



Article

# First Report of Three Tylenchidae Taxa from Southern Alberta, Canada

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**Abstract:** Members of the family Tylenchidae are highly abundant in soil habitats, including agricultural settings, where they play key ecological roles. In the present study, we identified three Tylenchidae species, namely *Basiria bhabi*, *Coslenchus acceptus*, and *Filenchus vulgaris*, using integrative taxonomy. The detailed morphological and morphometric characteristics, distribution, and host associations of each species were also discussed. Phylogenetic analyses of these populations with other Tylenchidae nematodes indicated the presence of divergent lineages in *Filenchus* and *Basiria*, whereas *Coslenchus* appeared to be a monophyletic genus. Herein, we aim to grow awareness about this common but least studied group of nematodes. The species reported in this study are new records for Canada, revealing that the identified nematode diversity in our cultivated areas is relatively underrepresented. Our analyses also provided greater taxonomic resolution and captured rare taxa that might have been missed or misidentified in prior nematode inventory surveys. These findings will add to our understanding of the nematofauna of southern Alberta, thereby providing a more complete picture of existing nematode diversity present in the fields of this highly cultivated region.

**Keywords:** morphology; morphometrics; nematode management programs; new record; soil health; plant-parasitic nematodes; taxonomy



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## 1. Introduction

The nematode family Tylenchidae Örley [1] is exceptionally diverse in soil habitats. Members of this family have high ecological significance and prominence in both agricultural and natural systems [2–4]. Based on their life cycle strategies, Tylenchidae species have been given a colonizer–persister (cp) value of 2 [5]. Nematodes in the cp-2 category have ecological relevance as soil health indicators [5]. The taxonomic placement of Tylenchidae in terms of feeding behavior is controversial [6,7], since the feeding habits and food preferences of the majority of tylenchid genera are unknown. Thus, members of this family are either regarded as root hair/epidermal feeders [3], fungivores [8], or herbivores [9], or are generally referred to as plant associates [10].

The majority of Tylenchidae nematodes have similar general appearances, slender bodies, high intraspecific variation, and insufficient molecular data, posing great challenges to their classification and taxonomy [6]. Among plant-parasitic nematodes, Tylenchidae members have what is considered a “primitive morphology”, including weak stylets, weakly developed pharyngeal components, and long filiform tails. Subsequently, this family of nematodes has been preconceived to be economically insignificant and therefore often excluded from nematode surveillance and management programs [3,6,11]. In contrast, we believe that the identification of these nematodes is important, as they share a habitat with plant-parasitic nematodes [12,13]. Therefore, we focused on this neglected group of nematodes and found three species belonging to the genera *Basiria* Siddiqi [14], *Coslenchus* Siddiqi [15], and *Filenchus* Andrassy [16] from cultivated areas of southern Alberta, Canada.

Previous studies of the family Tylenchidae from Canada were limited to genus level identification of *Basiria* and *Coslenchus* [17,18], and to a few *Filenchus* species from high arctic areas [19–21]. This indicates that Tylenchidae nematodes are underrepresented and rarely documented in Canada. In the present study, we carefully examined the three Tylenchidae species found in southern Alberta and performed morphological and molecular analyses to confirm their identities. Detailed morphological examination revealed the presence of *B. bhabi* Siddiqi [22], *C. acceptus* Andr assy [23], and *F. vulgaris* (Brzeski) Lownsberry and Lownsberry [24,25]. Since all three species are first reports from Canada, the objectives of the present study were to (1) provide detailed morphometric and molecular characterizations of these species; (2) examine their phylogenetic relationships with related tylenchid nematodes; and (3) update the taxonomic records of these species from the new location of southern Alberta.

Our study provides increased taxonomic resolution and captures rare taxa that may have been missed or misidentified in prior nematode inventory surveys. Furthermore, our report will aid in filling the gaps of the virtually unknown nematofauna of southern Alberta, thereby providing a more complete picture of the existing nematode diversity present in the region.

## 2. Materials and Methods

### 2.1. Isolation and Morphological Studies

Continuing our wider program of uncovering soil nematofauna of southern Alberta, we collected several root and soil samples from the Vauxhall–Bow Island region. Nematodes were extracted from soil samples using the modified Cobb sieving and flotation-centrifugation method [26]. *Basiria*, *Coslenchus*, and *Filenchus* taxa were collected individually from the mixture of soil nematodes and assigned the population numbers 55, 60, and 70. For preliminary examinations, fresh adults of each species were transferred to a drop of distilled water, heat relaxed, and observed under a Zeiss Axioskope 40 microscope. For morphometric studies, nematodes were fixed, and permanent slides were prepared according to the methods of Seinhorst [27] and De Grisse [28]. Photomicrographs of each specimen were acquired using a Zeiss Axioskope 40 microscope equipped with a Zeiss AxioCam 208 camera (Carl Zeiss Microscopy, Jena, Germany). Measurements from the images were performed using ZEN blue 3.1 imaging software (Carl Zeiss Microscopy).

### 2.2. DNA Extraction, PCR, and Sequencing

After microscopic examination, a single nematode of each taxon was transferred to a 0.2 mL PCR tube, and DNA was extracted as described in Maria et al. [29]. Three sets of DNA primers (Integrated DNA Technologies, Coralville, IA, USA) were used to amplify the 18S (small subunit or SSU), 28S (large subunit or LSU), and ITS1 of ribosomal RNA (rRNA) genes. The partial 18S rRNA gene was amplified with 1813F and 2646R primers [30]. The 28S rRNA gene was amplified using D2A and D3B primers [31], and the ITS1 gene was amplified using F194 [32] and AB28-R primers [33]. For 18S, 28S, and ITS1 genes, the PCR conditions were as described in Holterman et al. [30], De Ley et al. [31], and Ferris et al. [32], respectively. Amplified PCR products were resolved by electrophoresis in 1% agarose gels and visualized by staining with GelRed (Biotium, Fremont, CA, USA). PCR products containing amplified DNA fragments of interest were sent to Genewiz, Inc. for DNA sequencing (South Plainfield, NJ, USA).

### 2.3. Phylogenetic Analyses

In the present study, we obtained DNA sequences for the 28S rRNA (D2–D3 domains), ITS1 rRNA, and 18S rRNA genes of three tylenchid populations. These sequences and additional Tylenchidae DNA sequences from GenBank were used for phylogenetic analysis. The selection of outgroup taxa for each dataset were based on previously published studies [34–37]. Multiple nucleotide sequence alignments for the different genes were performed using the heuristics progressive method FFT-NS-2 algorithm of MAFFT

v.7.450 [38]. The BioEdit v7.2.5 program [39] was used for sequence alignment visualization. For alignment edition, we used Gblocks v0.91b [40] on the Castresana Laboratory server (available online: [http://molevol.cmima.csic.es/castresana/Gblocks\\_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html) (accessed on 24 August 2021)) with options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences + 1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). Phylogenetic analyses were performed using Bayesian inference (BI) in MrBayes v3.1.2 [41]. The best-fit model of DNA evolution was achieved using JModelTest v2.1.7 [42] with the Akaike Information Criterion (AIC). Accordingly, the selected models were TIM2 + I + G, TIM3 + I + G, and GTR + G for the D2–D3 segments of the 28S rRNA, partial 18S, and ITS1, respectively. The best-fit model, base frequency, proportion of invariable sites, gamma distribution shape parameters, and substitution rates in the AIC were then used in MrBayes for the phylogenetic analyses, which ran with four chains for  $4 \times 10^6$  generations in all datasets. A combined analysis of the three ribosomal genes was not undertaken due to several sequences not being available for all species. The sampling for Markov chains was carried out at intervals of 100 generations. For each analysis, two runs were conducted. After discarding burn-in samples of 30% and evaluating convergence, the remaining samples were retained for more in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. On each appropriate clade, posterior probabilities (PP) were calculated. FigTree software v1.42 [43] was used for visualization of phylogenetic trees from all analyses.

### 3. Results

#### 3.1. Description of *Basiria bhabi*

*Female:* Body shape slightly ventrally arcuate when heat relaxed. Cuticle finely annulated with lateral field having four lines, the outer lines not crenated. Lip region continuous, without striations, narrow with a weakly sclerotized framework. Stylet straight, slender, with fine lumen and small rounded knobs. Orifice of dorsal esophageal gland (DGO) close to stylet knobs. Median bulb oval and weakly developed, with inconspicuous valve plates situated at ca. 42–52% of pharyngeal length. Isthmus slender, surrounded with nerve ring gradually expanding into a cylindrical basal pharyngeal bulb. Excretory pore distinct, situated at the anterior region of pharyngeal bulb. Deirids present in the anterior region of basal pharyngeal bulb, only discernable in inverted specimen. Reproductive system mono-prodelphic, composed of a single, anteriorly outstretched gonad, with oocytes in one row; vulva smooth, vagina straight, weakly sclerotized; spermatheca not offset, irregular squarish-shaped, and mostly empty; post-vulval uterine sac shorter than vulval body diameter. Anus a minute pore, tail elongated and slender, ending in a rounded or clavate tip. Phasmid was not observed in any of the studied specimens (Figure 1, Table 1).

**Table 1.** Female morphometrics of *Basiria bhabi* from Canada and from the original description. All measurements are in  $\mu\text{m}$  and presented as mean  $\pm$  standard deviation (range).

Characters	Canadian Population	Bajaj and Bhatti [44]
n *	15	1
Body length	799.5 $\pm$ 58.2 (702.0–933.0)	760
a	37.1 $\pm$ 3.2 (32.7–41.4)	36
b	6.4 $\pm$ 0.3 (5.9–6.9)	7
c	7.8 $\pm$ 0.5 (7.1–9.0)	8
c'	8.1 $\pm$ 0.6 (7.2–9.0)	8
V	72.9 $\pm$ 0.9 (71.3–74.2)	78
MB	47.2 $\pm$ 2.8 (42.0–52.0)	–
Lip height	3.3 $\pm$ 0.3 (3.0–4.0)	3–4
Lip width	6.6 $\pm$ 0.3 (6.0–7.0)	6–8
Stylet length	11.9 $\pm$ 0.5 (11.0–13.0)	12

Table 1. Cont.

Characters	Canadian Population	Bajaj and Bhatti [44]
Pharynx length	124.2 ± 6.5 (116.0–141.0)	–
Anterior end to excretory pore	100.2 ± 7.7 (87.0–117.0)	97–100
Maximum body width	21.7 ± 2.2 (17.0–25.0)	–
Vulva body width	18.8 ± 1.5 (16.5–22.0)	–
Post-vulval uterine sac (PUS) length	11.2 ± 2.0 (9.0–14.0)	–
Distance from vulva to anus	114.2 ± 13.1 (95.0–150.0)	80–92
Distance from vulva to tail terminus	216.5 ± 15.4 (191.0–254.0)	–
Anal body width	12.7 ± 0.7 (11.0–14.0)	–
Tail length	102.3 ± 5.8 (95.0–112.0)	80

\* Abbreviations: n, number of specimens on which measurements are based; a, body length/greatest body diameter; b, body length/distance from anterior end to pharyngo-intestinal junction; c, body length/tail length; c', tail length/tail diameter at anus; V, distance from body anterior end to vulva expressed as percentage (%) of the body length; MB, distance between anterior end of body and center of median pharyngeal bulb expressed as percentage (%) of the pharynx length.

*Male:* Not found.

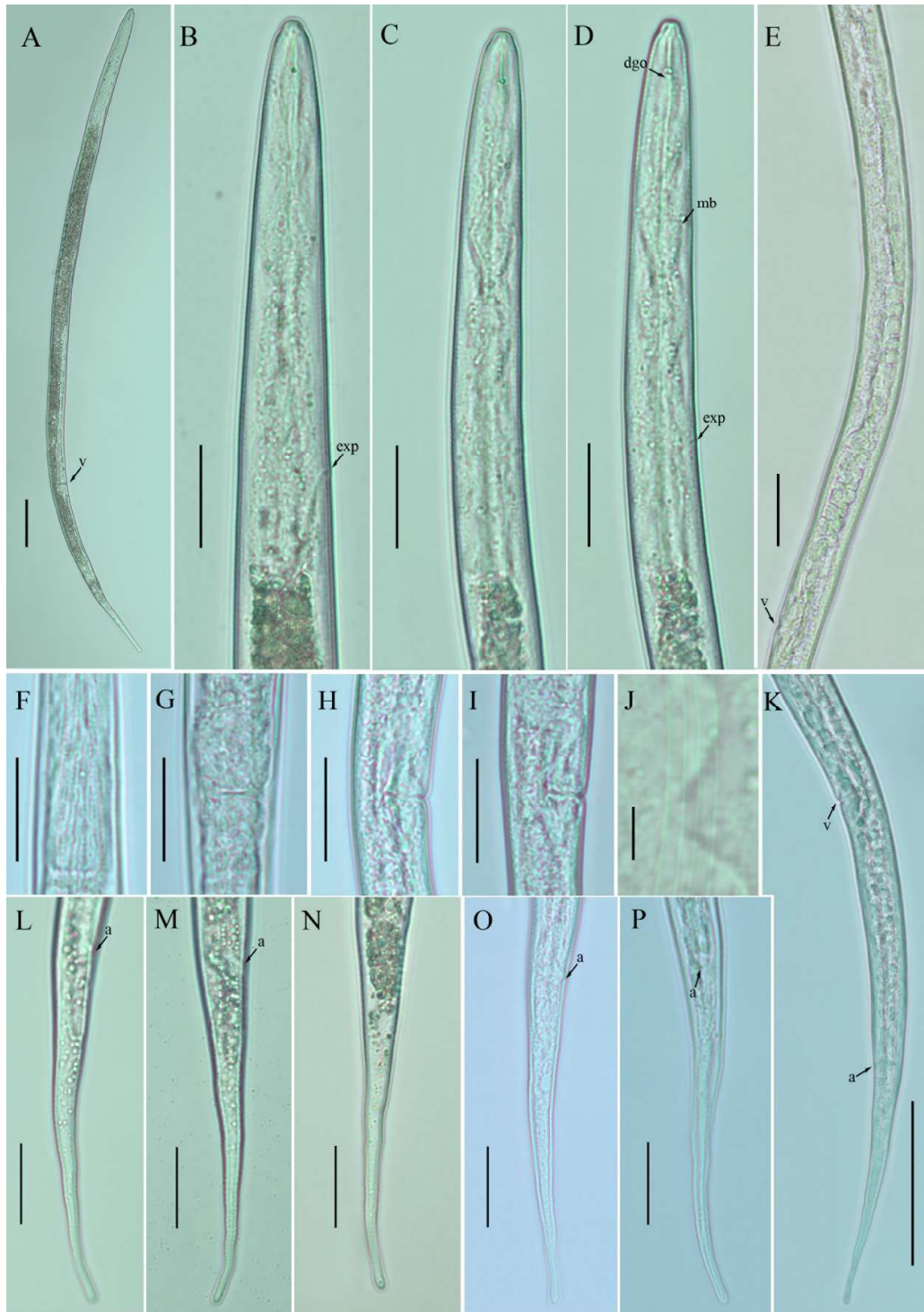
*Juveniles:* Present but not studied.

Remarks

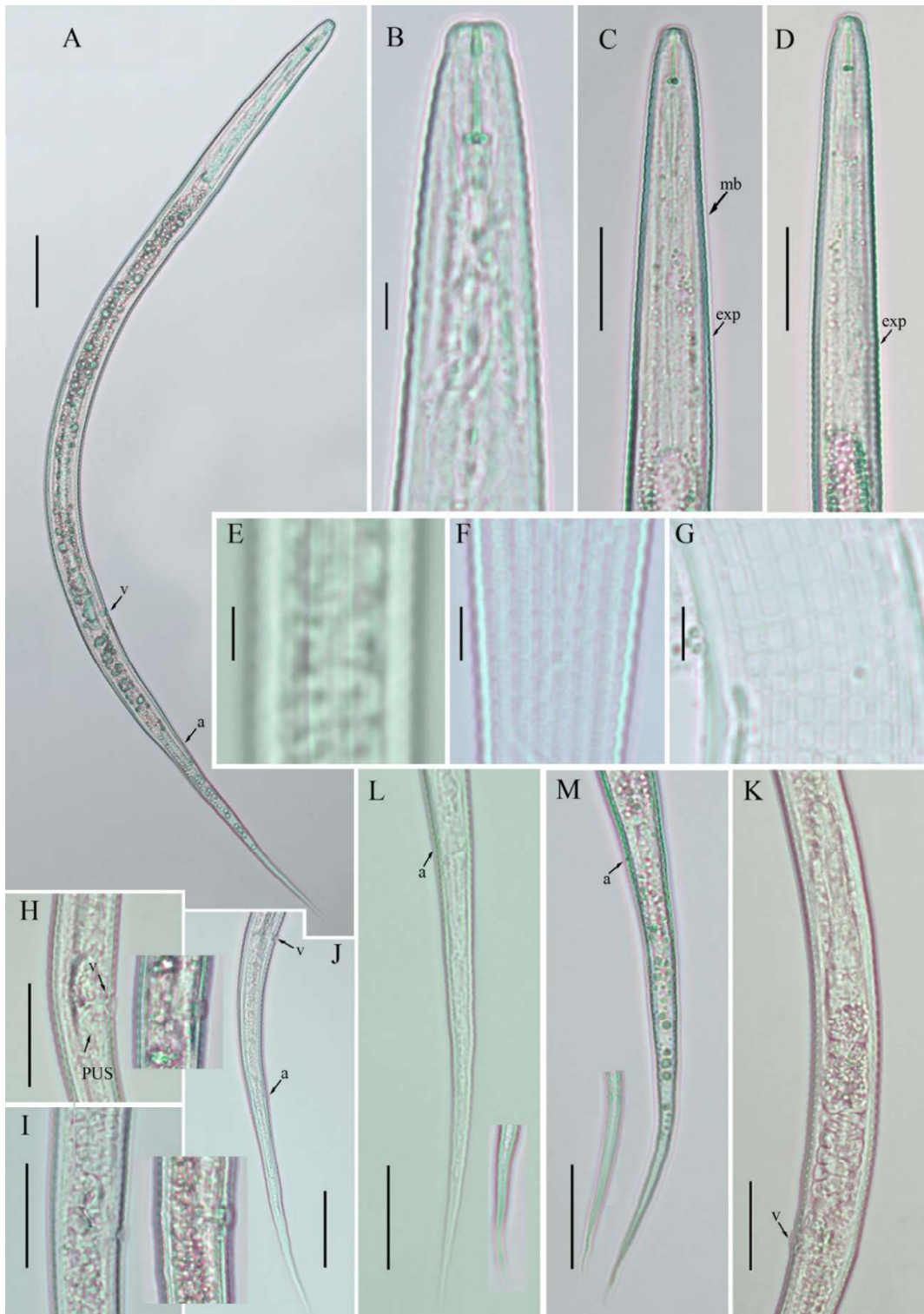
*Basiria bhabi* was first described as *B. indica* by Bajaj and Bhatti in 1979 [44]; however, Siddiqi [22] recognized it as a junior homonym (i.e., a name for a taxon that is identical in spelling to another such name, which belongs to a different taxon) of *B. indica* described by Chawla et al. [45], and therefore renamed it *B. bhabi*. The species was described from India and was never reported outside of its type locality. The morphology and morphometry of the Canadian population agreed well with the original description of *B. bhabi*, except for the longer body length and longer tails. Since the original description of *B. bhabi* is based on the characteristics of a single specimen, we cannot precisely determine intraspecific variation. Consequently, we refer to the Canadian population of *B. bhabi* as the reference population for future studies, until topotypes of this species can be sequenced. The species was first discovered in the rhizosphere of *Mangifera indica*. In the present study this species was found in the rhizosphere of dandelion and grass growing on the headland of a cultivated potato field.

### 3.2. Description of *Coslenchus acceptus*

*Female:* Body open C-shaped to ventrally bent when heat relaxed. Cuticle widely annulated with 14 longitudinal ridges. Lateral field with four incisures. Lip region slightly offset, squarish, anteriorly flattened, with three to four annuli. Stylet straight, slender, with rounded knobs. Median bulb oval with refractive valve plates, situated at ca. 42–50% of pharyngeal length. Isthmus slender, encircled with nerve ring gradually expanding into a small pyriform basal pharyngeal bulb. Excretory pore at anterior end of basal bulb. Reproductive system mono-prodelphic, composed of an outstretched ovary with oocytes mostly in two rows, reflexed ovary was observed in some specimens; vulva sunken in body with large vulval flaps, vagina straight, weakly sclerotized; spermatheca irregular round-shaped, filled with few sperm; post-vulval uterine sac shorter than vulval body diameter. Anus a minute pore. Tail elongated, filiform, ending in a finely attenuated tip (Figure 2, Table 2).



**Figure 1.** Photomicrographs of *Basiria bhabi* female. (A) Entire body; (B–D) pharyngeal regions; (E) entire gonad; (F) basal pharyngeal bulb; (G) vulval region (ventral view); (H,I) vulval region (lateral view); (J) lateral lines; (K) posterior body to tail terminus; (L–O) tail regions (lateral view); (P) tail region (ventral view). Scale bars: (A,K) 50  $\mu$ m; (B–I,L–P) 20  $\mu$ m; (J) 5  $\mu$ m. Arrowheads: (a) anus; (DGO) dorsal pharyngeal gland orifice; (exp) excretory pore; (mb) median bulb; (v) vulva.



**Figure 2.** Photomicrographs of *Coslenchus acceptus* female. (A) Entire body; (B) lip region; (C,D) pharyngeal regions; (E) lateral lines; (F,G) cuticular ridges; (H,I) vulval region; (J) posterior body to tail terminus; (K) entire gonad; (L,M) tail region. Scale bars: (A,J) 50  $\mu$ m; (C,D; H,I; K–M) 20  $\mu$ m; (B,E–G) 5  $\mu$ m. Arrowheads: (a) anus; (exp) excretory pore; (mb) median bulb; (PUS) post-vulval uterine sac; (v) vulva.

**Table 2.** Female morphometrics of *Coslenchus acceptus* from Canada and from the original description. All measurements are in  $\mu\text{m}$  and presented as mean  $\pm$  standard deviation (range).

Characters	Canadian Population	Andrássy [23]
n *	19	–
Body length	517.3 $\pm$ 24.8 (466.0–567.0)	460–540
a	31.8 $\pm$ 2.1 (27.5–35.0)	23–29
b	5.7 $\pm$ 0.2 (5.4–6.2)	–
c	4.6 $\pm$ 0.2 (4.4–5.0)	5–6
c'	12.2 $\pm$ 0.9 (10.1–14.6)	8–11
V	64.0 $\pm$ 1.0 (62.2–65.9)	64–67
MB	46.5 $\pm$ 2.0 (42.1–50.5)	48
G1	26.2 $\pm$ 3.9 (21.7–30.0)	–
Lip height	3.2 $\pm$ 0.2 (3.0–3.5)	–
Lip width	6.2 $\pm$ 0.2 (6.0–6.5)	6–7
Stylet length	11.7 $\pm$ 0.7 (10.0–13.0)	12
Dorsal pharyngeal gland orifice (DGO)	2.3 $\pm$ 0.4 (2.0–2.5)	–
Median bulb length	11.2 $\pm$ 1.3 (10.0–13.5)	–
Median bulb width	7.4 $\pm$ 0.6 (6.0–8.0)	–
Pharynx length	90.9 $\pm$ 2.7 (85.0–95.0)	92–96
Anterior end to excretory pore	75.6 $\pm$ 2.8 (72.0–82.0)	–
Maximum body width	16.3 $\pm$ 1.4 (14.0–19.0)	–
Vulva body width	14.9 $\pm$ 1.3 (13.0–18.0)	–
Post-vulval uterine sac (PUS) length	7.7 $\pm$ 1.2 (7.0–9.0)	–
Distance from vulva to anus	74.5 $\pm$ 4.3 (65.0–81.0)	79–92
Distance from vulva to tail terminus	185.9 $\pm$ 9.3 (168.0–204.0)	–
Anal body width	9.2 $\pm$ 0.6 (8.0–10.0)	–
Tail length	111.5 $\pm$ 6.9 (100.0–128.0)	92–105

\* Abbreviations: n, number of specimens on which measurements are based; a, body length/greatest body diameter; b, body length/distance from anterior end to pharyngo-intestinal junction; c, body length/tail length; c', tail length/tail diameter at anus; V, distance from body anterior end to vulva expressed as percentage (%) of the body length; MB, distance between anterior end of body and center of median pharyngeal bulb expressed as percentage (%) of the pharynx length; G1, % length of anterior female gonad in relation to body length.

*Male:* Not found.

*Juveniles:* Present but not studied.

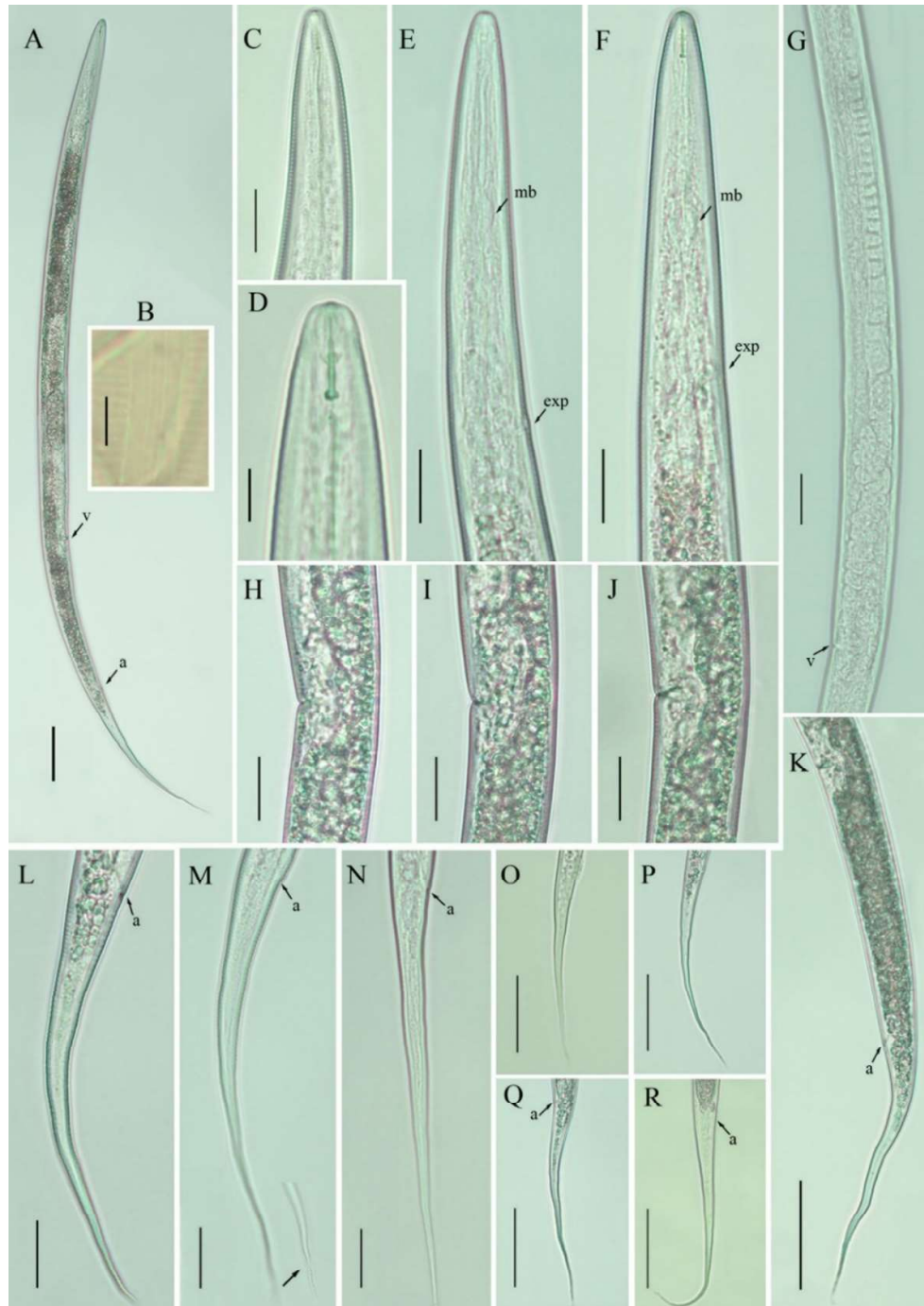
Remarks

This species was first described by Andr assy [23] from California, USA, in the rhizosphere of strawberry. The same author reported it from two other localities, namely Colorado, USA, and Ujsszentmargita, Hungary, in the rhizosphere of *Carex* sp. and *Artemisia* sp. Here, we found *C. acceptus* in the rhizosphere of grass growing on the headland of a cultivated potato field. To the best of our knowledge, after the formal description, the species was never reported again. The apparent morphology and morphometry of the *C. acceptus* original description agree well with the Canadian population except for the longer tail length (100–128 vs. 92–105  $\mu\text{m}$ ) and the presence (vs. absence) of a post-vulval uterine sac (PUS). In fresh specimens of the Canadian population, we were not able to observe any PUS; however, in permanent mounts we observed a small rudimentary PUS. The absence of a PUS in the original description could be related to the fixation technique used on specimens [23]. As for the longer tail of Canadian specimens, we agree with Brzeski [46], who stated that tail length in tylenchid nematodes cannot be accepted as a specific character because the filiform portion of the tail is attenuated and could easily break off during handling and processing of specimens.

### 3.3. Description of *Filenchus vulgaris*

*Female:* Body slightly ventrally arcuate when heat relaxed. Cuticle finely annulated with four lateral lines. Lip region hemispherical anteriorly flattened, continuous with body contour. Stylet straight, delicate, with rounded knobs. Median bulb oval with refractive valve plates, situated at ca. 39–45% of pharyngeal length. Isthmus slender, encircled with nerve ring gradually expanding into a small pyriform basal pharyngeal bulb. Excretory

pore at anterior end of basal bulb. Reprod+uctive system mono-prodelphic, composed of an outstretched ovary with oocytes mostly in a single row; vulva smooth, vagina straight to slightly inclined anteriorly; spermatheca offset, irregular rounded shaped filled with few sperm; post-vulval uterine sac shorter than vulval body diameter. Anus a minute pore. Tail elongated, filiform, ending in a finely attenuated tip (Figure 3, Table 3).



**Figure 3.** Photomicrographs of *Filenchus vulgaris* female. (A) Entire body; (B) lateral lines; (C,D) lip regions; (E,F) pharyngeal regions; (G) entire gonad; (H–J) vulval regions; (K) posterior body till tail terminus; (L–R) tail regions. Scale bars: (A,K,O–R) 50  $\mu$ m; (C,E–J,L–N) 20  $\mu$ m; (B,D) 5  $\mu$ m. Arrowheads: (a) anus; (exp) excretory pore; (mb) median bulb; (v) vulva.

*Male:* Not found.

*Juveniles:* Present but not studied.

*Remarks*

This species was described by Brzeski [24] in the rhizosphere of a vegetable crop from Poland. *Filenchus vulgaris* is considered to be a cosmopolitan species; it has been reported from Belgium, Germany, Iran, Korea, Poland, Russia, Slovakia, and the USA [9,24,25,47–50]. In the present study, the Canadian population of *F. vulgaris* was recovered from the rhizosphere of dandelion and grass growing on the headland of a cultivated potato field. Because of the widespread distribution of *F. vulgaris*, it has a broad host association. The species is known to occur in various biotypes, e.g., agricultural and forest soils and rhizospheres of grasses and vegetables [46,49]. Because *F. vulgaris* is a common species, morphometric studies were not always performed for the detected populations of this nematode (Table 3). Based on the available data, the morphological and morphometric details of the Canadian population are within the species boundaries of *F. vulgaris*.

**Table 3.** Female morphometrics of *Filenchus vulgaris* from Canada and retrieved from the original and subsequent published descriptions. All measurements are in  $\mu\text{m}$  and presented as mean  $\pm$  standard deviation (range).

Characters	Canadian Population	Brzeski [24]		Raski and Geraert [48]		Karegar and Geraert [49]
		Poland	Colorado	Belgium	Korea	Iran
n *	15	25	5	51	11	5
Body length	779.1 $\pm$ 61.8 (677.0–870.0)	520–810	650–690	490–750	560–740	580–730
a	29.9 $\pm$ 2.8 (25.3–36.5)	29–42	36–40	24–42.5	–	31.6–37.2
b	6.0 $\pm$ 0.3 (5.4–6.5)	4.7–7.0	5.6–5.9	4.7–6.4	5.1–6.9	5.9–6.4
c	5.3 $\pm$ 0.4 (4.7–6.2)	4.2–5.7	4.2–4.9	3.4–5.3	4.6–5.3	3.9–4.5
c'	9.7 $\pm$ 1.0 (8.2–11.7)	13	12–15	–	–	13.4–17.4
V	61.2 $\pm$ 1.4 (59.0–64.0)	53–65	58–61	53–64	60–65	54.6–60.2
MB	41.2 $\pm$ 1.4 (39.7–45.1)	44	40–43	40–48	39–44.5	38.1–45.8
Lip height	3.2 $\pm$ 0.3 (3.0–4.0)	–	–	–	–	–
Lip width	6.5 $\pm$ 0.4 (6.0–7.0)	–	–	–	–	–
Stylet length	10.5 $\pm$ 1.0 (9.0–12.0)	10–12	10–11	9–12	9.5–10.5	8.1–10.5
Dorsal pharyngeal gland orifice (DGO)	1.5 $\pm$ 0.2 (1.0–2.0)	–	–	–	–	–
Median bulb length	12.4 $\pm$ 0.9 (11.0–14.0)	–	–	–	–	–
Median bulb width	7.7 $\pm$ 0.7 (7.0–9.0)	–	–	–	–	–
Pharynx length	129.4 $\pm$ 5.8 (120.0–141.0)	114	111–123	89–123	98–124	96–115
Anterior end to excretory pore	98.9 $\pm$ 6.8 (87.0–110.0)	92–102	84–94	–	–	–
Maximum body width	26.3 $\pm$ 2.4 (22.0–30.0)	–	–	–	–	–

Table 3. Cont.

Characters	Canadian Population	Brzeski [24]		Raski and Geraert [48]		Karegar and Geraert [49]
		Poland	Colorado	Belgium	Korea	Iran
Vulva body width	24.4 ± 1.9 (21.5–27.5)	–	–	–	–	–
Post-vulval uterine sac (PUS) length	14.1 ± 1.8 (12.0–17.0)	–	–	–	–	–
Distance from vulva to anus	149.3 ± 12.4 (125.0–166.0)	–	–	–	–	105–122
Anal body width	15.3 ± 1.2 (13.0–17.0)	–	–	–	–	–
Tail length	146.9 ± 12.0 (133.0–175.0)	135	137–157	111–165	114–150	145–172

\* Abbreviations: n, number of specimens on which measurements are based; a, body length/greatest body diameter; b, body length/distance from anterior end to pharyngo-intestinal junction; c, body length/tail length; c', tail length/tail diameter at anus; V, distance from body anterior end to vulva expressed as percentage (%) of the body length; MB, distance between anterior end of body and center of median pharyngeal bulb expressed as percentage (%) of the pharynx length.

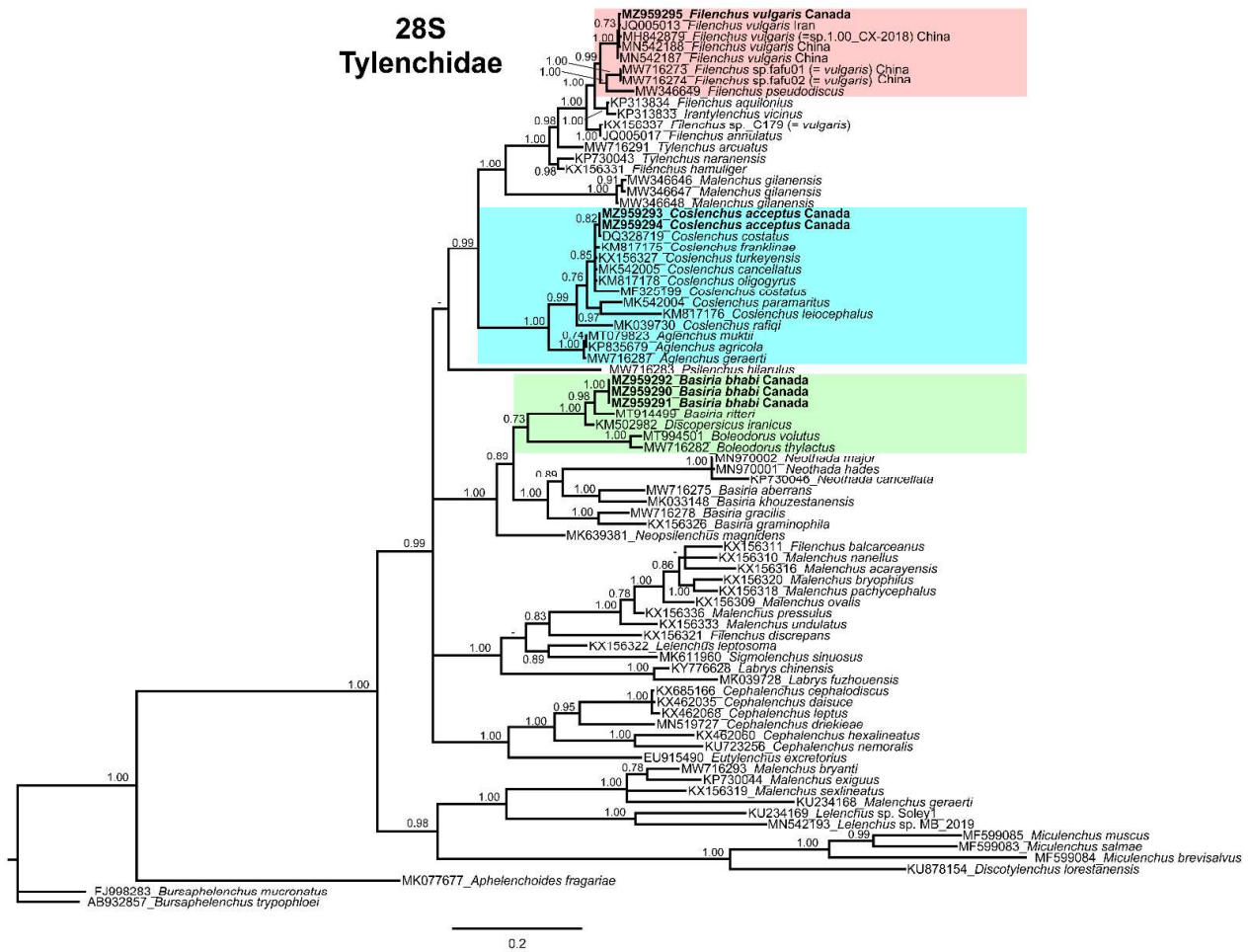
#### 3.4. Molecular Characterization and Phylogenetic Relationship of *Basiria bhabhi*, *Coslenchus acceptus*, and *Filenchus vulgaris* with Related Tylenchidae Species

The three Tylenchidae species recovered in this study were molecularly characterized using partial 18S, 28S, and ITS1 sequences. The newly obtained sequences were edited and submitted to NCBI under the following accession numbers: partial 18S (MZ959113 for *C. acceptus*); D2–D3 of 28S (MZ959290–MZ959292 for *B. bhabhi*; MZ959293–MZ959294 for *C. acceptus*; MZ959295 for *F. vulgaris*); and ITS1 (MZ959284 for *B. bhabhi*; MZ959285–MZ959286 for *C. acceptus*; MZ959287–MZ959288 for *F. vulgaris*).

In the 28S tree (Figure 4), *B. bhabhi* clustered with species of subfamily Boleodorinae Khan [51] in a highly supported molecular clade (PP = 1.00). This clade further divided into two subclades where *B. bhabhi* has a sister relationship with *B. ritteri* (Baqri and Jaira-jpuri) Bernard [52,53] (from China), and grouped with *Discopersicus iranicus* (Ghaemi, Pourjam, Atighi, Pedram, and Karssen) Yaghoubi et al. [54,55], *Boleodorus volutus* Lima and Siddiqi [56], and *Boleodorus thylactus* Thorne [57].

This clade further grouped with other species of *Basiria* (such as *B. aberrans* (Thorne) Siddiqi [58,59], *B. gracilis* (Thorne) Siddiqi [58,59], *B. graminophila* Siddiqi [14], *B. khouzes-tanensis* Eisvand, Farrokhi-Nejad, and Azimi [60]), *Neothada* Khan [61], and *Neopsilenchus* Thorne and Malek [62]. Our results support previous findings of Yaghoubi et al. [55] that monodelphic genera of Boleodorinae are monophyletic. Although *B. bhabhi* is in the Boleodorinae clade, it grouped distantly from other *Basiria* species. The sequence identity of our *B. bhabhi* with available *Basiria* species was 81–95% (38–149 nucleotide differences), with 0–6% indels.

In subfamily Atylenchinae Skarbilovich [63], the 28S-rRNA gene has been sequenced only for *Coslenchus* and *Aglenchus* Andrassy [16]. In our 28S tree, *C. acceptus* grouped with *C. cancellatus* (Cobb) Siddiqi [15,64]; *C. costatus* (de Man) Siddiqi [15,65]; *C. franklinae* Siddiqi [66]; *C. leiocephalus* Brzeski [67]; *C. oligogyrus* Brzeski [68]; *C. paramaritus* Hosseinvand, Eskandari, and Ghaderi [36]; *C. turkeyensis* Siddiqi [66]; *C. raftiqi* (Siddiqui and Khan) Siddiqi [22,69] and *A. agricola* (de Man) Andrassy [16,70]; *A. geraerti* Mizukubo [71]; and *A. muktii* Phukan and Sanwal [72]. The sequence identity of *C. acceptus* with the species of *Coslenchus* was 87–99%, with 87–99 nucleotide differences and 0–2% indels, whereas the identity with *Aglenchus* species was 90%, with 71–73 nucleotide difference and 2% indels.

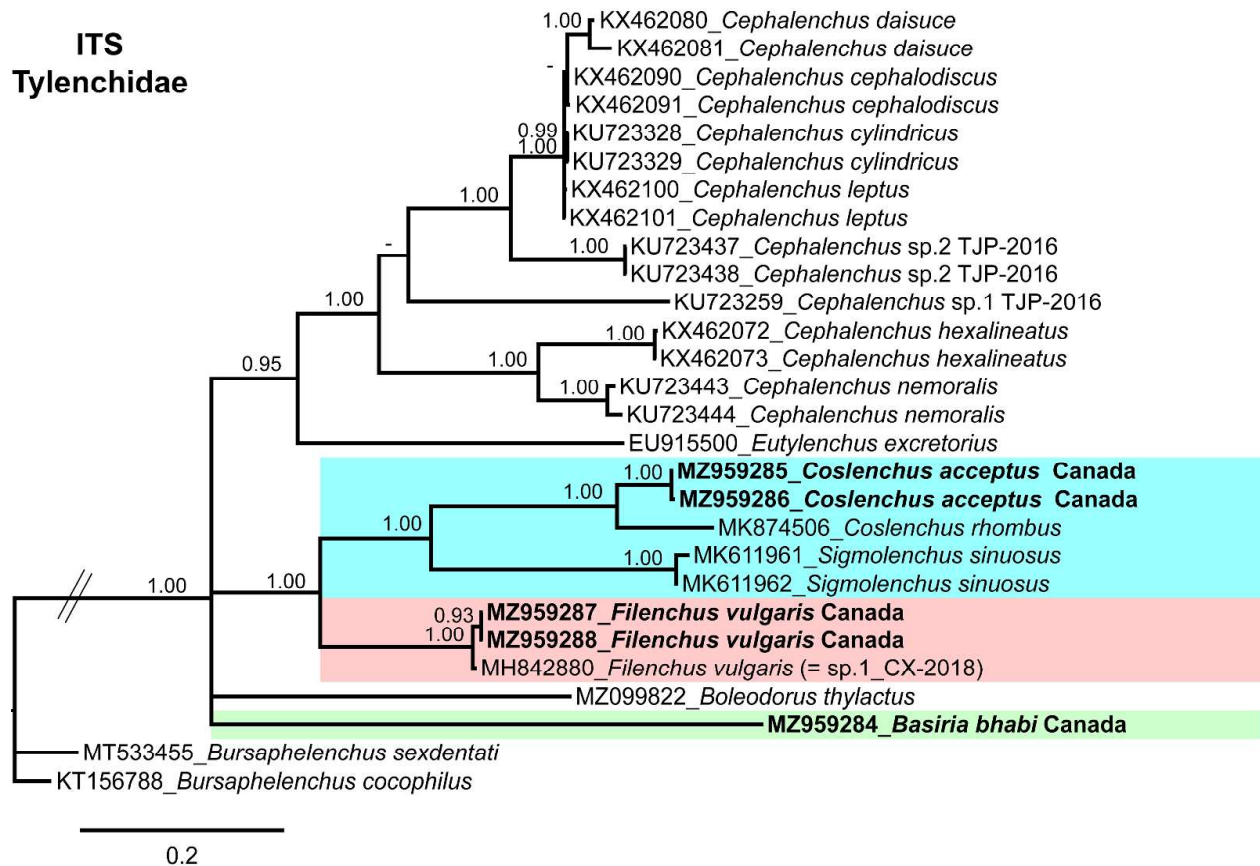


**Figure 4.** Phylogenetic relationships of *Basiria bhabi*, *Coslenchus acceptus*, and *Filenchus vulgaris* within Tylenchidae. Bayesian 50% majority rule consensus tree as inferred from D2–D3 expansion domains of the 28S rRNA sequence alignment under the transition rule model with invariable sites and a gamma-shaped distribution (TIM2 + I + G). Posterior probabilities of greater than 0.70 are given for appropriate clades. Sequences produced in this study are shown in bold, and colored boxes indicate the clade association of each species detected in this study.

In the 28S tree, members of subfamily Tylenchinae Örley [1] did not form a monophyletic group; species of *Filenchus*, *Discotylenchus* Siddiqui [73], *Malenchus* Andrassy [74], and *Miculenchus* Andrassy [75] were independently grouped at various positions of the tree. However, the Canadian population of *F. vulgaris* formed a clade with the sequences of *F. vulgaris* from the NCBI database and a few other species of *Filenchus* (namely, *F. pseudodiscus* Mortatazavi, Heydari, Abolafia, Castillo, and Pedram [37], *F. aquilonius* Wu [20], *F. annulatus* (Siddiqui and Khan) Siddiqui [22,69], *F. hamuliger* Brzeski [67] *Tylenchus* Bastian [76], and *Malenchus* as well. The tree also revealed that the sequence of *Filenchus* sp. deposited in the NCBI database under *F. xuelouensis* sp. n. (not published) is 99% identical (7 nucleotide difference) to the Canadian population of *F. vulgaris*; consequently, we refer to this as a population of *F. vulgaris*. In addition, the Canadian population of *F. vulgaris* showed 99% sequence identity (5–7 nucleotide difference and 0% indels) with *F. vulgaris* populations from China and Iran. The other supposed *F. vulgaris* populations from China (recovered from the rhizosphere of vegetables) showed 93% sequence identity (44 nucleotide difference and 1% indels) with our Canadian population of *F. vulgaris*. These sequences were deposited under *F. vulgaris*; however, no morphological or morphometric information is available to confirm their identity. Based on our results, we believe a detailed re-evaluation

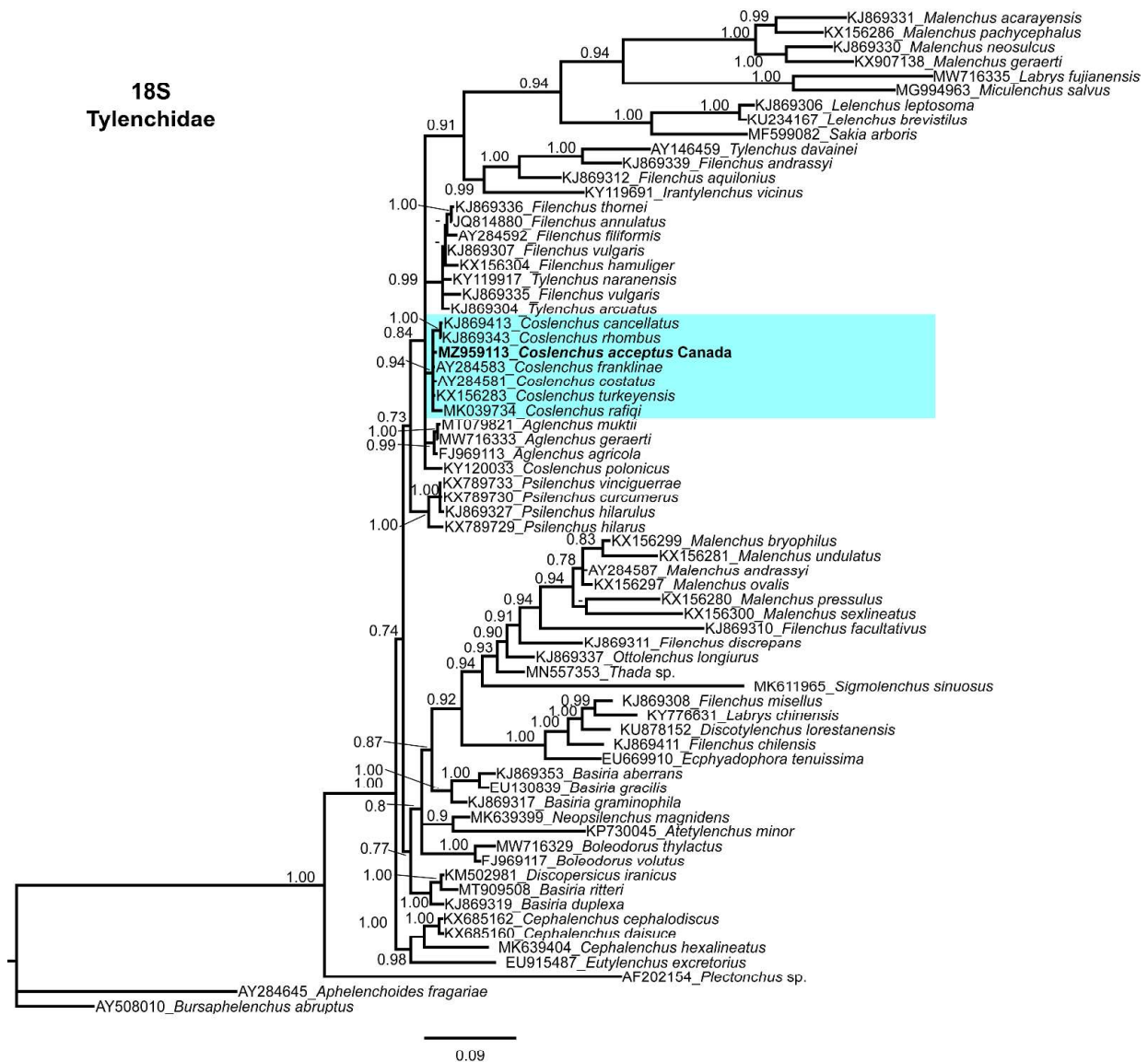
based on integrative taxonomy should be performed to determine the exact status of these species, which could have been previously misidentified.

The majority of studies conducted to understand the phylogenetic relationships of the family Tylenchidae is based on D2–D3 domains of the 28S gene. In contrast, very few Tylenchidae members were characterized by ITS1 sequences. Notably, no ITS1 sequence was found for the genus *Basiria*, and only a single ITS1 sequence was available for each of *Filenchus* and *Coslenchus* species. We constructed the ITS tree (Figure 5) with the available ITS sequences of *Cephalenchus* Goodey [77] and the Canadian populations of tylenchid nematodes. *Basiria bhabi* appeared independently and occupied a basal position in the tree, whereas *C. acceptus* clustered with *C. rhombus*, and *F. vulgaris* finally grouped with the *F. vulgaris* from China, initially identified as sp1 CX-2018. Due to the lack of ITS1 sequences, prediction of the phylogenetic relationships is uncertain; therefore, we omitted calculation of the sequence identity of the Canadian populations of tylenchid nematodes with related species.



**Figure 5.** Phylogenetic relationships of *Basiria bhabi*, *Coslenchus acceptus*, and *Filenchus vulgaris* within Tylenchidae. Bayesian 50% majority rule consensus tree as inferred from the ITS1 rRNA sequence alignment under the general time-reversible model and a gamma-shaped distribution (GTR + G). Posterior probabilities of more than 0.70 are given for appropriate clades. Sequences produced in this study are shown in bold, and colored boxes indicate clade association of each species detected in this study.

An 18S tree was constructed with the single sequence of *C. acceptus* and related Tylenchidae species sequences (Figure 6). As with the 28S and ITS trees, *C. acceptus* grouped within the *Coslenchus* clade. The sequence identity of *C. acceptus* with the *Coslenchus* clade species was 99%, with 4–9 nucleotide differences and 0–1% indels.



**Figure 6.** Phylogenetic relationships of *Coslenchus acceptus* within Tylenchidae. Bayesian 50% majority rule consensus tree as inferred from the 18S rRNA sequence alignment under the transition model with invariable sites and a gamma-shaped distribution (TIM3 + I + G). Posterior probabilities of more than 0.70 are given for appropriate clades. Sequences produced in this study are shown in bold, and colored box indicate clade association of *Coslenchus* species.

The Canadian populations of *B. bhabhi*, *C. acceptus*, and *F. vulgaris* obtained in this study were identified using an integrative taxonomical approach. Hence, the sequences of these populations could serve as reference sequences for future studies, until topotype specimens of these species can be sequenced.

#### 4. Discussion

Members of the family Tylenchidae are highly abundant in agricultural soil, representing at least 30% of soil nematofauna in any given sample [6]. In the present study, the Canadian populations of *B. bhabhi*, *C. acceptus*, and *F. vulgaris* were present at higher densities in headland vegetation (grasses and flowering plants) than those in field soils. The lower densities of these nematodes in field samples may be due to sampling depth, as Tylenchidae nematodes dominated in the upper 10 cm of soil, with their numbers declining

with depth [78]. Another reason may simply be that these nematodes have adapted better to the headland vegetation because the headland area is less disturbed—not vigorously tilled as are the cultivated fields.

The family Tylenchidae is notorious for its complicated taxonomy and morphology, whereby phenotypic convergence of morphologically similar but genetically distant genera cause problems in the delimitation of taxa [6,35]. However, implementation of molecular tools allows nematologists to better study the characters and utilize them to distinguish the species [6,7,11,34,35,79]. In our phylogenetic analysis, *B. bhabhi* exhibited a sister relationship with *B. ritteri* and clustered distant from the other species of *Basiria*. Such divergent phylogenetic placement suggests that *Basiria* is not a monophyletic genus. Currently, the genus contains over 40 nominal species [21,80], and the molecular information is only available for few species. In our view, genus-wide sequencing is needed to validate the true positioning and phylogenetic relationships of *Basiria*. Andr assy [23] did a thorough review of *Coslenchus* and concluded that the genus is homogenous. This close morphological affinity is also reflected in several phylogenetic studies [7,36,81] where all the *Coslenchus* species form a monophyletic clade. Our results are also in line with these studies, i.e., the Canadian population of *C. acceptus* was nested within the *Coslenchus* clade with very little sequence divergence. *Filenchus* is one of the most diverse genera in the subfamily Tylenchinae, currently representing over 90 species [21]. Phylogenetic studies have indicated the presence of divergent lineages in *Filenchus*; therefore, superficial observation of morphologically similar species can lead to misidentifications [82]. We also observed questionable sequences of *F. vulgaris* deposited in the NCBI database. Moreover, no morphological or morphometric details were associated with these supposed sequences. Therefore, future studies are well advised to meticulously examine species morphological characters and use the same specimen for morphological and molecular studies. Nevertheless, molecular studies of Tylenchidae genera are important to expand our knowledge of these microscopic, morphologically-reduced organisms [6].

The feeding habits of the majority of Tylenchidae nematodes are barely known; the species we detected in this study were all isolated from the rhizosphere of plants. We did not observe significant root damage or mycelial growths at the time of sampling, which suggests that Canadian populations of *B. bhabhi*, *C. acceptus*, and *F. vulgaris* are plant-feeding rather than plant-parasitic nematodes. The detection of these species in our cultivated areas updates the biogeography of these nematodes; however, there is still lack of knowledge on the diversity of tylenchid species from southern Alberta, Canada. It is likely that tylenchid species not directly implicated in plant diseases will remain undocumented for years to come.

## 5. Conclusions

In southern Alberta, the presence of phytoparasitic nematodes has been examined in several studies [12,13,83], but detection and identification of Tylenchidae-related nematodes had never been addressed. As a result, we know almost nothing about insignificant or mild parasitic nematodes that share the same habitat with economically important plant-parasitic nematodes. Herein, we aim to grow awareness about a less studied group of nematodes. We believe that including more species in nematode surveillance programs is both a step forward in understanding the nematofauna of agro-ecosystems and an essential component for the assessment of soil biodiversity in any management plan. It is also a promising direction for both taxonomy and education. In addition, our results suggest that the nematode diversity in our cultivated areas is underrepresented, since the species reported in this study are all new records for Canada. This study updates the biogeography of *B. bhabhi*, *C. acceptus*, and *F. vulgaris* from Canada.

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D.P.Y. and P.C.; visualization, M.M. and P.C.; supervision, M.M. and D.P.Y.; project administration, D.P.Y.; funding acquisition, D.P.Y. All authors have read and agreed to the published version of the manuscript.

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