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Description of *Hirschmanniella dicksoni* n. sp. (Nematoda: Pratylenchidae) from rhizosphere soil of limpograss from Florida, USA

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Abstract

Hirschmanniella dicksoni n. sp. isolated from the rhizosphere soil of limpograss is described and illustrated based on morphology and molecular characters. The new species is characterized by its body length, 1,582 to 1,929 µm, hemispherical lip region with 6 to 7 annules, stylet length 19 to 21 µm, irregularly areolated lateral fields, oval spermatheca filled with rounded sperm, intestine not overlapping the rectum, and tail length 103 to 149 µm, usually with ventral projection with subterminal notch. Males are similar to the females except for their shorter body length and presence of secondary sexual characteristics, with spicules length 19 to 30 µm. The new species is closely related with *H. mucronata, H. belli*, and *H. oryzae.* Compared with these three species, the new species has a relatively longer tail and a terminus shape tail with a ventral projection with a subterminal notch. Other morphological and morphometric characteristics that separate these species are as follows: lip region, number of lip annuli, and spicules length. Molecular sequence analysis using the D2–D3 expansion segments of 28S and the ITS rRNA sequences showed that the new species is genetically distinct. D2–D3 sequence of *H. dicksoni* n. sp. showed 99 and 95% sequence homology with an undescribed species of *Hirschmanniella* isolated from the Colorado River in Yuma, Arizona, and *H. oryzae,* respectively. ITS sequence of the new species also showed 88% sequence homology with *H. oryzae.* The phylogenetic analysis of D2–D3 and ITS region grouped *H. dicksoni* n. sp., as sister species, with *H. oryzae*.

Keywords

Diagnosis, D2–D3, Florida, *Hirschmanniella*, Limpograss, ITS, Morphology, Morphometric, New species, PCR, Phylogeny.

The University of Florida Nematode Assay Laboratory (NAL) received a rhizosphere soil sample for a routine diagnosis purpose from Beef Farm Prairie, Osceola County in Florida. The sample was taken from a site dedicated to limpograss (*Hemarthria altissima*). Limpograss is a warm-season perennial grass used by Florida beef cattle producers because of its high digestibility, adaptation to seasonally flooded soils, cool-season growth, and tolerance of light frost (Newman, 2014). This grass is well adapted to flatwoods sites and to similar areas characterized by a flat to gently sloping topography and poorly drained, sandy soils, where standing water is common during wet weather (Newman et al., 2014).

The owner of this property had recognized a patchy appearance of the field and yield reduction on the limpograss, and sent soil samples for plant-parasitic nematode diagnosis to the NAL in November 2015. We found several individuals belonging to the genus *Hirschmanniella* Luc and Goodey (1964). The

genus *Hirschmanniella* contains 29 valid species as revised by Khun et al. (2015). With the exception of *H. gracilis* (Luc and Goodey, 1964; de Man, 1880), the genus *Hirschmanniella* is considered a harmful pest and is a quarantine taxon for the European Union (Karssen, 2009). These species occur in diverse habitats and hosts in all continents except for Antarctica, and they appear to be well adapted to a broad range of moisture conditions, with a preference for periodically or permanently saturated soils and for graminaceous root systems (Tandingan De Ley et al., 2007).

Hirschmanniella parasitizes diverse plant groups ranging from paddy rice (Maung et al., 2010; Pascual et al., 2014; Khun et al., 2015), crops such as taro (Bridge et al., 1983), cabbage (Duan et al., 1996), maize, tomato, and sugarcane (Bridge et al., 1990) to a number of aquatic plants (Sher, 1968; Gerber and Smart, 1987; Tandingan De Ley et al., 2007). Several species of *Hirschmanniella* are a serious threat to lowland rice fields in Asia (Bridge et al., 1990; Prot et al., 1994; Maung et al., 2010; Win et al., 2013).

Only a handful of species in this genus have been reported in the USA, mainly from Florida and California. In Florida, Sher (1968) reported *H. marina* from a marine plant (*Diplanthera wrightii*), *H. microtyla* from eelgrass (*Vallisneria americana*), and *H. mexicana* (Chitwood, 1951, Sher, 1968) from aquatic rootless plants (*Ceratophyllum demersum*). *Hirschmanniella caudacrena* (Sher, 1968) was also recovered from a tissue of a submerged plant *Ceratophyllum demersum* by Gerber and Smart (1987) and the rice root nematode, *H. oryzae* (Van Breda de Haan, 1902; Luc and Goodey, 1964), was also reported in Florida (www.cabi.org/isc/datasheet/27867#20127201272).

In California, Golden (1957) reported *H. gracilis* from Greene's bur-reed (*Sparganium greenei*), prairie bulrush (*Scirpus paludosus*), and three-square bulrush (*S. americanus*). *Hirschmanniella spinicaudata* (Schuurmans Stekhoven, 1944; Luc and Goodey, 1964) from cattail (*Typha* sp.) and *H. belli* (Sher, 1968) from both cattail and spike rush (*Eleiocharis* spp.) were reported by Sher (1968). *Hirschmanniella pomponiensis* (Abdel-Rahman and Maggenti, 1987) was recorded from saltmarsh bulrush (*Scirpus robustus*) (Abdel-Rahman and Maggenti, 1987). Recently, Tandingan De Ley et al. (2007) reported *H. santarosae* (Tandingan De Ley et al., 2007) from cattail in vernal pools in the Santa Rosa Plateau Ecological Reserve, California.

There were very limited reports of *Hirschmanniella* species from other states in the USA. *H. caudacrena* and *H. oryzae* were isolated from rice fields in Louisiana (Atkins et al., 1955; Sher, 1968). In Hawaii, *H. diversa* (Sher, 1968) was reported from lotus (*Nelumbium nelumbo*). In Texas, *H. mexicana* and *H. oryzae* were recovered from an aquatic rootless plant and a rice field, respectively (Chitwood, 1951; Atkins et al., 1955). In Arkansas, *H. oryzae* and *H. spinicaudata* were isolated from pasture and cattails, respectively (Wehunt et al., 1989). Although it was not identified at the species level, one *Hirschmanniella* sp. was isolated from cattail (*Typha* spp.) along the Colorado River in Yuma, Arizona (Tandingan De Ley et al., 2007).

In this study, we report the results of our morphological, morphometric, and molecular characterization of the new species *Hirchmanniella dicksoni* n. sp. from the rhizosphere soil of limpograss (*Hemarthria altissima*) from Florida.

Materials and methods

Nematode extraction

Soil samples were collected from Beef Farm Prairie, Osceola County in Florida, with latitude 28.087400 and longitude 80.897847, and submitted to the NAL for nematode diagnosis in December 2015. Nematodes were extracted from 100 cm^3 of soil using centrifugation and sugar floatation techniques (Harrison and Green, 1976).

Morphological and morphometric characterization

In total, 20 females and 20 males were used for morphological and morphometric characterization. Nematodes were processed for permanent mounting according to Ryss (2003). Nematodes were picked and transferred to a PCR tube and killed with hot (95°C) 4% formalin. The PCR tube with heat-killed nematodes was immediately placed in a thermo cycler: 95°C for 2 min, 65°C for 10 min, 75°C for 10 min, 85°C for 10 min, and 95°C for 10 min. After the tube reached room temperature, the tube was rinsed with distilled water and the content was transferred to a staining dish. Nematodes were picked out and transferred to a glass slide with cavity filled with a mixture of glycerol and distilled water in proportion of 1:20. Nematodes were infiltrated in glycerin for permanent slide preparation by placing the cavity slide with the nematodes on a hot plate at 70°C for 15 to 20 min and mounted on glass slides following the method described by De Grisse (1969). The specimens were examined for morphological features and morphometrics using a camera installed on

ZEISS AXIO microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany). Measurements and pictures were taken using the ZEN lite software on ZEISS Axiocam ERc5s digital camera. Morphological and morphometric characteristics were compared with identification keys for genus *Hirschmanniella* revised by Khun et al. (2015).

Scanning electron microscopy

Adults and juveniles of the new species were collected individually by hand and washed with sodium phosphate buffered in 3% glutaraldehyde solution for 24 hr at 4°C. The specimens were then post fixed overnight in 3% Osmium tetraoxide solution and dehydrated in a concentration gradient of ethanol. Dehydrated nematodes were dried with a critical point dryer, attached to a self-adhesive stub, and coated with gold/palladium auto sputter coater. Specimens were studied with a Hitachi SU5000 electron microscope at the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, Gainesville, FL.

Molecular characterization

Total genomic DNA was extracted following the sodium hydroxide method (Wang et al., 1993). A single female was transferred to 20 µl of 0.25 NaOH in 1.5 ml eppendorf tubes, crushed in a sterile glass rod, and incubated overnight at 25°C. The tubes were then heated at 99°C for 3 min and cooled down to room temperature. In total, 5 µl of 1 M HCl, 10 µl of 0.5 M Tris-HCl (pH 8.0), and 5 µl of 2% Triton X-100 were added to the lysate and mixed briefly, and heated at 99°C for 3 min. The digest was cooled to room temperature and 1.5 µl of the digest was immediately used for polymerase chain reaction (PCR). DNA amplification was performed in 25 µl of reaction mix containing 12.5 µl 2× Hot Start Master mix (New England BioLabs, Ipswich, MA), 1.25 µl forward and reverse primers, 8.5 µl sterile nuclease free water (Thermo Fisher Scientific, Gainesville, FL), and 1.5 µl template DNA. For amplification of the ribosomal region spanning the internal transcribed spacer (ITS)1, 5.8 S gene, and ITS2, the primers TW81R (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28F (5′-ATATGCTTAAGTTCAGCGGGT-3′) were used. D2–D3 expansion segment of the large subunit (LSU) rDNA was amplified using the primers sets D2A (5′-CAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′). The thermocycling was carried out using a Master cycler nexus (Eppendorf AG, Hamburg, Germany). The thermocycling reactions for both DNA regions were as follows: 95°C for 15 min, followed by 40 cycles of 94°C for 30 sec, 50°C for 45 sec, and 72°C for 2 min, and a final extension step of 72°C for 7 min.

DNA fragments were separated by electrophoresis in 1XTAE buffer and 1.2% agarose gel, stained with ethidium bromide, and visualized under UV light using the ChemiDOC XRS One 4.5.2 program (Bio-Rad Laboratories, Life Science Group, Hercules, CA). The DNA fragments were purified using QIAquick PCR Purification kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The purified DNA was quantified using a NanoDrop 1000 (Thermo Science, Grand Island, NY) spectrophotometer and sequenced at the ICBR, University of Florida, Gainesville, FL. To identify the species, raw sequences were checked and edited manually using BioEDIT, v. 7.2.5 (Hall, 1999). Consensus sequences obtained were compared with those deposited in the GenBank database using BLAST engine search for sequence homology. The newly obtained consensus sequences were submitted to the GenBank database under accession numbers KY773577 for D2–D3 of 28S gene and KY773578 for ITS rRNA.

Phylogenetic analysis

To perform the phylogenetic analysis, D2–D3 LSU, ITS1-5.8S-ITS2 rDNA sequences obtained from *H. dicksoni* n. sp. identified in this study and those retrieved from the GenBank and NCBI databases (Tables 1, 2) were aligned over the same length in MUSCLE using MEGA v. X (Kumar et al., 2018). The alignment was analyzed to get the base substitution model for these sequences using MEGA X (Kumar et al., 2018). For D2–D3 LSU sequences, the evolutionary history was inferred by using the Kimura 2-parameter model (Kimura, 1980). For ITS1-5.8S-ITS2 rDNA sequences, the evolutionary history was inferred by using the Tamura 3-parameter model (Tamura, 1992). Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log-likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The phylogram was generated using base substitution model selected for each alignment and running maximum likelihood model with 1,000 bootstrap replicates using MEGA X (Kumar et al., 2018) to assess the degree of support for each branch on the tree (Landa et al., 2008). Unique data sequences obtained from this

Table 1. List of 28S ribosomal RNA gene partial sequences (D2–D3 segment) of *Hirschmaniella* and outgroup species used for phylogenetic analysis.

Species	Accession No.	Location
H. dicksoni n. sp.	KY773577	Florida
Hirschmanniella sp.	EU620464	South Africa
Hirschmanniella sp.	KY794704	Iran
Hirschmanniella sp.	JX261958	Iran
Hirschmanniella sp.	EU620465	South Africa
H. diversa	JX144359	Japan
H. santarosae	EF029859	California
H. pomponiensis	DQ077795	California
H. kuazuna	EU620466	South Africa
H. kuazuna	EU620467	South Africa
H. loofi	EU620468	Belgium
H. loofi	EU620469	Belgium
H. mucronata	KF201167	The Philippines
H. mucronata	KP179327	Cambodia
H. mucronata	KP179333	Cambodia
H. mucronata	KY424320	China
H. mucronata	MK241938	Indonesia
Hirschmanniella sp. Yuma	EF029861	Arizona
H. belli	EF029860	California
H. oryzae	JX291142	Myanmar
H. oryzae	KF201165	The Philippines
Hirschmanniella sp.	DQ328686	Vietnam
Hirschmanniella sp	KP671713	Belgium
Pratylenchus vulnus	JX047010	USA

study were submitted to GenBank under accession numbers KY773577 and KY773578 for D2–D3 LSU and ITS1-5.8S-ITS2 rDNA sequences, respectively. The D2–D3 sequence from *Pratylenchus vulnus* was used as outgroup for constructing the phylogenetic tree based on the sequences form D2–D3 expansion segment of 28S rRNA, whereas *P. bolivianus* was used as outgroup in the case of phylogenetic tree based on ITS1-5.8S-ITS r DNA sequence.

Results

Systematics

Hirschmanniella dicksoni n. sp.
(Figs. 1–3; Table 3).

Females

They are characterized by slender body, slightly curved to irregularly shaped, 1,582 to 1,929 µm long. The maximum body diameter is 26 to 31 µm. Lip region is hemispherical (Figs. 1C, 3A), with diameter 9.1 to 11.2 µm and height 3.7 to 4.8 µm, and six to seven transverse annules without longitudinal lines, continuous with body contour. Some lip region annules are incomplete. Cephalic framework is moderately sclerotized. Stylet is 1.9 to 2.1 times as long as lip region diameter; stylet knobs are round, clearly offset, and stylet cone is 1.07 to 1.1 times as long as shaft. The position of excretory pore (EP) is 124 to 146 µm from the anterior end. Oesophageal glands are elongated, tapering posteriorly

Table 2. List of 18S ribosomal RNA gene partial sequences (ITS rRNA) of *Hirschmaniella* and outgroup species used for phylogenetic analysis.

Species	Accession No.	Location
H. dicksoni n. sp.	KY773578	Florida
H. mucronata	KP179328	Belgium
H. mucronata	KF201166	The Philippines
H. mucronata	DQ309589	Taiwan
H. mucronata	KP179329	Belgium
H. mucronata	KJ923642	China
H. mucronata	KP179331	Belgium
H. loofi	EU620472	South Africa
H. loofi	EU620473	South Africa
H. kuazuna	EU620471	South Africa
H. kuazuna	EU620470	South Africa
H. oryzae	KF201164	The Philippines
H. oryzae	KF201163	The Philippines
H. oryzae	DQ309588	Taiwan
Pratylenchus bolivianus	FR692329	Italy

and overlapping intestine ventrally. Hemizoid is anterior to EP. Lateral incisures on lateral fields begin about 26 annules from anterior end as two fully areolated lines and becoming four lines towards posterior end. Areolation is irregular and incomplete in the middle of lateral field. Female genital branches are opposed and outstretched. Vulva is located at 50 to 56% of the body length. Spermatheca is oval and filled with rounded sperm. Intestine is not overlapping rectum. The shape of tail terminus is variable, with a ventral projection usually with subterminal notch (Figs. 1B, 3F).

Males

They are similar to females in general morphology except for secondary sexual character and shorter body length, ranging 1,235 to 1,772 µm. Male genital system is monorchic and outstretched. Spicules are medium-sized, 19 to 30 µm long, well developed and arcuate ventrally. Gubernaculum is simple, straight or curved, not protruding, ca 7.4 to 11.3 µm or 27 to 38.9% of spicules length. Bursa is leptoderan. The shape of tail terminus is variable, with pointed terminus without a projection and round terminus without a projection (Fig. 2). Tail terminus with subterminal notch is rare in males.

Type habitat and locality

Soil samples were collected from Beef Farm Prairie, Osceola County, Florida with latitude 28.087400 and longitude 80.897847. The new species was collected from a waterlogged wetland rhizosphere soil associated with limpograss.

Type material

Holotype female and Paratypes (NemaLAB 0001) and paratypes (NemaLAB 0002) are deposited at the NAL, University of Florida nematode collection.

Etymology

The species is named in honor of Dr Donald W. Dickson for his over 50 years of outstanding service as a mentor, trainer, and researcher at the University of Florida.

ZOOBANK Code: zoobank.org:pub:6DD36DA0-6544-406D-8F4C-371EE443E115.

Diagnosis and relationships

The main diagnosis characteristics of *Hirschmanniella dicksoni* n. sp. are as follows: body length less than

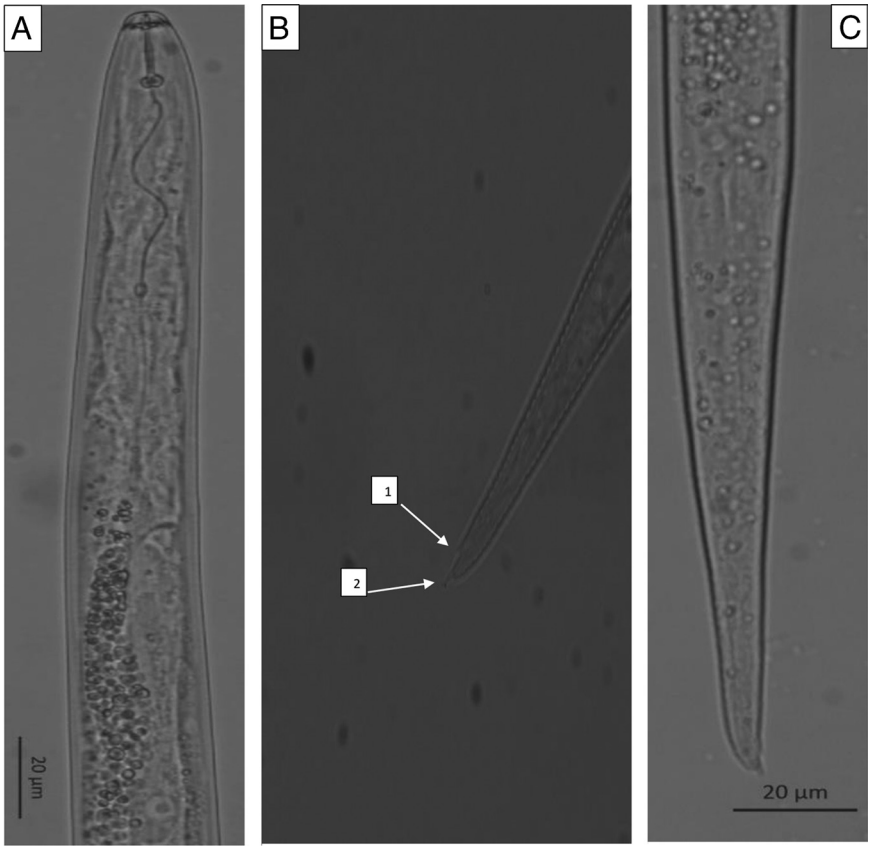

Figure 1: *Hirschmanniella dicksoni* n. sp. female: (A) Anterior region; (B) tail region (arrow 1: subterminal notch, arrow 2: ventral projection); (C) tail region. Scale bars in A, B, C = 20 µm.

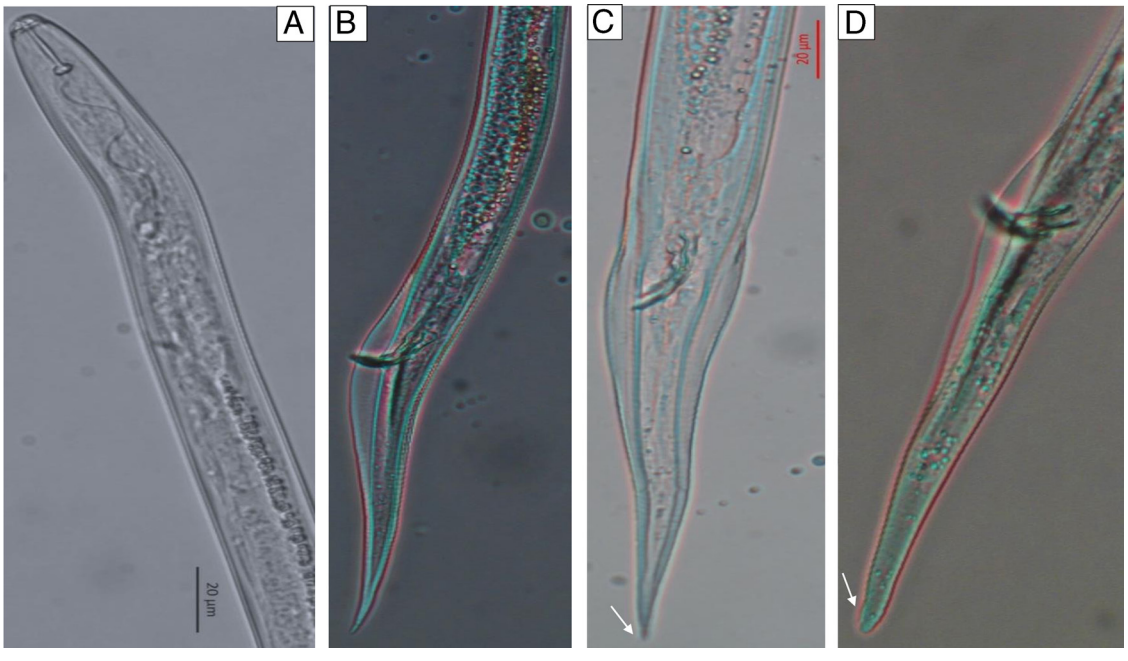

Figure 2: *Hirschmanniella dicksoni* n. sp. male: (A) Anterior region; (B, C, D) tail region (arrows: pointed and round tail terminus). Scale bars in A, B, C, D = 20 µm.

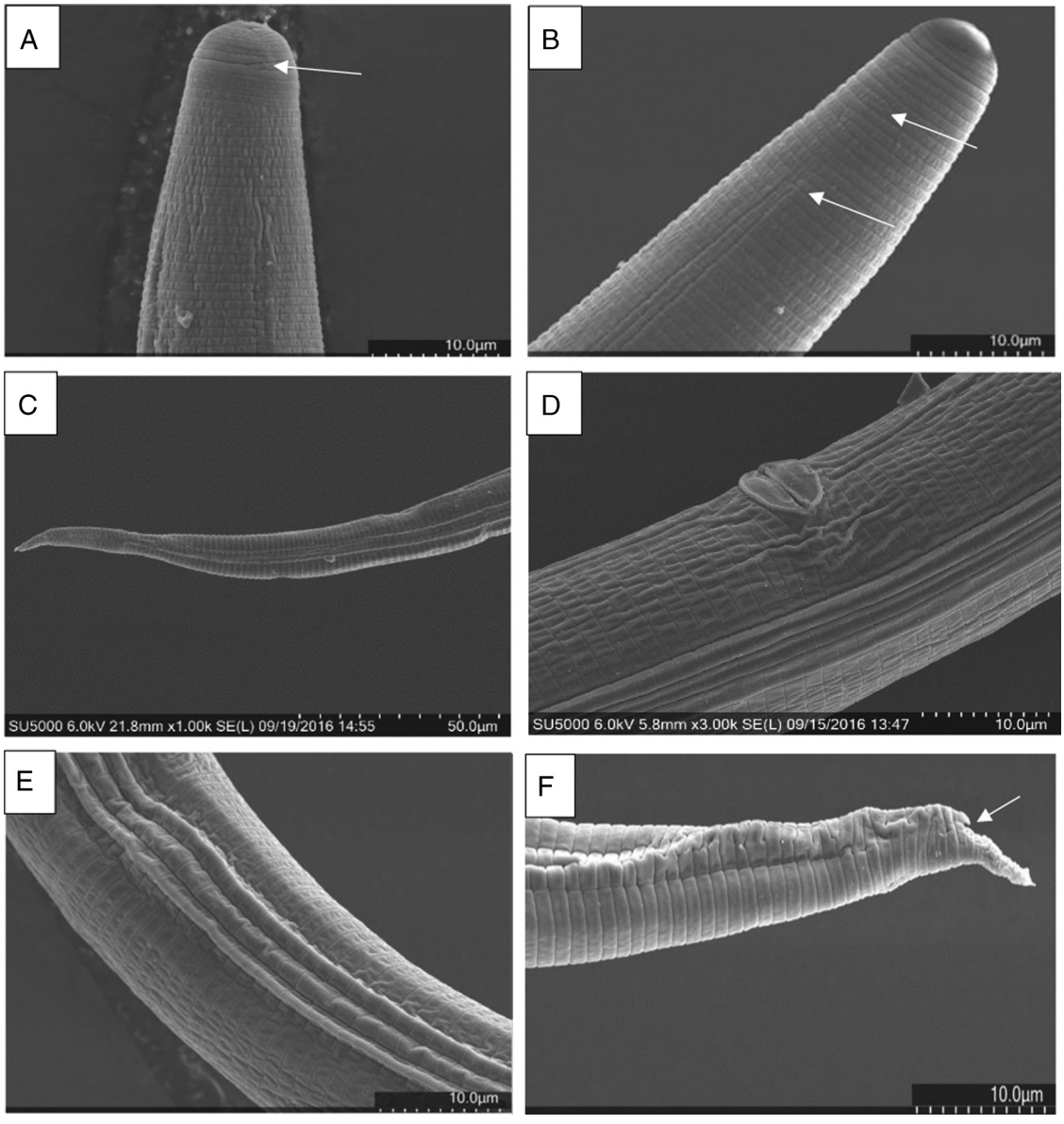

Figure 3: SEM photomicrographs of *Hirschmanniella dicksoni* n. sp. female: (A) Lip region (arrow: incomplete lip annules); (B) lateral field in anterior region (arrows: incomplete annules; rings and incisures on the beginning on lateral field); (C) posterior region; (D) vulva and lateral fields; (E) lateral field in middle body region showing incomplete aerolations; (F) tail region with subterminal notch (arrow: subterminal notch).

2 mm, hemispherical lip region with 6 to 7 lip annuli, stylet length 19 to 21 µm with round knobs, excretory pore at or anterior of oesophageal–intestinal junction (OIJ) up to 32 µm anterior to the latter, irregularly areolated lateral fields, spermatheca oval and filled with rounded sperm, intestine not overlapping the rectum. Tail length is 103 to149 µm, and tail terminus has a ventral projection usually with subterminal notch. Males are present and are similar to the females except for shorter body length and secondary sexual characters. Male spicules are 19 to 30 µm in length.

Female *Hirschmanniella dicksonsi* n. sp. closely resembles *H. mucronata* (Das, 1960; Luc and Goodey, 1964) in morphology and morphometry, but it can be distinguished from *H. mucronata* by the following characteristics: it has a relatively longer tail (103–149 vs 60–160 µm). Usually the tail terminus has a ventral projection and subterminal notch vs

Table 3. Morphometrics of *Hirschmanniella dicksoni* n. sp.

	Female		Male
Character	Holotype	Paratypes	Paratype
n	–	19	20
L	1,906	1714 ± 152 (1,582–1,929)	1506 ± 143 (1,235–1,772)
a	61	59 ± 4.9 (50–69)	60 ± 6.3 (47–72)
b	11	11 ± 1.1 (10–14)	9.6 ± 0.9 (8.6–11.3)
b′	4.1	4.2 ± 0.4 (3.8–4.8)	4.3 ± 0.4 (3.8–5.1)
c	13	14 ± 0.9 (13–15)	15 ± 1.5 (13–18)
c′	6.7	6.2 ± 0.5 (5.1–6.7)	6.2 ± 0.8 (4.8–7.2)
G1	22	23 ± 2.3 (20–26)	–
G2	20	20 ± 1.8 (17–24)	–
T	–	–	37 ± 5 (29–45)
V	55	53 ± 1.7 (50–56)	– –
M	51	52 ± 1.7 (51–55)	54 ± 1.9 (51–57)
o	23	22 ± 2.2 (19–27)	21 ± 2.9 (17–27)
DGO	4.5	4.3 ± 0.4 (3.8–5.1)	4 ± 0.6 (3.3–5.1)
Lip region diameter	10	10 ± 0.7 (9.1–11.2)	9.9 ± 0.4 (9.2–11)
Lip region height	3.7	4.1 ± 0.3 (3.7–4.8)	4.3 ± 0.3 (4.0–5.1)
Stylet length	19	20 ± 0.6 (19–21)	19 ± 0.5 (18–20)
Conus length	9.8	10 ± 0.5 (9.7–11)	10 ± 0.4 (9.6–11)
Shaft length	9.5	9.3 ± 0.4 (8.6–9.9)	8.7 ± 0.4 (8.2–9.6)
Stylet knob height	3	2.7 ± 0.4 (2–3.4)	2.7 ± 0.2 (2.4–3.2)
Stylet knob width	4.6	4.5 ± 0.4 (3.8–5.3)	4.3 ± 0.5 (3.4–5.0)
Procorpus length	80	63 ± 13.8 (50–87)	70 ± 7.2 (54–80)
Median bulb length	17	17 ± 1.4 (15–20)	16 ± 1.3 (14–18)
Median bulb diam.	13	12 ± 0.8 (11–13)	11 ± 1.4 (7.5–13)

Median bulb valve length	5.8	5 ± 0.7 (3.5–6.3)	4.7 ± 0.7 (3.8–6)
Median bulb valve width	3.6	2.9 ± 0.4 (2.3–3.6)	2.9 ± 0.5 (2.1–3.6)
Anterior end to oesophageal-intestinal junction	177	154 ± 12 (142–177)	158 ± 13 (129–179)
Anterior end to excretory (EP) pore	146	133 ± 7 (124–146)	119 ± 11 (87–134)
EP pore to oesophageal-intestinal junction	31	25 ± 4.3 (20–32)	39 ± 8.9 (24–50)
Metacorpus valve from anterior end	89	85 ± 4.1 (80–94)	80 ± 4.7 (72–87)
Nerve ring from anterior end	116	113 ± 5 (105–122)	102 ± 10 (73–114)
Oesophageal gland overlap	294	257 ± 34 (204–311)	193 ± 23 (150–223)
Oesophagus length	470	411 ± 38 (330–470)	351 ± 30 (303–392)
Max body length	31	29 ± 1.6 (26–31)	26 ± 3.1 (21–30)
Length from phasmids to terminus	32	29 ± 2 (25–32)	32 ± 2.9 (27–35)
Tail length	149	125 ± 11 (103–149)	102 ± 11 (84–119)
Anal body width	22	20 ± 1 (18–22)	17 ± 1.8 (15–20)
From cloaca to anterior most part of testis		–	559 ± 92 (420–708)
Vulva to anterior end	1045	912 ± 90 (803–1056)	–
Anterior genital branch	420	391 ± 57 (305–472)	–
Posterior genital branch	385	333 ± 39 (289–433)	–
Ant. Spermatheca length	26	28 ± 4.2 (20–35)	–
Ant. Spermatheca diam	15	16 ± 2.6 (9.3–19)	–
Post. Spermatheca length	24	24 ± 3.9 (17–30)	–
Post. Spermatheca diam	14	14 ± 2 (8.9–17)	–
Bursa	–	–	70 ± 15 (45–94)
Cloaca to anterior tip of bursa	–	–	25 ± 5.8 (15–33)
Cloaca to posterior tip of bursa	–	–	45 ± 9.5 (27–61)
Gubernaculum	–	–	8.9 ± 1.1 (7.4–11)
Spicules	–	–	25 ± 2.9 (19–30)

–, data not available. All measurements are in µm and in the form: mean ± SD (range).

usually pointed or round with or without a projection, but no subterminal notch; relatively smaller *c* (13–15 vs 13–30 µm), but relatively greater *c'* (5.1–6.7 vs 2.5–5.5 µm), relatively longer procorpus (50–87 vs 31–62 µm), relatively longer distance of OIJ from anterior end (142–177 vs 91–150 µm), excretory pore (124–146 vs 83–141 µm), and nerve ring position (105–122 vs 74–107 µm) (Table 3) (Khun et al., 2015).

The new species *H. dicksoni* n. sp. differs from *H. oryzae* and *H. belli* by having a hemispherical lip region with six to seven lips annuli vs no hemispherical lip region with three to four lips annuli and a tail terminus with ventral projection and subterminal notch vs a tail terminus with projection or mucron. *Hirschmanniella dicksoni* n. sp. also differs in spicules length (19–30 vs 31–36 µm) from *H. belli*, but has similar spicules length (19–30 vs 18–34 µm) as *H. oryzae*. However, *H. dicksoni* n. sp. is similar in stylet length to *H. belli* (19–21 vs 18–22 µm) and *H. oryzae* (19–21 vs 13–21 µm), as well as body length (1,582–1,929 µm), (1,420–2,220 µm), (900–1,850 µm), respectively (Khun et al., 2015).

Hirschmanniella dicksoni n. sp. has a longer body (1,582–1,929 µm) than *H. brassicae* (Duan et al., 1996) (920–1,420 µm), *H. microtyla* (930–1,450 µm), and *H. shamimi* (Ahmad, 1972) (1,190–1,540 µm). The new species has a shorter body (1,582–1,929 µm) than *H. furcate* (Razjivin et al., 1981) (2,830–2,990), *H. imamuri* (Sher, 1968) (2,150–272 µm), *H. obesa* (Razjivin et al., 1981) (3,240–3,410 µm) and *H. truncata* (Razjivin et al., 1981) (2,180–2,430 µm). *Hirschmanniella dicksoni* n. sp. possesses greater *c'* ratio (5.1–6.7) than *H. areolata* (Ebsary and Anderson, 1982) (3.1–4.6), *H. mexicana* (6.2 vs 4.8), *H. microtyla* (3.0–4.9), *H. miticausa* (Bridge et al., 1983) (3.4–4.9), and *H. thornei* (Sher, 1968) (3.2–4.8), but smaller *c'* ratio (6) than *H. zostericola* (Allgén, 1934; Luc and Goodey, 1964) (9) (Khun et al., 2015).

The hemispherical lip region in *H. dicksoni* n. sp. is similar to *H. areolate*, *H. behningi* (Micoletzky, 1923; Luc and Goodey, 1964), *H. diversa*, *H. furcate*, *H. imamuri*, *H. loofi* (Sher, 1968), *H. mucronata*, *H. obesa*, *H. pisquidensis* (Ebsary and Pharoah, 1982), *H. spinicaudata*, *H. thornei,* and *H. truncate*. Based on stylet length, *H. dicksoni* n. sp. (19–21 µm) is close to *H. belli* (18–22 µm), *H. caribbeana* (Van den Berg and Quénéhervé, 2000) (17–20 µm), *H. caudacrena* (17–23 µm), *H. gracilis* (20–24 µm), *H. kwazuna* (Van den Berg et al., 2009) (18–22.5 µm), *H. marina* (20–25 µm), *H. mexicana* (20 µm), *H. microtyla* (17.6–21.5 µm), *H. miticausa* (Bridge et al., 1983) (18.5–21 µm), *H. oryzae* (13–21 µm), *H. pomponiensis* (17–23 µm), and *H. santarosae* (17–21 µm) (Khun et al., 2015).

Out of the 29 nominal species (Khun et al., 2015), *H. dicksoni* n. sp. has a longer stylet (19–21 µm) than *H. brassicae* (14–16 µm) and *H. zostericola* (15 µm) and shorter stylet than *H. areolata* (24–27 µm), *H. behningi* (24–28 µm), *H. diversa* (22–25 µm), *H. furcate* (38.0–38.5 µm), *H. imamuri* (29–32 µm), *H. loofi* (31–41.6 µm), *H. obesa* (40.9–41.5 µm), *H. pisquidensis* (23–25 µm), *H. spinicaudata* (38–50 µm), *H. thornei* (26–30 µm), and *H. truncata* (35.7–38.5 µm) (Khun et al., 2015).

Hirschmanniella dicksoni n. sp. has a tail terminus shape with single ventral projection usually with a subterminal notch, which is similar to *H. caudacrena, H. furcate, H. halophile, H. marina, H. mexicana*, and *H. obesa,* but it can be differentiated from these species as follows: *H. dicksoni* n. sp. differs from *H. caudacrena* with its hemispherical lip region and ventral tail projection vs no hemispherical lip region and axial tail projection. *Hirschmanniella dicksoni* n. sp. differs from *H. furcate* with its shorter body length (1,582–1,929 vs 2,830–2,990 µm), smaller *c* (12.6–15.3 vs18.3–20.4), shorter stylet length (19–21 vs 38.0–38.5 µm), greater *a* (50.3–68.6 vs 45.3–47) and *b* (10.0–14.0 vs 5.9–6.4). *Hirschmanniella dicksoni* n. sp. differs from *H. halophila* with its hemispherical lip region and EP anterior to OIJ vs no hemispherical lip region and EP posterior to OIJ. *Hirschmanniella dicksoni* n. sp. differs from *H. marina* with its hemispherical lip region, shorter spicules (19–30) vs no hemispherical lip region and longer spicules (34–36 µm). *Hirschmanniella dicksoni* n. sp. is differentiated from *H. mexicana* with its greater *a* (59 vs 37), smaller *b'* (4.2 vs 5.5) and *c* (14 vs 17) ratios, V value (53 vs 49) and its hemispherical lip region and EP anterior to OIJ vs no hemispherical lip region and EP posterior to OIJ. *Hirschmanniella dicksoni* n. sp. differs from *H. obesa* with its shorter body length (1,582–1,929 vs 3,240–3,410 µm), smaller *c* (13.0–15.0 vs 18.2–20.4), shorter stylet length (19–21 vs 40.9–41.5 µm), and greater *a* (50.0–69.0 vs 39.4–41), *b* (9.8–13.6 vs 6.3–6.6), and *c'* (5.1–6.7 vs 3.1–4.9).

Hirschmanniella dicksoni n. sp. differs from *H. spinicaudata* with the presence of subterminal notch and ventral projection, EP anterior to OIJ vs absence of subterminal notch and axial mucron and EP posterior to OIJ. *Hirschmanniella dicksoni* n. sp. also differs from *H. spinicaudata* with its smaller *c* (12.6–15.3 vs 17–28), shorter stylet (19–21 vs 38–50), and shorter spicules (22–30 vs 41–54).

Molecular characterization and phylogenetic analysis

The amplification of D2–D3 expansion segments of 28S rRNA yielded a single fragment of approximately 782 bp based on direct fragment sequencing. Sequences from other species of *Hirschmanniella*

obtained from NCBI were used for further phylogenetic studies (Table 1). Based on the BLAST analysis, D2–D3 expansion segments of 28S rRNA of *H. dicksoni* n. sp. showed 99% similarity (91% query cover) with an undescribed species of *Hirschmanniella* isolated from the Colorado River in Yuma, Arizona (EF 029861), whereas they showed 96% similarity (90% query cover) with *H. belli* (EF029860) and 95% similarity (89% query cover) with *H. oryzae* (JX291141).

Although D2–D3 expansion segments of 28S rRNA from *H. dicksoni* n. sp. species showed 99% similarity over 100% query cover with two isolates identified as *H. mucronata* from Jiangsu, China (KR780066 and KR780067), these sequences showed closer similarity with *H. oryzae* than other isolates of *H. mucronata* from the Philippines (KF201167) and Cambodia (KP179327 and KP179333). Therefore, we did not include these sequences in the phylogenetic analysis because we did not consider these sequences as *H. mucronata*. The phylogenetic analysis excluding these sequences from China isolates indicates that the sequences from *H. dicksoni* n. sp. and other species of *Hirschmanniella* obtained from GenBank were grouped into three different clades (Fig. 4). The D2–D3 expansion segments of 28S rRNA from *H. dicksoni* n. sp. clustered together with the undescribed species of *Hirschmanniella* from Yuma (Arizona) and Vietnam, *H. belli* from California, and *H. oryzae* in Clade I. The second clade included *H. loofi*, *H. kwazuna*, and *H. mucronanta* from the Philippines and Cambodia. The isolates of *H. halophila*, *H. diversa*, *H. santarosae*, and *H. pomponiensis* were grouped in Clade III.

The amplification of ITS1-5.8S-ITS2 r DNA yielded a single fragment of approximately 787 bp based on sequencing. Sequences from other species of *Hirschmanniella* obtained from NCBI were used for further phylogenetic studies (Table 2). The similarity search using BLAST tool showed that the ITS

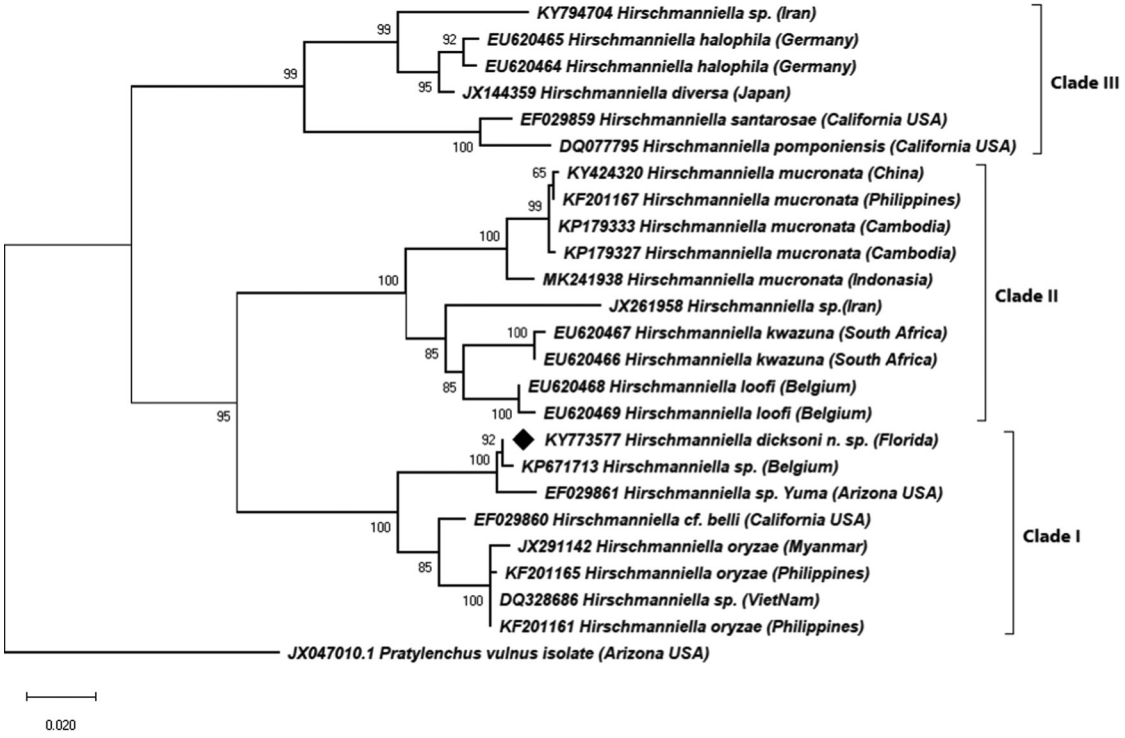

Figure 4: Phylogenetic relationship based on D2–D3 expansion segments of 28S rRNA gene sequences within the genus *Hirschmanniella*. The evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model. The tree with the highest log likelihood (–3,442.77) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3519)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 25 nucleotide sequences. There were a total of 795 positions in the final data set. Accession preceded by ♦ is a new sequence.

sequence from *H. dicksoni* n. sp. was 88% similar to *H. oryzae* (Vietnam isolate) over 99% query cover. As in the case of D2–D3 sequence analysis, the BLAST search using ITS sequence from *H. dicksoni* n. sp. also showed 96% similarity with sequences from an isolate of *H. mucronata* (KR780069) from Jiangsu, China, over 100% query cover. However, KR780069 showed higher similarity with *H. oryzae* sequence (88%) than with other isolates of *H. mucronata* sequences (84%) over 100% query cover. In contrast to this, another sequence (KJ923642) identified as *H. mucronata* from the same study (Jiangsu, China) showed only 85% similarity with *H. dicksoni* n. sp. over 74% query cover. The sequence KJ923642 showed 99% similarity with sequences from other isolates of *H. mucronata*, whereas the sequence KR78069 showed only 84% with *H. mucronata* over 100% query cover. Thus, since the identity of the China isolate KR78069 as *H. mucronata* was not certain based on the sequence analysis, we did not include this sequence in the phylogenetic analysis. The phylogenetic analysis excluding KR78069 showed that the sequences from *H. dicksoni* n. sp. and other isolates from GenBank were grouped into two clades. *Hirschmanniella dicksoni* n. sp. was grouped, as a sister species, with isolates of *H. oryzae* in Clade I, which was similar to the phylogenetic analysis based on D2–D3 sequence. The second clade included *H. loofi*, *H. kwazuna* and *H. mucronata* from the Philippines, Belgium, Taiwan, and China.

Discussion

We have characterized *H. dicksoni* n. sp. based on morphological, morphometric, and molecular data. Our analysis confirmed that *H. dicksoni* n. sp. from Florida is a distinct species in the genus *Hirschmanniella*. Morphology and morphometric characteristics of *H. dicksoni* n. sp. closely resemble *H. mucronata*. However, *H. dicksoni* n. sp. has smaller *c*, but greater *c'* and procorpus length compared to *H. mucronata* (Sher, 1968; Sivakumar and Khan, 1982; Liao et al., 2000; Chen et al., 2006). The new species also has longer distance from anterior end to OlJ compared to *H. mucronata* (Khun et al., 2015). The nerve ring position was more positerior in *H. dicksoni* n. sp. than *H. mucronata*. The other prominent deviation of *H. dicksoni* n. sp. from *H. mucronata* is a longer tail compared to *H. mucronata* and the tail terminus usually with subterminal notch (Ahmad, 1972; Chen et al., 2006; Khun et al., 2015).

Our data show the presence of interaspecific variation in the female tail shape in *H. dicksoni* n. sp., with some of the individuals having a tail terminus with ventral projection and others having a tail terminus with a ventral projection and subterminal notch (Fig. 3). Variation in male tail shape is also observed, with some individuals having a pointed tail terminus without a projection and others having a round terminus without a projection (Fig. 2). Our observation was in line with the intraspecific variations observed among *H. mucronata* populations from different localities in Cambodia (Khun et al., 2015).

Previous studies have shown that a slight difference in the morphology and morphometrics might lead to the description of new species, resulting in over estimation of species in the genus *Hirschmanniella*. However, these species were later considered as synonyms of a well-characterized species (Khun et al., 2015). However, *Hirschmanniella* also comprises cryptic species such as *H. santarosae* and *H. pomponiesis* that can be overlooked based on morphological observation alone (Tandingan De Ley et al., 2007). It is very likely that the genus includes cases of synonymous species, as well as cryptic species that are morphologically confounding, but nevertheless genetically distinct (Tandingan De Ley et al., 2007). Hence, a combination of morphological, morphometric and molecular data would provide accurate species identification and diversity studies of *Hirschmanniella* sp.

The new species showed 99% D2–D3 sequence homology with 94% query coverage to undescribed species of *Hirchmanniella* sp. from Yuma, Arizona (EF029861). However, we could not compare the morphology and morphometric characters of these isolates due to the lack of morphology and morphometric data from Yuma isolate (Tandingan De Ley et al., 2007). D2–D3 expansion segments of 28S rRNA of *H. dicksoni* n. sp. showed 98% (KR780066) and 95% (KR780067) similarity with two isolates of *H. mucronata* from Jiangsu, China. Despite the high similarity with *H. dicksoni* n. sp., these sequences were not included in the phylogenetic analysis because of the uncertainty in their identification as *H. mucronata*. In our sequence analysis, KR780066 and KR780067 showed closer similarity with *H. oryzae* than other isolates of *H. mucronanta* from the Philippines (KF201167) and Cambodia (KP179327 and KP179333) (Hui et al., 2016). Similarly, our initial phylogenetic analysis including these sequences showed that the isolates from China were clustered with *H. oryzae*, whereas the other isolates of *H. mucronata* were grouped together with *H. loofi* and *H. kwazuna* in a different clade as observed in the original study by Hui et al. (2016). Our D2–D3 phylogenetic tree is generally similar to Khun et al. (2015) D29D3 phylogentic tree

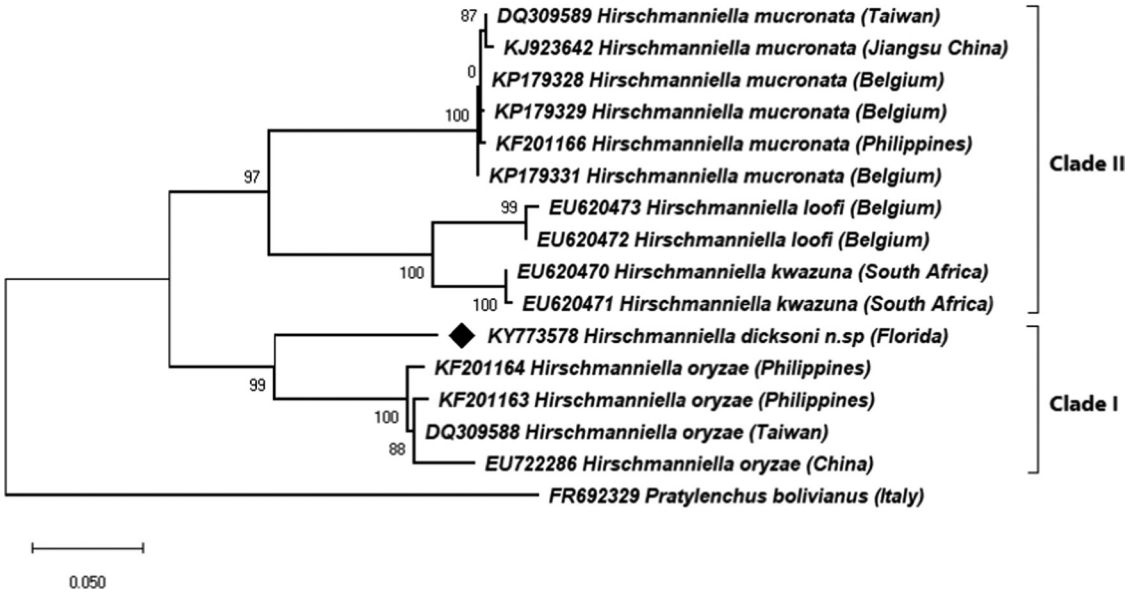

Figure 5: Phylogenetic relationship based on ITS rRNA sequences within the genus *Hirschmanniella*. The evolutionary history was inferred by using the maximum likelihood method and Tamura 3-parameter model. The tree with the highest log likelihood (–4,485.75) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.9061)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 16 nucleotide sequences. There were a total of 1,486 positions in the final data set. Accession preceded by ♦ is a new sequence.

where the *H. dicksoni* n. sp. fell in Clade III as sister taxon with *H. oryzae*.

As in the case of D2–D3 sequence analysis, the BLAST search using ITS sequence from *H. dicksoni* sp. n. showed 96% similarity with an isolate of *H. mucronata* sequences (KR780069) from China over 100% query cover. However, KR780069 showed higher similarity with *H. oryzae* sequence (88%) than with other isolates of *H. mucronata* sequences (84%) over 100% query cover. In contrast, another sequence (KJ923642) identified as *H. mucronata* from the same study showed only 85% similarity with *H. dicksoni* n. sp. over 74% query cover. The sequence KJ923642 showed 99% similarity with the sequences from other isolates of *H. mucronata*. As in the case of D2–D3 sequence, KR780069 was not included in the authors' phylogenetic analysis, but KJ923642, which has only 85% similarity with *H. dicksoni* n. sp., was included. In this analysis, KJ923642 clustered with *H. mucronata* (Hui et al., 2016). Thus, the identity of the China isolate, KR780069, as *H. mucronata* was not very certain based on the sequence analysis, and we did not include this sequence in the phylogenetic analysis.

Apart from the inconsistences in the molecular data, we studied the morphology and morphometric data of the China isolates from Hui et al. (2016). We found differences between *H. mucronata* from China and *H. dicksoni* n. sp. from Florida. Morphologically, most *H. dicksoni* n. sp. female isolates possess tail terminus with ventral projection with subterminal notch (Figs. 1B, 3F), but no subterminal notch was reported from China isolates (Fig. 1D,G,J; Hui et al., 2016). Morphometrically, *H. dicksoni* n. sp. has shorter stylet length (19–21 vs 24–27 μm) for females and (19–21 vs 24–25 μm) for males, smaller *b* (10–14 vs 16–21) for females and *c* (13–15 vs 22–29) for females and (13–18 vs 16–20) for males, and greater *c'* (5–7 vs 4–5) for females and (5–7 vs 4.6–5) for males.

This is a first report on the occurrence of *Hirschmanniella* associated with limpograss from Florida. Limpograss is adapted only to moist flatwoods soils and is recommended for use on land that is too moist to grow other grasses in north and south Florida (Miller and Lord, 2012). The adaptation of *H. dicksoni* n. sp. to same moist soil environment as that of the limpograss shows the potential negative impact on the Florida cattle and beef industry that uses limpograss

on moist flatwood soils where other grasses cannot be grown. Up to 60% tiller growth and 25% yield reduction were observed due to the presence of *H. oryzae* in the soil before rice seedlings were transplanted (Ou, 1985; Jairajpuri and Baqri, 1991). Similarly, rice fields infested with *H. mucronata* caused brown and orange lesions on the roots that could cause a 20 to 30% loss of rice (https://vtnews.vt.edu/articles/2016/10/outreach-oiredsurveycambodia.html, 2016). We have also witnessed the presence of limpograss root lesion and damage at the study site that could cause damage to productivity of this grass.

The identification of *H. dicksoni* n. sp. from limpograss warrants the need for additional surveys to understand the distribution and biology, and to explore the potential economic damage on livestock production in Florida. In addition, host suitability tests of *H. dicksoni* n. sp. to common grasses that can be used for forage and lawn will be required to take appropriate management strategies to prevent spread of this pest to other grasses (Fig. 5).

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