isolate and weakly anteriorly tapering
410 tube-like in the previous isolate (Sachs, 1950; Ragsdale, 2015; Susoy *et al.*, 2015). In
411 addition, the extent of overlap of chailostom and gymnostom is greater in the new
412 isolate; and the width-depth ratio of stoma, deeper than diameter in the original isolate,
413 but the diameter is larger than the depth in the new isolate (Sachs, 1950; Andrásy,
414 1984; Ragsdale, 2015; Susoy *et al.*, 2015).

415 Phylogenetically, new species did not form well-supported clade with new
416 isolate of '*S. zurstrasseni*' (Fig. 1, Suppl. Figs S1, S2). Comparing with the previous
417 study, the phylogenetic tree topology is a little different, *i.e.*, in a subtree, *T. foetida* is
418 located to be basal position, subsequently *P. stresemanni* and *E. levidentum* branched

419 out, and *P. micrura* and '*S. zurstrasseni*' form a well-supported derived clade in Susoy
420 *et al.* (2015). On the other hands, in the present study, although the position of *T. foetida*
421 is consistent to Susoy *et al.* (2015), the others are separated into two clade, two
422 *Paroigolaimella* spp. + '*S. zurstrasseni*' and *S. putridicola* n. sp. + *E. levidentum* (Fig.
423 1, Suppl. Figs S1, S2). We consider the difference between these two analyses is
424 derived from the number of available sequences to be compared, *i.e.*, the phylogeny was
425 constructed based on multiple genes obtained from draft genome sequences, and only
426 SSU and D2-D3 LSU were available for new species. However, considering the PP
427 values (100%) for these clades in the present study, *S. putridicola* n. sp. is clearly
428 separated from '*S. zurstrasseni*' as different genus.

429 Therefore, the new isolate of '*S. zurstrasseni*' is assumed to be an undescribed
430 genus sharing similar typological characters with *Sachsia*. For this species, reisolation
431 and detailed typological observation are necessary to describe it as a new genus.

432

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434

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442

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596

597 **Figure legends**

598

599 **Fig. 1.** Phylogenetic status of *Sachsia putridicola* n. sp. among closely related
600 diplogastrids.

601 A subtree was cropped from family-wide tree provided in Supplementary Fig. S1. The
602 Bayesian tree inferred from near full length of SSU and D2-D3 LSU of ribosomal RNA
603 genes. The GTR + G + I model was applied to both loci, and the parameters are as
604 follows: AIC = 51622.23; lnL = -25567.8; freqA = 0.25, freqC = 0.21, freqG = 0.27,

605 freqT = 0.27; R(a) = 0.89, R(b) = 2.47, R(c) = 2.08; R(d) = 0.89, R(e) = 3.65, R(f) =
606 1.00; Pinva = 0.39; Shape = 0.58 for SSU, and AIC = 54978.481; lnL = -27247.532;
607 freqA = 0.21, freqC = 0.22, freqG = 0.32, freqT = 0.25; R(a) = 0.46, R(b) = 1.7, R(c) =
608 0.91, R(d) = 0.44, R(e) = 3.54, R(f) = 1.00; Pinva = 0.21; Shape = 1.01 for D2-D3 LSU.
609 Posterior probability (PP) values exceeding 50% are given on appropriate clades.

610

611 **Fig. 2.** *Sachsia putridicola* n. sp. adults. A: Male; B: Female; C: Anterior part in right
612 lateral view; D: Surface of male lip region in left lateral view showing labial and
613 cephalic sensilla and amphid; E: Stomatal region in left lateral view; F: Stomatal region
614 in ventral view; G: Body surface structure of deirid region in left lateral view.

615

616 **Fig. 3.** *Sachsia putridicola* n. sp. adults. A: Anterior gonad of female in right lateral
617 view; B: Female vulval region in ventral view; C: Female body surface of post-deirid
618 region in right lateral view; D: Female whole tail in right lateral view; E: Female
619 rectum-anal region in right lateral view; F: Close-up of female anal region showing
620 surface striations around phasmid in right lateral view; F: Female anal region in ventral
621 view; G: Male gonad in right lateral view; H: Male tail region in right lateral view; I:
622 Surface of male tail region in right lateral view where lateral field is indicated by grey
623 colour; J: Male tail region in ventral view; K: Spicule and gubernaculum in left lateral
624 view.

625

626 **Fig. 4.** Differential interference contrast micrographs of the anterior region of *Sachsia*
627 *putridicola* n. sp. A: Stoma and anterior pharynx of male in four different focal planes;
628 B: Posterior pharynx of female in three different focal planes. Abbreviations are as

629 follows: am = amphid; bb = basal bulb; cs = cephalic sensilla; dr = deirid; ep =
630 secretory-excretory pore; ls = labial sensilla; mb = median bulb (metacarpus); nr =
631 nerve ring.

632

633 **Fig. 5.** Differential interference contrast micrographs of the adult male tail of *Sachsia*
634 *putridicola* n. sp. All images are in right lateral view. A: Anterior part of tail in four
635 different focal planes; B: Posterior part of tail in three different focal planes. Genital
636 papillae are labeled with the suffix “d” indicates papillae that open laterally or
637 subdorsally. Abbreviations are as follows: ph = phasmid; v + number, ad, pd = genital
638 papillae according to the labelling of Sudhaus & Fürst von Lieven (2003); vs = ventral
639 single papilla.

640

641 **Fig. 6.** Differential interference contrast micrographs of the female of *Sachsia*
642 *putridicola* n. sp. All images are in right lateral view. A: Vulval region in three different
643 focal planes; B: Anal region in three different focal planes. Abbreviations are as
644 follows: a = anus; lf = lateral field; m = vulval muscle; ph = phasmid; rg = rectal gland;
645 vg = vulval gland; vo = vulval opening.

646

647 **Supplementary Figure S1.** Phylogenetic relationship among 116 diplogastrids.
648 *Rhabditoides inermis* was used for an outgroup species. Analytical parameters are same
649 as Fig. 1.

650

651 **Supplementary Figure S2.** Phylogenetic relationship among 26 diplogastrids. The
652 OTUs appeared in Fig. 1 were phylogenetically re-analyzed using *Micoletzkyia japonica*

653 as an outgroup species. The Bayesian tree inferred from near full length of SSU and D2-
654 D3 LSU of ribosomal RNA genes. The GTR + G + I model was applied to both loci,
655 and the parameters are as follows: AIC = 13199.87; nL= -6540.85; freqA = 0.25, freqC
656 = 0.22, freqG = 0.27, freqT = 0.26, R(a) = 0.68, R(b) = 2.35, R(c) = 1.74, R(d) = 0.79,
657 R(e) = 3.64, R(f) = 1.00; Pinva = 0.57; Shape = 0.46 for SSU, and AIC = 12252.50; lnL
658 = -6067.04; freqA = 0.20, freqC = 0.22, freqG = 0.33, freqT = 0.25; R(a) = 0.29, R(b) =
659 1.61, R(c) = 0.91, R(d) = 0.47, R(e) = 3.70, R(f) = 1.00; Pinva = 0.34; Shape = 1.06 for
660 D2-D3 LSU. Posterior probability (PP) values exceeding 50% are given on appropriate
661 clades.
662

Table 1. Morphometric values of *Sachsia putridicola* n. sp.

| | Male | | Female |
|--|----------|----------------------------|----------------------------|
| | Holotype | Paratypes | paratypes |
| n | - | 9 | 10 |
| L | 546 | 554 ± 39 (486-607) | 651 ± 66 (575-746) |
| a | 14.7 | 15.5 ± 2.0 (12.7-19.0) | 13.2 ± 0.7 (11.8-14.0) |
| b | 7.5 | 7.4 ± 0.6 (6.5-8.2) | 8.3 ± 0.9 (7.2-10.2) |
| c | 7.0 | 6.5 ± 0.4 (5.8-7.2) | 4.7 ± 0.5 (4.0-5.6) |
| c' | 3.9 | 4.2 ± 0.3 (3.7-4.7) | 6.2 ± 0.9 (4.8-7.7) |
| T or V | 70.7 | 72.5 ± 5.2 (60.8-78.1) | 45.6 ± 2.2 (41.1-48.3) |
| Stoma diam. | 3.6 | 3.6 ± 0.3 (3.2-3.9) | 3.6 ± 0.3 (3.2-3.9) |
| Stoma length | 6.4 | 6.8 ± 0.4 (6.4-7.5) | 6.9 ± 0.4 (5.7-7.1) |
| Stoma length / diam. ratio | 1.8 | 1.9 ± 0.2 (1.6-2.2) | 1.9 ± 0.2 (1.5-2.1) |
| Median bulb diam. | 10.0 | 9.8 ± 0.8 (8.6-10.7) | 10.9 ± 0.6 (10.0-12.1) |
| Basal bulb diam. | 8.2 | 8.4 ± 0.8 (7.1-10.0) | 9.5 ± 0.6 (8.9-10.7) |
| Anterior pharynx length | 33 | 34 ± 1.8 (31-36) | 35 ± 2.9 (32-40) |
| Posterior pharynx length | 34 | 35 ± 2.2 (31-38) | 37 ± 2.8 (33-41) |
| Anterior / posterior pharynx lengths ratio | 0.95 | 0.96 ± 0.06 (0.86-1.05) | 0.95 ± 0.05 (0.87-1.05) |
| Nerve ring from anterior end | 56 | 59 ± 2.5 (55-62) | 59 ± 3.9 (55-66) |

| | | | |
|---|------|---------------------------|---------------------------|
| Secretory-excretory pore from anterior end | 79 | 86 ± 4.5 (78-94) | 88 ± 6.2 (80-101) |
| Maximum body diam. | 37 | 36 ± 5.0 (25-40) | 49 ± 5.9 (43-60) |
| Cloacal or anal body diam. | 20.0 | 20.2 ± 0.8 (19.0-21.4) | 22.4 ± 1.8 (19.0-25.0) |
| Tail length ¹⁾ | 79 | 85 ± 8.1 (75-95) | 140 ± 25 (102-188) |
| Entire gonad length ²⁾ | 386 | 403 ± 50 (295-463) | - |
| Reflexed part of testis | 85 | 83 ± 14 (63-100) | - |
| <i>Vas deferens</i> length | 115 | 119 ± 15 (96-143) | - |
| Ratio of <i>vas deferens</i> to total gonad length in % | 29.9 | 29.6 ± 2.4 (25.6-32.7) | - |
| Spicule length in curve | 25.7 | 24.6 ± 1.4 (22.1-26.4) | - |
| Spicule length in chord | 24.3 | 23.4 ± 1.4 (21.4-25.0) | - |
| Gubernaculum length in chord | 17.5 | 16.8 ± 1.4 (13.9-18.6) | - |
| Tail spike length | 43 | 45 ± 5.9 (36-54) | - |
| Ratio of tail spike to total tail length in % | 55.2 | 52.4 ± 2.4 (47.6-56.3) | - |
| Vulval body diam. ³⁾ | - | - | 49 ± 5.7 (43-60) |
| Anterior ovary length | - | - | 242 ± 85 (100-371) |
| Posterior ovary length | - | - | 21 ± 84 (105-348) |

| | | | |
|---|---|---|---------------------------|
| Anus-phasmid distance | - | - | 12.6 ± 2.5 (7.1-15.5) |
| Anus-phasmid distance / anal body diam. ratio ⁴⁾ | - | - | 0.56 ± 0.1 (0.38-0.67) |
| Phasmid position to total tail length in % ⁵⁾ | - | - | 9.1 ± 2.4 (6.1-14.0) |

664

665 1) Tail length including tail spike.

666 2) Gonad length including reflexed part and *vas deferens*.

667 3) Body diam. is maximum at vulval position in and female (vulval body diam. = maximum
668 body diam.) in the most cases.

669 4) Calculated as anus-phasmid distance / anal body diam.

670 5) Calculated as 100 x anus-phasmid distance / whole tail length.

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672 **Supplementary Table S1.** The molecular sequences of 115 diplogatrid species
 673 compared in the present study. *Rhabditoides inermis* served as outgroup species.

| Species | 28S (LSU) | 18S (SSU) |
|--|------------------|-----------|
| <i>Rhabditoides inermis</i> (Outgroup species) | EU195981 | AF082996 |
| <i>Sachsia putridicola</i> n. sp. | LC773616* | |
| <i>Acrostichus floridensis</i> | LC374587 | LC374587 |
| <i>Acrostichus halicti</i> | AB455818 | AB455817 |
| <i>Acrostichus megaloptae</i> | AB477074 | AB477077 |
| <i>Acrostichus palmarum</i> RGD194 | LC374584 | LC374584 |
| <i>Acrostichus puri</i> | AB477076 | AB477079 |
| <i>Acrostichus rhynchophori</i> | LC374583 | LC374583 |
| <i>Acrostichus</i> sp. "femorata" | LC530747 | LC530748 |
| <i>Acrostichus ziaelasi</i> | LC530735 | LC530736 |
| <i>Allodiplogaster hylonii</i> | KJ877266 | KJ877224 |
| <i>Allodiplogaster</i> cf. <i>lucani</i> | AB597244 | AB597233 |
| <i>Allodiplogaster seani</i> | JX163970 | JX163979 |
| <i>Allodiplogaster josephi</i> | EU195999 | EU196025 |
| <i>Allodiplogaster sudhausi</i> | KJ877267 | KJ877226 |
| <i>Butlerius</i> sp. VS-2014 | KJ877247 | KJ877204 |
| <i>Cutidiplogaster manati</i> | MT160762 | MT160758 |
| <i>Cutidiplogaster</i> sp. "LT" | MT160763 | MT160759 |
| <i>Demaniella</i> sp. NKZ367 | LC210628 | LC210625 |
| <i>Diplogasteriana schneideri</i> | KJ877246 | KJ877203 |
| <i>Diplogasteriana</i> sp. RS9000 | KJ877245 | KJ877202 |

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|--|-----------|----------|
| <i>Diplogasteroides (Fuchsnema) halleri</i> | KJ877253 | KJ877227 |
| <i>Diplogasteroides (Fuchsnema) sp. RS5537</i> | KJ877254 | KJ877228 |
| <i>Diplogasteroides (Pseudodiplogaster) magnus</i> | KJ877270 | KJ877214 |
| <i>Diplogasteroides (Pseudodiplogaster) nasuensis</i> | LC0276755 | LC027674 |
| <i>Diplogasteroides (Pseudodiplogaster) sp. RS5444</i> | KJ877271 | KJ877215 |
| <i>Diplogasteroides (Rhabdontolaimus) andrassyi</i> | AB808723 | AB808722 |
| <i>Diplogasteroides (Rhabdontolaimus) asiaticus</i> | LC027673 | LC027672 |
| <i>Diplogasteroides (Rhabdontolaimus) luxuriosae</i> | LC099974 | LC099973 |
| <i>Diplogasteroides nix</i> | LC145090 | LC145091 |
| <i>Diplogastrellus gracilis</i> | KJ877249 | KJ877216 |
| <i>Diplogastrellus metamasius</i> | EU419762 | EU419758 |
| <i>Diplogastrellus (Metadiplogaster) sp. RS5608</i> | KJ877248 | KJ877205 |
| <i>Diplogastrellus (Metadiplogaster) sp. "Tadami"</i> | AB597250 | AB597239 |
| <i>Eudiplogasterium levidentum</i> | KJ877258 | KJ877206 |
| <i>Fictor platypaillata</i> | - | MW621342 |
| <i>Fictor stercorarius</i> | KJ877282 | KJ877235 |
| <i>Fictor sp. RS9001</i> | KJ877280 | KJ877233 |
| <i>Fictor sp. RS9002</i> | KJ877281 | KJ877234 |
| <i>Koerneria cf. luziae</i> | AB597243 | AB597232 |
| <i>Koerneria sp. RS9004</i> | KJ877283 | KJ877239 |
| <i>Leptojacobus dorci</i> | KJ877277 | KF924399 |
| <i>Levipalatum texanum</i> | KJ877257 | KJ877221 |
| <i>Mehdinema alii</i> | KJ877285 | KJ877213 |
| <i>Micoletzkyia buetschlii</i> | KJ877252 | JX163973 |

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|--------------------------------------|----------|----------|
| <i>Micoletzkyia calligraphi</i> | KJ531092 | KJ531036 |
| <i>Micoletzkyia hylurginophila</i> | KJ531102 | KJ531046 |
| <i>Micoletzkyia inedia</i> | KJ531104 | KJ531048 |
| <i>Micoletzkyia japonica</i> | JX163967 | JX163976 |
| <i>Micoletzkyia masseyi</i> | JX163968 | JX163977 |
| <i>Micoletzkyia palliati</i> | JX163965 | JX163974 |
| <i>Micoletzkyia sexdentati</i> | KJ531094 | KJ531038 |
| <i>Mononchoides compositicola</i> | - | GU943511 |
| <i>Mononchoides kanzakii</i> | MW763063 | MW649133 |
| <i>Mononchoides macrospiculum</i> | LN827617 | LN827618 |
| <i>Mononchoides striatus</i> | - | AY593924 |
| <i>Mononchoides</i> sp. RS5441 | KJ877262 | KJ877210 |
| <i>Mononchoides</i> sp. RS9007 | KJ877263 | KJ877209 |
| <i>Mononchoides</i> sp. RS9008 | KJ877264 | KJ877211 |
| <i>Mononchoides</i> sp. NK2017 | LC210629 | LC210626 |
| <i>Neodiplogaster acaloleptae</i> | LC107878 | LC107877 |
| <i>Neodiplogaster crenatae</i> | AB326309 | AB326310 |
| <i>Neodiplogaster</i> sp. RGD904 | AB478641 | AB478640 |
| <i>Neodiplogaster</i> sp. RS9009 | KJ877265 | KJ877212 |
| <i>Neodiplogaster unguispiculata</i> | MH048998 | MH049001 |
| <i>Neodiplogaster unguispiculata</i> | MH048996 | MH048999 |
| <i>Neodiplogaster unguispiculata</i> | MH048997 | MH049000 |
| <i>Oigolaimella attenuata</i> | KJ877276 | KJ877219 |
| <i>Oigolaimella</i> sp. RS9010 | KJ877275 | KJ877218 |

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| <i>Oigolaimella</i> sp. RGD844 | AB478631 | AB478630 |
| <i>Oigolaimella</i> sp. RGD884 | AB478633 | AB478632 |
| <i>Onthodiplogaster japonica</i> | LC721118* | |
| <i>Parapristionchus giblindavisi</i> | JX163972 | JX163981 |
| <i>Parasitodiplogaster citrinema</i> | AY840555 | AB901285 |
| <i>Parasitodiplogaster maxinema</i> | AB810253 | AB901283 |
| <i>Parasitodiplogaster nymphanema</i> | LC109318 | LC109317 |
| <i>Parasitodiplogaster obtusinema</i> | LC101737 | LC101736 |
| <i>Paroigolaimella micrura</i> | KJ877259 | KJ877207 |
| <i>Paroigolaimella stresemanni</i> | KJ877261 | KJ877230 |
| <i>Pristionchus aerivorus</i> | KJ705000 | KJ704996 |
| <i>Pristionchus americanus</i> | KJ704999 | KJ704995 |
| <i>Pristionchus arcanus</i> | KT188878 | KT188848 |
| <i>Pristionchus bucculentus</i> | AB852582 | AB852581 |
| <i>Pristionchus entomophagus</i> | KT188873 | KT188843 |
| <i>Pristionchus exspectatus</i> | KT188879 | KT188849 |
| <i>Pristionchus fissidentatus</i> | KJ877273 | KT188855 |
| <i>Pristionchus japonicus</i> | KT188880 | KT188850 |
| <i>Pristionchus lheritieri</i> | KT188876 | KT188846 |
| <i>Pristionchus marianneae</i> | KT188866 | KT188836 |
| <i>Pristionchus maupasi</i> | LC011449 | LC011448 |
| <i>Pristionchus pacificus</i> | EU195982 | U81584 |
| <i>Pristionchus racemosae</i> | KT188888 | KT188859 |
| <i>Pristionchus sycomori</i> | KT188886 | KT188857 |

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|---|----------|----------|
| <i>Pristionchus triformis</i> | KT188884 | KT188854 |
| <i>Pristionchus uniformis</i> | KJ877272 | KJ877236 |
| <i>Pseudodiplogasteroides</i> cf. <i>compositus</i> | AB597248 | AB597237 |
| <i>Pseudodiplogasteroides</i> sp. SB257 | KJ877250 | KJ877217 |
| <i>Pseudodiplogasteroides</i> sp. 'Luc8' | AB597249 | AB597238 |
| <i>Rhabditoides aegus</i> | AB597251 | AB597240 |
| <i>Rhabditoides humicolus</i> | AB440322 | LC095813 |
| <i>Rhabditoides</i> sp. RS5443 | KJ877251 | KJ877229 |
| <i>Rhabditolaimus anoplophorae</i> | AB849949 | AB849946 |
| <i>Rhabditolaimus leuckarti</i> | JQ005870 | JQ005865 |
| <i>Rhabditolaimus</i> sp. RS5442 | KJ877255 | KJ877220 |
| <i>Rhabditolaimus</i> sp. RS5414 | JQ005871 | JQ005866 |
| <i>Rhabditolaimus</i> sp. RSA134 | JQ005872 | JQ005867 |
| <i>Rhabditolaimus</i> sp. "Episcapha" | AB849950 | AB849947 |
| <i>Rhabditolaimus</i> sp. "Euwallacea" | AB849951 | AB849948 |
| <i>Sachsia zurstrasseni</i> | KJ877260 | KJ877208 |
| <i>Sudhausia aristotokia</i> | KJ877278 | KJ877231 |
| <i>Sudhausia crassa</i> | KJ877279 | KJ877232 |
| <i>Sudhausia floridensis</i> | LC214842 | LC214841 |
| <i>Teratodiplogaster fignewmani</i> | AB440311 | AB440308 |
| <i>Teratodiplogaster</i> sp. 1 VS-2014 | KJ877268 | KJ877225 |
| <i>Teratodiplogaster</i> sp. 2 VS-2014 | KJ877269 | KJ877223 |
| <i>Teratodiplogaster variegatae</i> | LC004468 | LC004467 |
| <i>Tylopharynx foetidus</i> | - | EU306343 |

674 * Long sequence including near full SSU, ITS and D1-D4 regions of LSU

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