



# Comparative morphometrics, molecular characterization, and dynamic population of *Zeldia punctata* associated with mustard crops from India

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## Abstract

A survey was conducted in the agricultural fields of mustard of Haridwar district, Uttarakhand, India, where a heavy population of *Zeldia* was recovered. The morphological, molecular, and phylogenetic characterization confirmed the nematode as *Zeldia punctata*. The phylogenetic analysis based on 18 and 28S rDNA placed the studied population of *Z. punctata* close to other populations of the same species. The principal component analysis (PCA) data revealed the high intraspecific and interspecific variations between the populations of *Zeldia punctata* from different geographical regions. The result indicated a variation between the Indian populations of *Z. punctata*. Additionally, the selected ecological factors indicated an impact of EC, temperature, and phosphorus on *Z. punctata* during the flowering and podding time of mustard crops in India. However, the PCA revealed no effect of ecological factors on the nematode diversity indices. In conclusion, *Z. punctata*, with its high individual numbers on mustard, may affect crop production due to activating the bacterial community of the soil and less space for plant-parasitic nematodes.

**Keywords** Bacterivores · Crop · Dynamic · Free-living · Morphology · Mustard · Nematode

## Introduction

Mustard (*Brassica rapa*) is one of the important cash crops grown in India, belonging to the family Brassicaceae. The primary production of mustard occurred in the states of northern India. The mustard grown in the areas of Uttarakhand requires 120–155 days to get a mature harvest (Shekhawat et al. 2012; Dwivedi and Mishra 2020). The extensive application of fertilizers favors the growth of bacterivores in agricultural fields (Wasilewska 1979; Yeates 1984). The first description of the *Zeldia punctata* was given by Thorne (1925), as the species was classified as *Acrobeles punctata* under the genus *Acrobeles* von Linstow, 1877. Later, a new

genus *Zeldia* Thorne, 1937 was erected in the family Cephalobidae, and the species was transferred under this new genus as a type species. The species was again described as *Acrobeles glaphyrus* Steiner, 1935; *Z. serrata* Heyns, 1962; *Z. paucipunctata* Andrassy, 1967 which was synonymized under *Z. punctata* by Allen and Noffsinger (1972). The distinctive morphological characteristics, including the presence of teeth-like structures on the cheilorhabdions of the genus *Zeldia* were observed by Steiner (1938) with a description of *Z. odontocephala*. Yeates (1967) described *Zeldia punua* from the sand under the *Ammophilia arenaria* (L.) link under this genus. Furthermore, seven new species were described under the genus *Zeldia*, namely *Z. feria*, *Z. minor*, *Z. tridentata*, *Z. spinula*, *Z. acuta*, *Z. neoacuta*, and *Z. solata* by Allen and Noffsinger (1972). However, due to overlapping morphological characteristics, *Z. minor* was synonymized under *Z. punctata* by Rashid et al. (1985). *Zeldia spannata* Waceke et al., 2005 recovered from the Mojave deserts, were described under this genus; however, the species transferred to genus *Chiloplacus* as *C. spannatus* (Abolafia and Peña-Santiago 2018). So far, the genus *Zeldia* comprises nine valid species and two species as

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*species inquirenda*, namely *Z. punctulata* Thorne, 1925 and *Z. setosa* Cobb, 1914 (Allen and Noffsinger 1972; Rashid et al. 1985).

*Zeldia punctata* has been reported in the USA (Thorne 1925; Allen and Noffsinger 1972), Mexico (Steiner 1935), South Africa (Heyns 1962), Brazil (Rashid et al. 1985), Namibia (Rashid and Heyns 1990), Senegal (De Ley et al. 1990), Greece (Boström 1992), Botswana (De Bruin and Heyns 1993), India (Tahseen et al. 1999), Pakistan (Tabasum and Shahina 2002), Spain (Abolafia and Peña-Santiago 2003), Hungary (Andrássy 2005) and Iran (Mehdizadeh and Shokoohi 2013). The description of the *Z. punctata* was found to be based on morphological studies with fewer studies on molecular characterization. Although molecular description of the species was provided by De Ley et al. (1999), Nadler et al. (2006), Kiontke et al. (2007), Holovachov et al. (2015), Abolafia and Veechi (2021), and Shokoohi et al. (2022) in the construction of phylogenetic trees to resolve the Rhabditida group without providing detailed description of the species. Furthermore, bacterial communities associated with *Z. punctata* from South Africa have been studied (Shokoohi et al. 2022). Therefore, the study's aims were (1) to study *Z. punctata* based on morphology and molecular analyses and (2) to study the dynamic populations of *Z. punctata* using principal component analysis (PCA).

## Materials and methods

The present investigation was carried out in the fields of Haridwar district, Uttarakhand. The soils were collected from the mustard (*Brassica rapa*) fields of district Haridwar, Uttarakhand (29°54'29" N, 78°6'58" E; 265 m a.s.l.) viz., Bahadarabad, Khanpur, Laksar, Roorkee, Bhagwanpur and Narsan at a minimum depth of 5–30 cm (Southey 1974). Soil samples (250 g) were collected in transparent polyzipper bags labeled with the date, host plant, soil type, and locality. Further, the soil was brought to the Nematology Laboratory, Department of Zoology, Chaudhary Charan Singh University, Meerut, India, for further processing. The nematodes were extracted from 250 g of the soil, using modified Cobb's (1918) sieving method and Baermann's funnel techniques (Goodey 1957; Southey 1985).

## Morphology and morphometry analysis

The adults were heat-killed in Ringer's solution and then fixed in 50% TAF for 24 h. They were then fixed in 100% TAF for 5–7 days (Courtney et al. 1955); dehydrated in Seinhorst I and II (Seinhorst 1959). Further processing was

done by following the Sharma and Chaubey (2023) description. The nematodes were permanently fixed on glass slides. The phase contrast microscope's inbuilt software was used to perform observations and measurements in micrometers ( $\mu\text{m}$ ). De Manian indices (de Man 1880) and other ratios were calculated. Images were captured with a Nikon Digital Sight camera and DIC optics (Y-TV55) on a Nikon microscope. Adobe®Photoshop®CS was used to edit the micrographs.

## Scanning electron microscopy (SEM)

Some specimens preserved in glycerin were selected for their observation with a scanning electron microscope (SEM) according to the protocol described by Abolafia (2015). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point-dried, coated with gold, and observed with a Zeiss Evo scanning electron microscope (5 kV) (Model: EVO 18 Research, Country: India, Company: ZEISS).

## Molecular characterization

The DNA was extracted from single virgin females (not having a plug at the vulva). The single virgin female was first washed with Ringer's solution and then in Phosphate buffer saline (pH 7.2). Then, the nematode was transferred into a sterile Eppendorf tube (500  $\mu\text{l}$ ) with extraction buffer 20  $\mu\text{l}$  (ddH<sub>2</sub>O 17.7  $\mu\text{l}$ , 10X Taq buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2  $\mu\text{l}$ , 1% tween 2  $\mu\text{l}$ , and proteinase K 0.1  $\mu\text{l}$ ). This buffer was frozen at -80°C for several days before being incubated at 65°C for 1 hour and subsequently at 95°C for 10 minutes. The lysates were chilled on ice, then centrifuged at 6000  $\times$  g for 2 minutes, and 3  $\mu\text{l}$  of supernatant was used for PCR (Bharti et al. 2020). Partial sequence of 28S rDNA gene, D2–D3 domains were amplified using primers, D2A(5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') (De Ley et al. 1999) and partial sequence of 18S rDNA was amplified using primers NEM18SF: 5'-CGCGAATRGCTCATTACAACAGC-3'' (forward) and NEM18SR: 5'-GGGCGGTATCTGATCGC C-3'' (reverse) (Floyd et al. 2005). The 25  $\mu\text{l}$  PCR product consisted of 7.5  $\mu\text{l}$  nuclease-free distilled water, 1  $\mu\text{l}$  each forward and reverse primer, 3  $\mu\text{l}$  DNA extract, and 12.5  $\mu\text{l}$  Dream Taq Green PCR master mix 2X (Thermo Scientific). The PCR amplifications were carried out using Verti 96 well fast thermocycler (AP Scientific) with heated lid pre-set at 95 °C and subjected to the following cycling profile; as for the 18 S rDNA region, PCR conditions included 1 cycle of initial denaturation at 94 °C for 3 min; followed by 40 cycles of 94 °C (denaturation) for 30 s, 54 °C (annealing) for 30 s

and 72 °C (primer extension) for 60 s, followed by a final extension at 72 °C for 10 min. For the D2-D3 rDNA region, PCR conditions included 1 cycle of initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C (denaturation) for 45 s, 55 °C (annealing) for 45 s and 72 °C (primer extension) for 1 min, followed by a final extension at 72 °C for 10 min to confirm all of the PCR products are complete length. The amplified products were electrophoresed in 1% agarose (w/v) gel using 1x TAE buffer buffered agarose gel stained with ethidium bromide (45 min, 100 V) was used to assess amplification success. Bioserve Pvt. Ltd. Hyderabad (India) purified and sequenced the amplified PCR products. BioEdit was used to edit the newly discovered sequences (Hall 1999).

### Phylogenetic analyses

The 18S rDNA and D2-D3 sequences were BLAST (Basic Local Alignment Search Tool) for % similarity matches with the previously submitted sequences in the NCBI (National Centre for Biotechnology Information) (Altschul et al. 1990). For each amplified rDNA region (18S rDNA and 28S rDNA), an alignment of our data with sequences from other closely related species was generated using default ClustalW parameters in MEGA 7.0 (Kumar et al. 2016) and manually optimized in BioEdit (Hall 1999). Bayesian inference (BI) was used to infer the phylogenetic trees from the datasets. *Macrolaimus* sp. (DQ145639) and *Diastolaimus grossus* (DQ145636) for 28 S and *Psilenchus hilarulus* (MK639403), and *Aphelenchus avenae* (JQ348399) for 18S were utilized as outgroup taxa and, with all characteristics being equally weighted and gaps being treated as missing data (Nadler et al. 2006; Mehdizadeh and Shokoochi 2013; Holovachov et al. 2015; Abolafia and Vecchi 2021). MrBayes 3.2.7 was used to produce Bayesian phylogenetic reconstructions (Ronquist et al. 2012). The best-fit nucleotide substitution models used for the phylogenetic analysis were selected using jModel-Test 2.1.10 (Darriba et al. 2012). The General Time Reversible substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites (GTR+G+I) was used as the optimal nucleotide substitution model for the analyses. One tree was preserved per 1000 generations after running Metropolis-coupled Markov chain Monte Carlo generations (MCMC) for  $1 \times 10^7$  cycles. The partial sequences of 18S rDNA (ON739024), and 28S rDNA (OM280054) were deposited in NCBI GenBank.

### Population dynamics

The population densities of *Z. punctata* in the samples were calculated by applying the formulae proposed by Norton (1978)

to determine the ecological index. The samples were collected during the podding (September–October) and flowering period (December–February) from the rhizosphere of mustard fields. The calculations are as follows:

1- Absolute frequency = (No. of samples containing a species)/ (No. of samples collected)  $\times$  100.

2- Relative frequency = (Frequency of a species)/ (Sum of frequencies of all spp.)  $\times$  100.

3- Relative density = (No. of individuals of a species in a sample)/ (Total of all individuals in a sample)  $\times$  100.

4- Absolute density = (No. of individuals of a species in a sample)/ (Volume or mass or units of the sample)  $\times$  100.

5- Prominence value = (Absolute density  $\times \sqrt{\text{Absolute frequency}}$ )/10.

### Soil physiochemical properties

The pH of collected soil samples was measured by pH meter (Systronics pH system 362). For measuring the pH of each sample, 20 g of soil sample was dissolved in 100 ml of distilled water. The pH meter was calibrated before taking the readings. For proper dissolution, the solution was stirred with a magnetic stirrer (Spinot), and the reading was measured with a pH meter. For the estimation of electrical conductivity (EC), the conductivity cell was first calibrated with 0.01 M KCl. After calibrations, reading was taken from the digital TDS/ Conductivity meter (model: MK-509). For the available phosphorus (AP), 0.5 M NaHCO<sub>3</sub> used as extractant (Olsen et al. 1954) and reading was obtained from the Spectrophotometer.

### Statistical analyses

Principal component analysis (PCA) was done to find out the morphological variations between the isolated nematodes in the present investigation and closely related species of the nematodes using XLSTAT (Addinsoft 2007). The measurements of the *Zeldia* species were collected from their original descriptions. The 2D plot was formed by using the PC1 and PC2 values of each isolate based on eigenvalues given by the software. The relationship of the population indices, including AF, RF, AD, RD, and PV, with the environmental factors (temperature) and edaphic (pH, available phosphorus, and EC) were determined through canonical correspondence analysis (CCA) using XLSTAT.

## Results

### *Zeldia punctata* (Thorne, 1925) Thorne, 1937

Measurements - see Table 1 for 20 individuals of females, males, and juveniles in good state of preservation.

*Female* Lateral field with three incisures (Fig. 2d, f). Lip region with six lips amalgamated in pairs; labial probolae

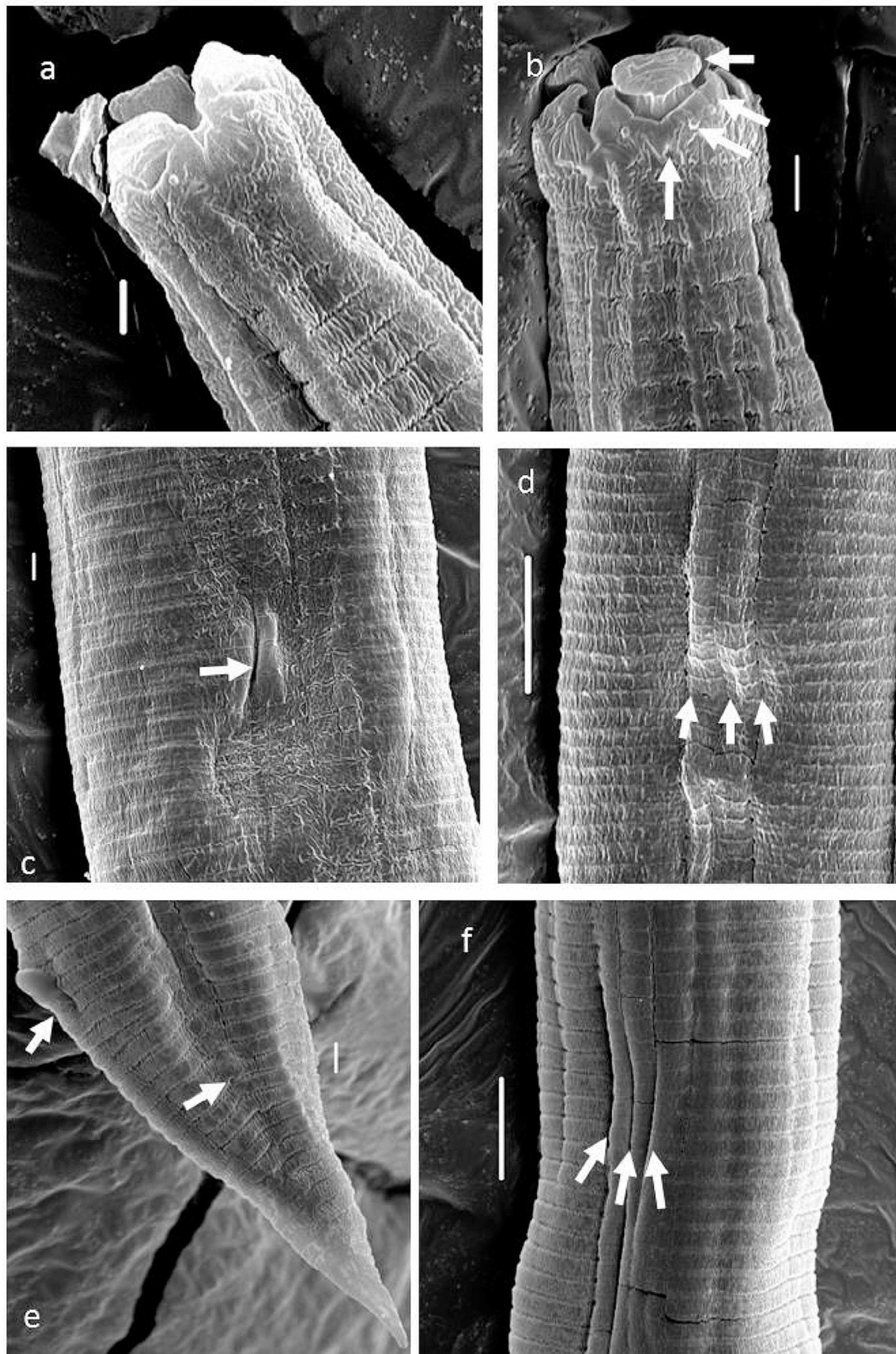
bifurcate, 2–5  $\mu\text{m}$  long; primary axils U-shaped, secondary axils V-shaped (Figs. 1b, c, 2a, b). Pharyngeal corpus cylindrical (4–8 times isthmus length). Excretory pore at level of isthmus (Fig. 1a). Ovary reflexed at oviduct, posteriorly reflexed to vulva usually straight without flexures (Fig. 1g). Vagina short, straight and distally thick walled 5.15–7.91  $\mu\text{m}$  long, occupying 14–19% of body diameter (Figs. 1e, 2c). Postvulval uterine sac distinct with 19.08–28.14  $\mu\text{m}$  long.

**Table 1** Morphometrics of *Zeldia punctata* HN3 (Thorne, 1925) Thorne, 1937 from Uttarakhand, India

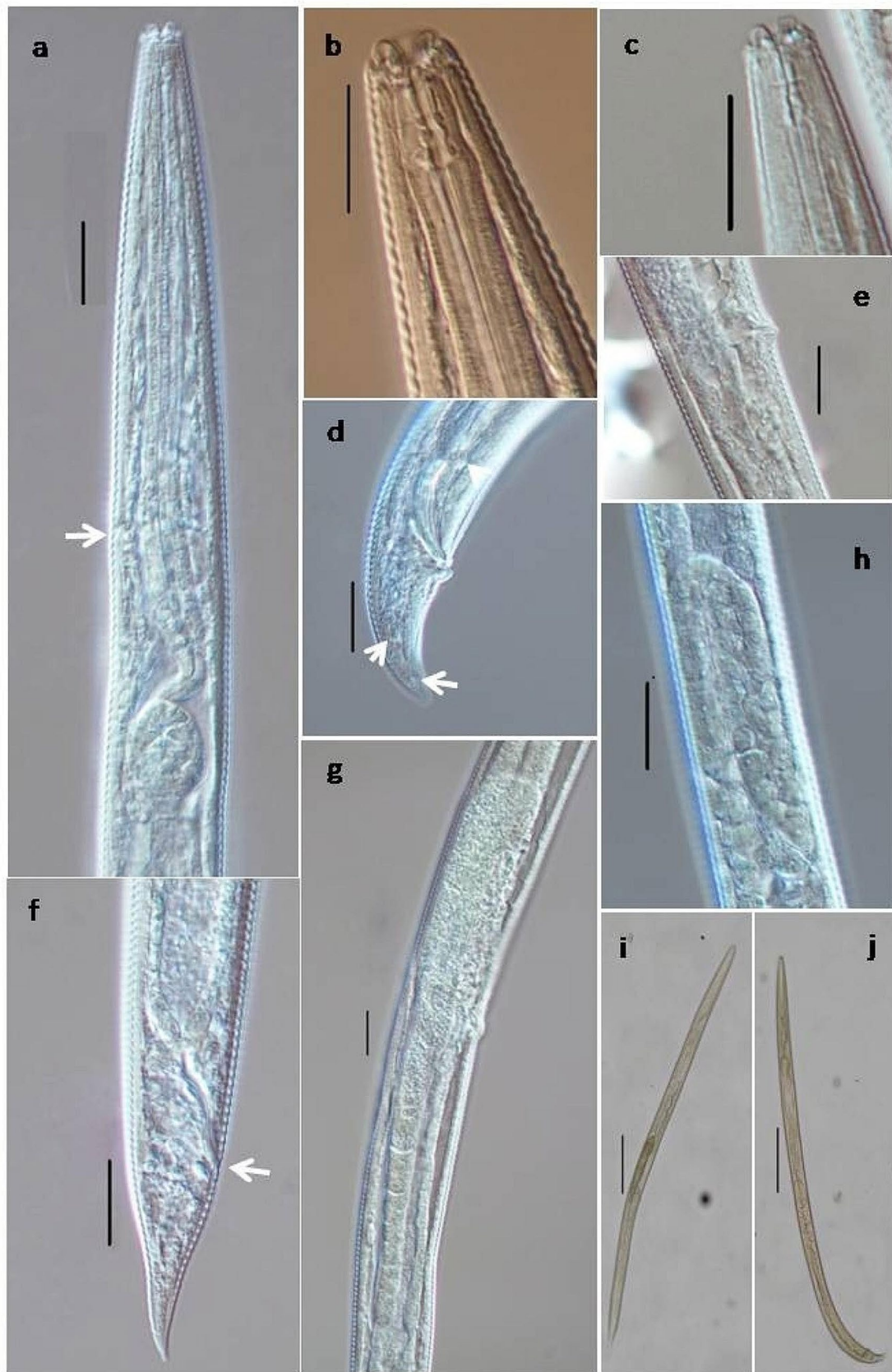
Characters	Female	Male	Juveniles
n	20	20	20
Body length (L)	809.1 $\pm$ 30 (745–867)	760.3 $\pm$ 28.7 (700–810)	519.3 $\pm$ 40.1 (430–651)
a (L/GBD)	18.8 $\pm$ 1.3 (16.3–20.9)	22.7 $\pm$ 2.2 (18.9–28.7)	23.3 $\pm$ 6.1 (16.8–35.0)
b (L/neck)	3.8 $\pm$ 0.2 (3.5–4.2)	4.0 $\pm$ 0.2 (3.7–4.4)	3.2 $\pm$ 0.5 (2.6–4.2)
c (L/T)	16.1 $\pm$ 0.8 (14.6–18.2)	15.9 $\pm$ 1.0 (14.3–17.1)	14.6 $\pm$ 3.5 (10.8–20.0)
c' (T/ABW)	2.2 $\pm$ 0.2 (1.9–2.5)	1.8 $\pm$ 0.1 (1.7–2.1)	2.9 $\pm$ 0.8 (1.6–4.8)
V% (AV/L * 100)	63.8 $\pm$ 1.4 (60–66)	-	-
Labial probolae length	3.7 $\pm$ 0.8 (2.2–5.4)	2.9 $\pm$ 0.5 (2.1–4.1)	2.8 $\pm$ 0.5 (2.1–3.9)
Lip region width	10.9 $\pm$ 0.6 (10–12)	10.5 $\pm$ 0.9 (8–12)	9.1 $\pm$ 0.8 (7–11)
Stoma length	10.7 $\pm$ 1.0 (9–13)	10.3 $\pm$ 1.3 (8–13)	8.9 $\pm$ 0.8 (7–10)
Pharyngeal corpus length	150.9 $\pm$ 9.6 (132–172)	135.4 $\pm$ 5.2 (125–150)	116.3 $\pm$ 5.7 (100–130)
Isthmus length	21.7 $\pm$ 5.0 (15–39)	19.1 $\pm$ 3.7 (14.7–26.9)	17.2 $\pm$ 3.7 (9–23)
Bulb length	23.8 $\pm$ 2.9 (19–30)	23.5 $\pm$ 1.6 (20–26)	14.8 $\pm$ 3.5 (13–21)
Bulb width	20.4 $\pm$ 1.5 (18–22)	18.1 $\pm$ 1.0 (16–20)	10.9 $\pm$ 1.9 (9–13)
Pharynx length	196.5 $\pm$ 12.6 (171–213)	178.1 $\pm$ 10.5 (161–204)	148.3 $\pm$ 7.9 (124–156)
Nerve ring from anterior end (NR)	116.6 $\pm$ 3.8 (108–128)	112.9 $\pm$ 6.1 (103–122)	89.8 $\pm$ 4.2 (67–116)
Excretory pore from anterior end (EP)	121.2 $\pm$ 5.0 (114–132)	117.6 $\pm$ 3.9 (109–125)	95.9 $\pm$ 6.1 (69–127)
Body width at excretory pore (WEP)	33.2 $\pm$ 2.0 (30–37)	28.9 $\pm$ 2.4 (25–34)	23 $\pm$ 3.9 (17–27)
Deirid from anterior end	-	-	?
Annuli width	2.4 $\pm$ 0.3 (2.0–2.8)	2.2 $\pm$ 0.3 (1.8–2.5)	-
Neck length (stoma + pharynx)	207.2 $\pm$ 12.3 (184–223)	188.4 $\pm$ 11.8 (169–216)	157.2 $\pm$ 7.8 (134–166)
Body width at neck base	38 $\pm$ 4.1 (34–41)	30.1 $\pm$ 2.0 (29–32)	24.6 $\pm$ 5.3 (18–28)
Body diameter at midbody (BD)	43 $\pm$ 3.2 (36–49)	33.7 $\pm$ 2.8 (28–37)	26.3 $\pm$ 1.4 (21–29)
Lateral field width	4 $\pm$ 0.7 (3.5–4.2)	3.4 $\pm$ 0.4 (3.0–3.7)	-
Vagina	6.6 $\pm$ 0.8 (5–8)	-	-
Ovary length	133.1 $\pm$ 5.6 (123–145)	-	-
Spermatheca	13 $\pm$ 2.1 (11–16)	-	-
Postuterine sac length (PUS)	24.3 $\pm$ 2.9 (19–28)	-	-
Vulva from anterior end (AV)	516.5 $\pm$ 23.2 (468–553)	-	-
Vulva from posterior end (PV)	292.6 $\pm$ 14.8 (265–325)	-	-
Rectum length	27.9 $\pm$ 2.7 (21–33)	-	12.6 $\pm$ 4.0 (8–22)
Anal body diameter (ABD)	22.9 $\pm$ 1.8 (20–26)	26.2 $\pm$ 1.4 (23–29)	13.4 $\pm$ 3.1 (8–19)
Tail	50.2 $\pm$ 2.6 (43–56)	48 $\pm$ 2.7 (44–53)	36.6 $\pm$ 6.2 (28–49)
Phasmid-anus distance	13.7 $\pm$ 3.1 (11–16)	11.1 $\pm$ 2.1 (9–13)	-
Spicule length (SL)	-	35.3 $\pm$ 2.2 (30–39)	-
Gubernaculum length (GL)	-	16.7 $\pm$ 1.0 (15–19)	-

\* All measurements are in  $\mu\text{m}$  (except ratio and percentage) and in the form of mean  $\pm$  standard deviation (range)





**Fig. 2** *Zeldia punctata* (Thorne, 1925) Thorne, 1937 (SEM). **a, b** Lip region (arrows pointing to the labial probolae, cephalic probolae, papillae, amphid). **c** Vulva region. **d, f** Lateral field. **e** Female posterior end (arrow pointing to the anus and phasmid). Scale bars: a–c, e = 3  $\mu$ m; d, f = 10  $\mu$ m



**Fig. 1** *Zeldia punctata* (Thorne, 1925) Thorne, 1937 (LM). **a** Neck. **b, c** Lip region. **d** Male posterior end. **e** Vulva. **f** Female posterior end. **g** Female reproductive system. **h** Male reproductive system. **i** Entire female. **j** Entire male. Scale bars: a, c, d-h = 20  $\mu$ m; b = 15  $\mu$ m; i, j = 100  $\mu$ m

Post uterine sac 0.5–0.6 times the corresponding body diameter in length. Female tail conical (Fig. 2e), with concave ventral side (Fig. 1f, i).

**Male** Body 0.70–0.81 mm long, ventrally curved, appearing J-shaped (Fig. 1j). Reproductive system monarctic, with testis ventrally reflexed anteriorly located on the left side of the intestine (Fig. 1h). Spicules curved ventrally, long, broad, 2.0–2.5 times gubernaculum's length, somewhat rounded and reduced manubrium, conical calamus, lamina slightly curved with a hump with a ventral velum, and a thin cylindrical tip (Fig. 1d). Gubernaculum well developed with manubrium straight, crura with a tip (Fig. 1d). Genital papilla observed, eight pairs consisting of three pairs of preanal papillae, four pairs of caudal papillae, and one pair of subdorsal papillae and phasmid. The preanal papillae present sub-ventrally, the caudal papillae where two pairs observed near tail terminus and two pairs arranged near the phasmid. Phasmids were observed anterior to the caudal papillae at 20–25% of tail length. Tail conical in shape, short with a pointed terminus.

**Third stage juveniles (J3)** Body 0.43–0.65 mm long becomes straight after fixation. Cuticle showed similarity with the adults. Lip region 7.37–10.62  $\mu\text{m}$  wide with a narrow stoma. Distinct cephaloboid pharynx differentiated into three sections viz., corpus, isthmus and basal bulb. Nerve ring visible at the corpus level opposite to excretory pore. Deirids obscure. Rectum distinct, straight 1.0–1.2 of anal body diameter. Prominent anus. Tail conical in shape with a pointed terminus. General morphology showed similarity with adult female, except for the absence of reproductive system.

## Remarks and morphometric comparison

*Zeldia punctata* is a widespread species that has been reported in different countries worldwide (Shokoohi and Abolafia 2019). The morphological and morphometrical analysis revealed the present isolate belongs to *Z. punctata*. The Indian population, compared with the American population (Thorne 1925), differs in tail length (43–56 vs. 33  $\mu\text{m}$ ). The Indian population of *Z. punctata* is similar to the specimens studied from India by Tahseen et al. (1999). Compared with Pakistani population of the same species (Tabassum and Shahina 2002), they differ in tail length (43–56 vs. 32–40  $\mu\text{m}$ ). Compared with the Spanish material examined by Abolafia and Peña-Santiago (2003), they differ in body length (0.74–0.86 vs. 0.62–0.82 mm), and shorter neck (184–223 vs. 202–216  $\mu\text{m}$ ) and spermatheca (11–16 vs. 37  $\mu\text{m}$ ). In comparison to the material examined from Hungary by Andrassy (2005), Indian specimens have shorter body length (0.74–0.86 vs. 0.64–0.94 mm) and shorter neck (184–223 vs. 210–230  $\mu\text{m}$ ). The comparative

morphometrics for *Z. punctata* is given in Tables 2 and 3, respectively.

## Molecular characterization

Pairwise distances (Kimura 2-parameter) based on the D2-D3 region revealed the genetic distances between the populations of *Z. punctata* described from other geographical regions. The Indian population showed the lowest genetic distance (0.0081) from the USA population (DQ145662). The highest genetic distance (0.7717) was observed between the USA (EU195988) and Sweden (KU180685) populations. The average overall genetic distance between the different populations of *Z. punctata* was 0.0579, ranging from 0.001 to 0.7717. The lowest genetic distance (0.001) was observed between the USA (DQ145662) and Sweden (KU180685) population (Table 4). The Indian population showed the lowest distance (0.0081) with a population from the USA (DQ145662) and the highest distance (0.0185) with a population from Sweden (KU180685).

## Phylogenetic relationships

The phylogenetic analysis based on 18S and 28S rDNA confirmed the species as *Z. punctata*. Based on the phylogenetic analysis inferred from 18S rRNA sequences; the Indian population formed a clade with the Netherlands population with a 1.00 posterior probability (Fig. 3). The phylogenetic tree based on the 28S rDNA sequences showed that the present Indian population placed close to the Swedish population of *Z. punctata* (MZ254892) with a 1.00 posterior probability. The Indian population was also placed close to other molecularly identified *Z. punctata* from the USA (AF147070; DQ145662; EU195988) and Sweden (KU180685) (Fig. 4).

## Principal component analysis (PCA)

The principal component analysis based on the morphometric data of *Z. punctata* reported from different geographical locations, including India (Tahseen et al. 1999), USA (Allen and Noffsinger 1972), Brazil (Rashid et al. 1985), Namibia (Rashid and Heyns 1990), Senegal (De Ley et al. 1990), Greece (Bostrom 1992), Botswana (De Bruin and Heyns 1993), Pakistan (Tabassum and Shahina 2002), Spain (Abolafia and Peña-Santiago 2003), Iran (Mehdizadeh and Shokoohi 2013) and the present study revealed the morphometric variations (Table 5).

The Indian populations showed variations in the morphometry from the populations reported from other countries; however, the result indicated that the present population from India was close to the Iranian and Mexican populations of *Z. punctata*. The PCA based on the

**Table 2** Comparative morphometrics of females from populations of *Zeldia punctata* described from different regions (all measurements are in µm)

Characters	<i>Z. punctata</i>														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
HN3 (Present study)	India (Uttarakhand)	USA	Mexico	South Africa	USA	Brazil	Namibia	Senegal	Greece	Botswana	India (Uttar Pradesh)	Pakistan	Spain	Hungary	Iran
n	20	1	1	10	74	6	40	10	3	7	6	9	4	-	3
L	745–867	700–900	710	640–850	600–940	650–780	500–920	598–743	689–800	570–690	790–912	656–880	629–824	640–940	618–736
a	16.3–20.9	21.8	13.8	15.5–22.0	15.8–27.3	18.0–25.0	19.2–27.5	18.0–19.9	20–22	21.9–28.2	17–25	21.0–30.8	18.5–20.5	16–26	19.2–24.4
b	3.5–4.2	3.8	3.4	3.4–4.4	3.3–4.5	3.4–4.0	2.8–4.9	2.9–3.6	3.8–3.9	3.3–3.6	3.6–4.3	3.4–4.4	3.1–3.9	3.3–4.5	3.3–3.4
c	14.6–18.2	21.2	18.5	13.4–20.9	13.2–23.6	17.5–20.0	13.7–30.5	17.1–21.2	18–19	12.6–18.6	13–17	16.6–22	17.5–20.5	13–20	15.6–16.6
c'	1.9–2.5	?	?	?	?	?	1.1–2.5	1.6–2.0	1.9–2.1	2.2–3.1	2.4–3.0	2.5–3.3	1.6–1.9	1.8–3.0	1.8–2.1
V%	60–66	67	59	64–67	62–68	65–66	58–67	61–71	65–66	64–68	57–65	58–67	64–65	57–65	64–66
Lip region diameter	10–12	?	?	11*	10*	11*	11*	?	?	12*	10*	6–8	11–12	10–12	9–10
Stoma	9–13	?	?	12.2*	13*	14.5–15.5	10.0–13.5	11–13	12–13	10–13	16–18	10.4–12.0	11–15	14–18	12–14
EP	114–132	?	?	107*	100–159	112.0–28.0	87.5–146.5	91–130	?	83*	131*	100–120	86–127	?	112–130
NR	108–128	100	160	120*	?	110*	87–143.5	87–125	?	85–108	120*	112–124	110–131	?	106–123
Bulb length	19–30	?	?	26*	?	22*	23*	23–32	26–29	23–27	22–32	20.0–28.0	26–28	?	27–29
Pharynx length	171–213	?	?	181*	?	171*	124–235.5	192–220	182–203	152*	213–238	169*	188*	?	172–192
Neck length	184–223	180	200	187*	?	191–200	189*	?	?	168–200	227*	180–200	202–216	210–230	184–212
Tail	43–56	33	39	46*	41*	37–41	32–51	30–40	38–44	33–47	48–63	32–40	36–40	?	38–47
ABW	20–26	21	25	20*	21*	16*	19*	18–23	?	14*	19–24	12–14	20–23	?	20–25
Rectum	21–33	?	?	21*	?	20*	17–29	21–23	18–25	16–22	25–28	24*	24–28	?	25–28
PUS	19–28	?	?	?	?	14.5–15.0	10–37	?	12–23	9*	15–25	8–10	10	?	10–16
Spermatheca	11–16	?	?	?	?	11*	20*	?	12–17	20*	31*	17*	37	?	12–14

\*extracted from drawing of the original drawing

1 - Thorne (1925), 2 - Steiner (1935), 3 - Heyns (1962), 4 - Allen and Noffsinger (1972), 5 - Rashid et al. (1985), 6 - Rashid and Heyns (1990), 7 - De Ley et al. (1990), 8 - Bostrom (1992), 9 - De Bruin and Heyns (1993), 10 - Tahseen et al. (1999), 11 - Tabassum and Shahina (2002), 12 - Abolafia and Peña-Santiago (2003), 13 - Andrassy (2005), 14 - Mehdizadeh and Shokoolhi (2013)



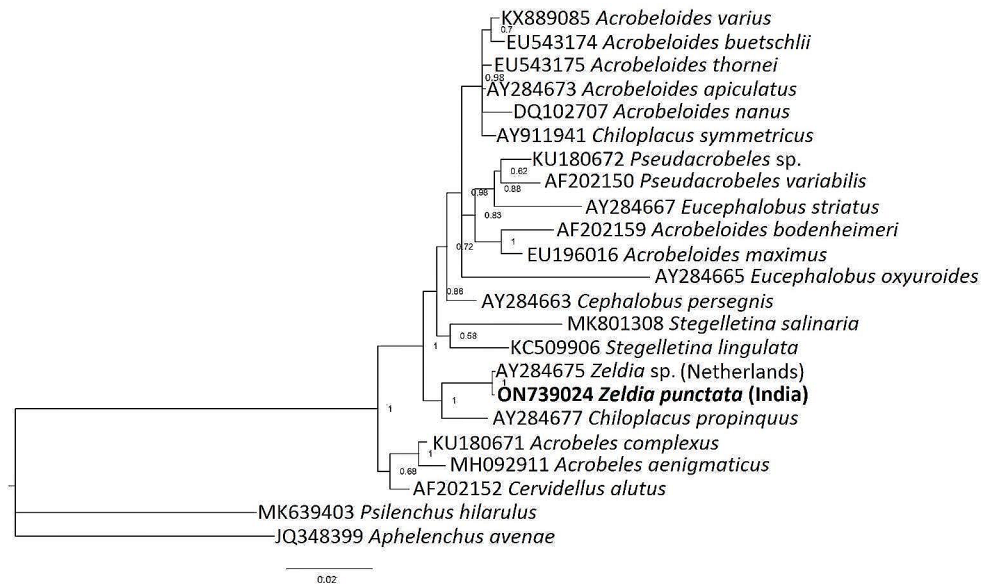
**Table 3** Comparative morphometrics of males and juveniles from populations of *Zeldia punctata* described from different regions (all measurements are in  $\mu\text{m}$ )

Characters	<i>Zeldia punctata</i>					
	Males			Third stage juveniles		
	HN3 (Present study)	Allen and Noffsinger (1972)	Rashid et al. (1985)	Andrássy (2005)	HN3 (Present study)	De Bruin and Heyns (1993)
	India (Uttarakhand)	USA	Brazil	Hungary	India (Uttarakhand)	Botswana
n	20	17	1		20	12
L	700–810	820–1010	710	820–1000	430–651	470–640
a	18.9–28.7	19.1–33.6	21.6	19–34	16.8–35.0	20.4–29.5
b	3.7–4.4	3.9–4.8	4	3.9–4.8	2.6–4.2	3.1–4.4
c	14.3–17.1	16.5–19.0	16	?	10.8–20.0	12.1–16.9
c'	1.7–2.1	?	?	2.0–2.5	1.6–4.8	2.2–3.2
Lip region diameter	8–12	?	10*	?	7–11	?
Stoma	8–13	?	11*	?	7–10	?
EP	109–125	129–172	129–172	?	69–127	?
NR	103–122	?	?	?	67–116	?
Bulb length	20–26	?	?	?	13–21	?
Pharynx	161–204	?	?	?	124–156	?
Neck	169–216	?	?	?	134–166	?
Tail	44–53	33.0–57.0	44	40–55	28–49	33–53
Anal body diameter	23–29	?	25*	?	8–19	?
Spicule	30–39	29.0–40.0	32	29–40	?	?
Gubernaculum	15–19	13.0–21.0	13	15–20	?	?

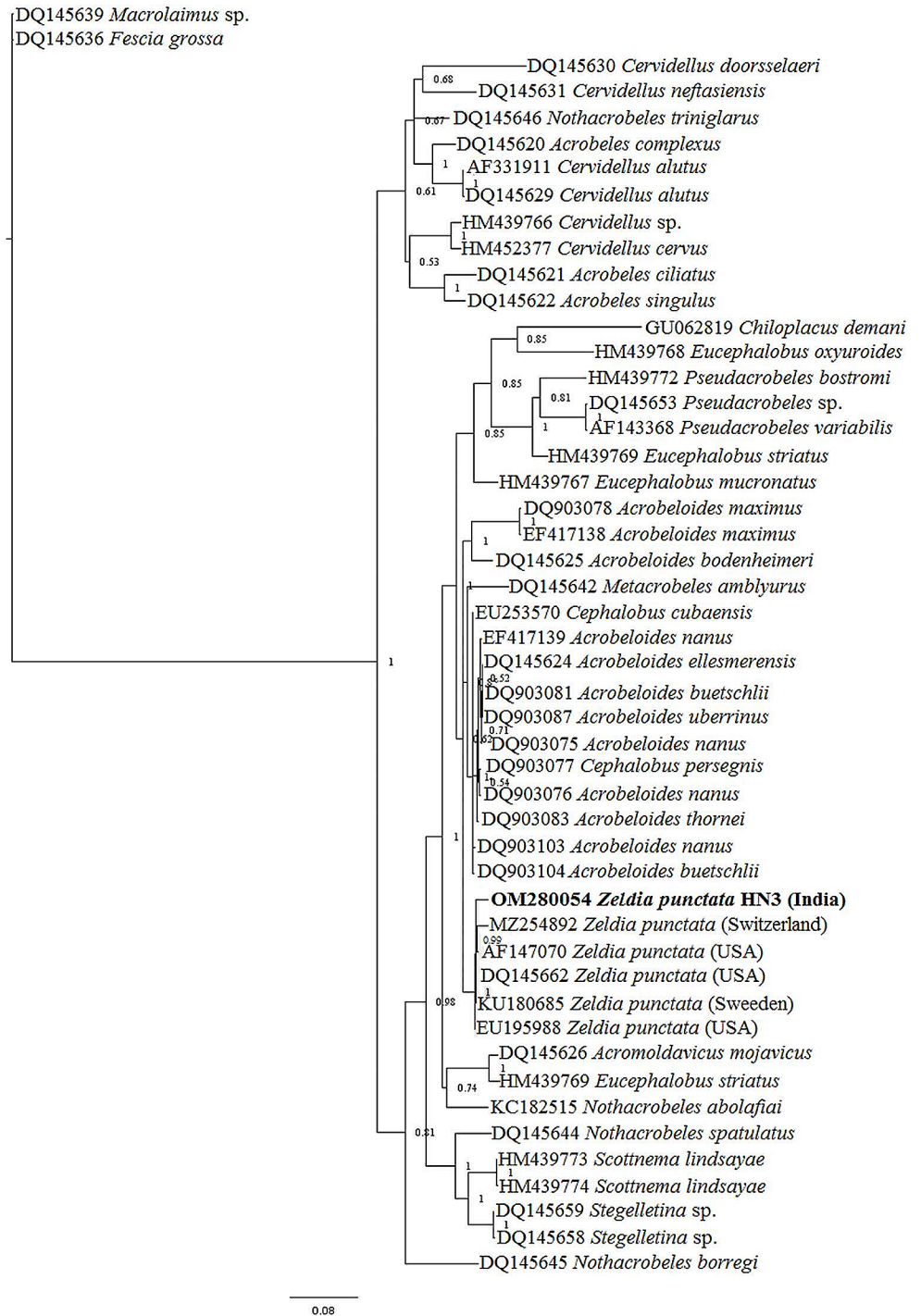
**Table 4** Pairwise distance (Kimura 2-parameter) for different populations of *Zeldia punctata* based on 28S rDNA.

			1.	2.	3.	4.	5.	6.
	Accession numbers	Locality	OM280054	MZ254892	AF147070	DQ145662	EU195988	KU180685
1.	<b>OM280054</b>	<b>India</b>		<b>0.0127</b>	<b>0.0098</b>	<b>0.0081</b>	<b>0.0123</b>	<b>0.0185</b>
2.	MZ254892	Switzerland	<b>0.0127</b>		0.0029	0.0057	0.0057	0.0056
3.	AF147070	USA	<b>0.0098</b>	0.0029		0.0028	0.0055	0.0027
4.	DQ145662	USA	<b>0.0081</b>	0.0057	0.0028		0.0032	0.0010
5.	EU195988	USA	<b>0.0123</b>	0.0057	0.0055	0.0032		0.7717
6.	KU180685	Sweeden	<b>0.0185</b>	0.0056	0.0027	0.0010	0.7717	

**Fig. 3** Phylogenetic position of *Zeldia punctata* based on 18S rDNA, including the sequenced population from India



**Fig. 4** Phylogenetic position of *Zeldia punctata* based on 28S rDNA, including the sequenced population from India



morphometry data of the female revealed an accumulated variability of 46.71% (Fig. 5; Table 5). The contribution of variation among the characters displayed in PC1 and PC2 was found to be 25.71% and 21.0%, respectively (Fig. 5). Three parameters, a ( $r = 0.606$ ), and V% ( $r = 0.782$ ) showed a positive correlation across populations of *Z. punctata* in PC1. Whereas neck length ( $r = -0.757$ ), and tail length ( $r = -0.754$ ) showed a negative correlation across populations

of *Z. punctata* in PC1 (Fig. 5). In addition, L ( $r = 0.725$ ), b ( $r = 0.728$ ), and c' ( $r = 0.738$ ) showed positive correlation across populations of *Z. punctata* in PC2. The result also showed that some populations of *Z. punctata* were placed close together (Fig. 5; Table 6). The result grouped different populations of *Z. punctata* into four groups, including (1) formerly studied population from India (Tahseen et al. 1999), USA (Allen and Noffsinger 1972), and South Africa,

**Table 5** Factor loading of studied morphometric characters for *Zeldia punctata*

	PC1	PC2
L	-0.255	0.725
a	0.606	0.494
b	-0.109	0.728
c	0.469	-0.292
c'	-0.044	0.738
V	0.782	-0.048
Stoma length	-0.213	0.547
EP	-0.247	-0.074
NR	-0.698	-0.319
Neck length	-0.757	-0.384
Tail length	-0.754	0.460
Rectum length	-0.484	-0.139
PUS	-0.310	-0.115

L=Body length, a (body length/ body diameter at midbody), b (body length/ neck length), c (body length/ tail), c' (tail/ anal body diameter) V% (vulva from anterior end /total body length) × 100, PUS=postuterine sac length, EP= excretory pore from anterior end, NR=nerve ring from anterior end, neck length (stoma + pharynx)

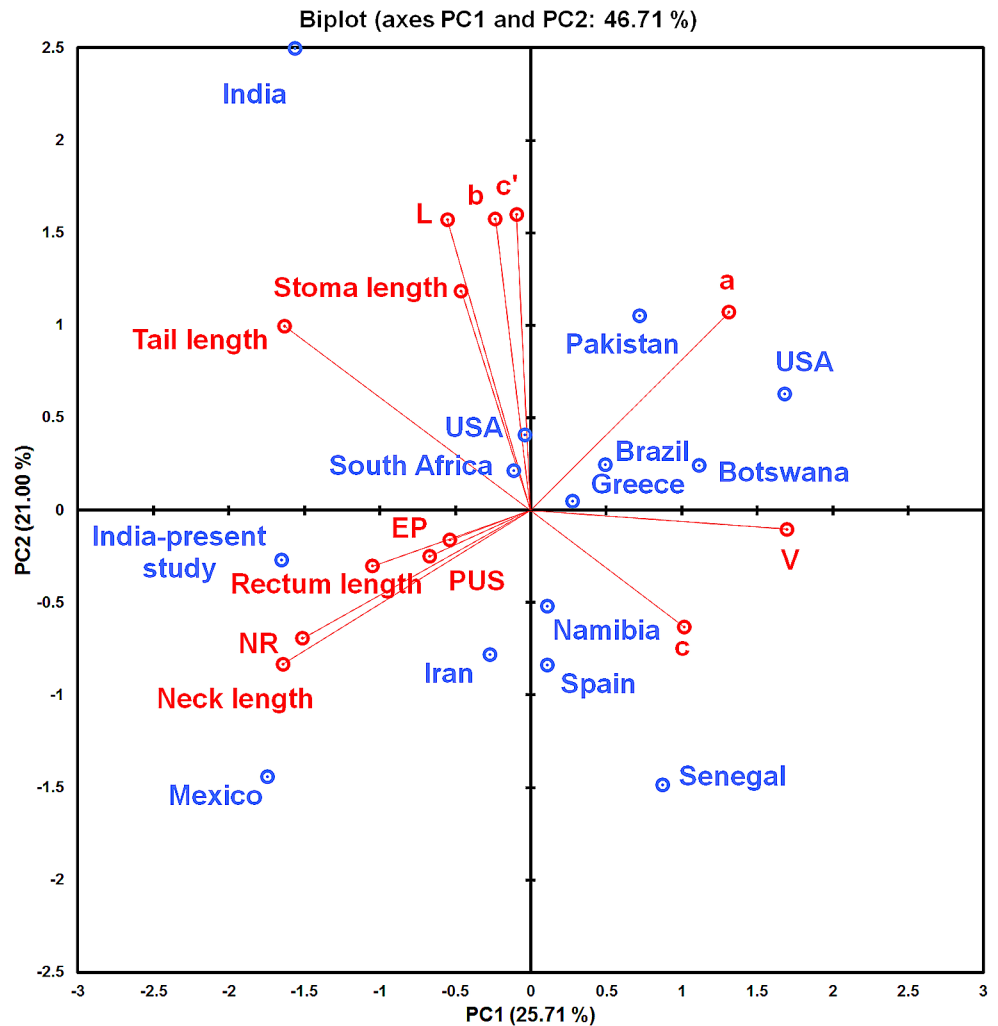
**Table 6** Factor score of studied morphometric characters for *Zeldia punctata*

Observation	Reference	PC1	PC2
India	HN3 (Present study)	-3.021	-0.444
USA	Thorne (1925)	3.074	1.037
Mexico	Steiner (1935)	-3.187	-2.379
South Africa	Heyns (1962)	-0.202	0.351
Africa			
USA	Allen and Noffsinger (1972)	-0.075	0.673
Brazil	Rashid et al. (1985)	0.905	0.408
Namibia	Rashid and Heyns (1990)	0.199	-0.860
Senegal	De Ley et al. (1990)	1.597	-2.454
Greece	Bostrom (1992)	0.507	0.079
Botswana	De Bruin and Heyns (1993)	2.039	0.400
India	Tahseen et al. (1999)	-2.856	4.126
Pakistan	Tabassum and Shahina (2002)	1.319	1.738
Spain	Abolafia and Peña-Santiago (2003)	0.196	-1.382
Iran	Mehdizadeh and Shokoohi (2013)	-0.493	-1.292

(2) Pakistan, Brazil, Botswana, Greece, and USA (Thorne

1925), (3) Namibia, Spain, and Senegal, and (4) Mexico, Iran, and present population from India (Fig. 5). The result indicated that two populations from Indian placed separately

**Fig. 5** PCA plot of the morphometric variation among the populations of *Zeldia punctata*



based on the female morphometrics. Additionally, the result revealed the high interspecific and intraspecific variations in the populations of *Z. punctata* studied.

### Population dynamics

The highest prominence value was observed in the Narsan and Laksar (88.2; 85.4), whereas the lowest was in the Bahadarabad (64.6) during the podding stages in 2021. The 2022 sampling revealed the highest prominence value in the Narsan and Bhagwanpur (91.6; 83.1) and the lowest in the Roorkee (48.9) (Table 7). The PCA analysis revealed that 77.36% of the variation, PC1 contributed 58.37%, and PC2 contributed 18.99%, where environmental factors like temperature and edaphic factors like pH, available phosphorus (AP) and electrical conductivity (EC) played a role in the population dynamics of *Z. punctata* (Fig. 6). The available phosphorus showed a positive correlation with the population dynamics of *Z. punctata* in Bhagwanpur, Bahadrad, and Khanpur regions during flowering. The edaphic factor EC positively correlated with Roorkee and Khanpur in 2021 during the podding of mustard. The result showed no correlation between the present study's diversity indices and ecological factors.

### Discussion

The description of *Z. punctata*, recovered from sewage, based on six females from India was first provided by Tahseen et al. (1999); however, the description lacks male morphology and molecular data of the species. Furthermore, this

species was described by Tabassum and Shahina (2002), Abolafia and Peña-Satiago (2003), and Mehdizadeh and Shokoohi (2013) describing females. Allen and Noffsinger (1972), Rashid et al. (1985), and Andrassy (2005) provided a description of males with limited morphology and morphometry. Likewise, the morphology of the juveniles was given by De Bruin and Heyns (1993). The present study is the first report on the molecular data of 18 and 28 S rDNA of *Z. punctata* from the Indian subcontinent with a detailed description of male. The morphological and morphometric data were found to be consistent with the type species (Thorne 1925) and redescribed by Thorne (1937), and Allen and Noffsinger (1972). *Zeldia feria* Allen and Noffsinger, 1972 can be distinguished from *Z. punctata* in terms of conspicuous punctuation, body length (0.77–0.98 vs. 0.74–0.86 mm), excretory pore (obscure vs. present), and tail (rounded vs. pointed). *Zeldia punctata* can be differentiated from *Zeldia punua* Yeates, 1967 by having shorter margins at labial probolae, phasmid (middle of tail vs. at the level of anus), and a longer tail. The description of *Zeldia punua* Yeates, 1967 is based on only three specimens, and the redescription given by Allen and Noffsinger (1972) is based on one specimen. *Zeldia punctata* can be distinguished from *Z. odontocephala* Steiner, 1938; *Z. tridentata* Allen and Noffsinger, 1972; *Z. spinula* Allen and Noffsinger, 1972; *Z. acuta* Allen and Noffsinger, 1972; *Z. neoacuta* Allen and Noffsinger, 1972 and *Z. solata* Allen and Noffsinger, 1972 as all the species possess accessory tooth associated with cheilorhabdions which is absent in the studied population. The species has been reported from sugarcane fields, sewage, beach sand, marginal soil, grapes, citrus tree, grasses, volcanic sandy soil, bulrush, date palm, sweet orange, tuberose,

**Table 7** The population dynamics of *Zeldia punctata* associated with mustard during podding and flowering period in Uttarakhand, India

Sampling sites	Growing season 2021/2022					Growing season 2021/2022				
	During podding					During flowering				
	AF% 1 <sup>1</sup>	RF 1 <sup>2</sup>	AD 1 <sup>3</sup>	RD 1 <sup>4</sup>	PV 1 <sup>5</sup>	AF% 2	RF 2	AD 2	RD 2	PV 2
Bahdarabad	53/76	0.66/0.81	545/616	56.5/65.9	64.6/73.8	61/79	0.71/0.84	613/689	66.5/71.2	74.6/75.8
Roorkee	66/72	0.75/0.84	775/816	68.3/85.5	72.5/48.9	78/90	0.79/0.88	801/887	74.6/88.4	78.6/47.5
Bhagwanpur	75/56	0.84/0.66	654/765	88.4/72.6	84.2/83.1	77/44	0.72/0.51	620/715	80.1/69.03	81.8/84.2
Narsan	83/76	0.89/0.85	713/848	85.6/86.8	88.22/91.6	92/94	0.91/0.86	727/917	75.5/86.4	88.32/90.01
Laksar	80/75	0.92/0.83	785/906	76.1/88.4	85.4/87.8	86/96	0.93/0.85	826/949	89.4/87.5	86.7/89.4
Khanpur	78/65	0.74/0.69	748/777	65.2/38.7	73.44/54.3	75/58	0.69/0.70	696/728	51.3/39.1	79.5/56.7

\*The left and right numbers are the 2021 and 2022 scores, respectively

AF% 1, RF 1, AD 1, RD 1, PV 1 representative during podding

AF% 2, RF 2, AD 2, RD 2, PV 2 representative during flowering

1- Absolute frequency (AF%) = (No. of samples containing a species)/ (No. of samples collected) × 100

2- Relative frequency (RF) = (Frequency of a species)/ (Sum of frequencies of all spp.) × 100

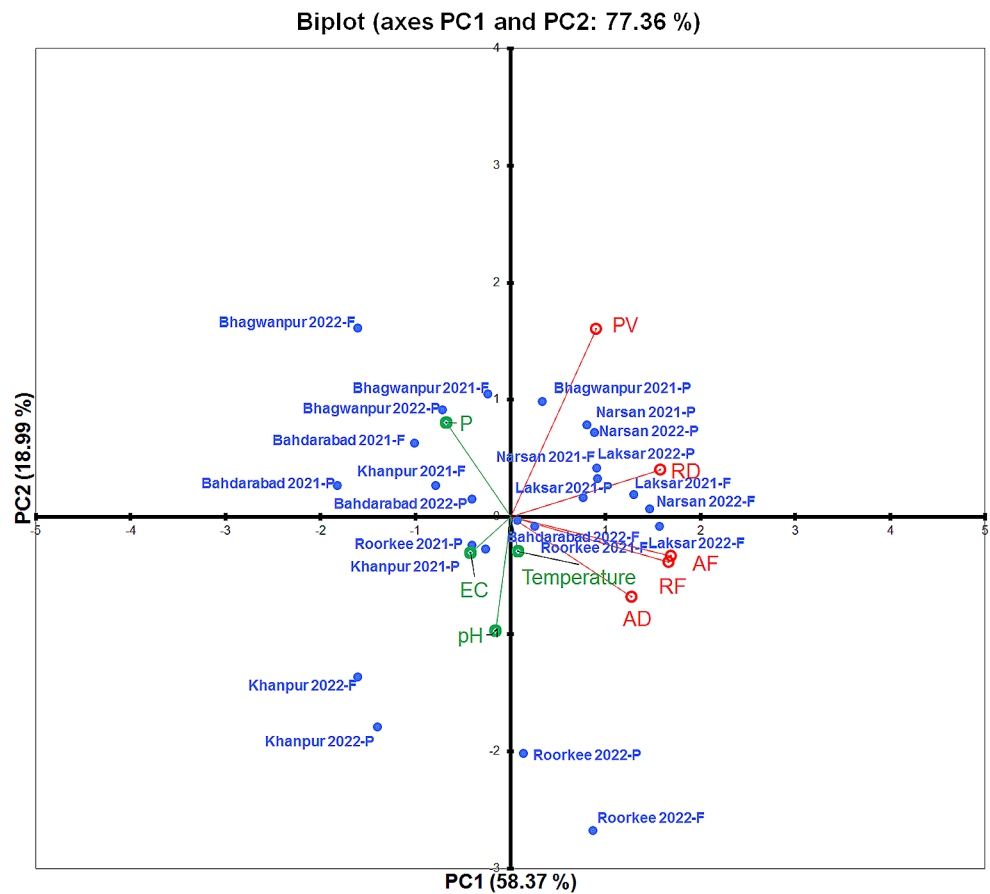
3- Relative density (RD) = (No. of individuals of a species in a sample)/ (Total of all individuals in a sample) × 100

4- Absolute density (AD) = (No. of individuals of a species in a sample)/ (Volume or mass or units of the sample) × 100;

5- Prominence value (PV) = (Absolute density × √ (Absolute frequency))/10



**Fig. 6** PCA plot of the relationship of the *Zeldia punctata* and selected ecological factors during podding and flowering times in mustard crops in India



and maize fields. The present study also revealed mustard fields as a new host to *Z. punctata*.

The presence of available phosphorus was found to increase the population of nematodes. The use of fertilizers in agricultural fields has led to an increase in available phosphorus and nitrogen (Wasilewska 1979; Yeates 1984). During the flowering season, the *Z. punctata* population in the regions of Bahadarabad, Bhagwanpur, and Khanpur was positively correlated with the available phosphorus. The PV (81.8/84.2) of Bhagwanpur increased as the availability of phosphorus ( $158.5 \pm 1.1$ ;  $175.1 \pm 1.07$ ) increased in this area. Similarly, the highest available phosphorus ( $189.5 \pm 1.0$ ;  $192.3 \pm 1.1$ ) was observed in the Bahadarabad region, which stimulated bacterial populations and recorded PV (74.6/75.8). During the podding season in 2021, the population of Khanpur with a recorded PV (73.44) was found to be positively correlated with EC ( $0.174 \pm 0.1$ ). Likewise, the EC ( $0.224 \pm 0.2$ ) of Roorkee also showed a positive correlation with the *Z. punctata* population (PV = 78.6) during podding seasons. In 2021, the EC of Roorkee ( $0.224 \pm 0.2$ ) and Bhagwanpur ( $0.256 \pm 0.2$ ) was observed to be the highest during the podding season, which stimulated the *Z. punctata* population with a recorded PV of 78.6 and 81.8, respectively.

The phylogenetic analyses were found consistent with the results of Abolafia and Veechi (2021), and Kiontke et al. (2007). However, due to the few sequences available for *Zeldia*, molecular comparison with other species is not possible. In conclusion, *Z. punctata* is a free-living bacterivores nematode distributed worldwide. Because of their feeding behavior, they may impact crop production through microbial changes in the soil. In addition, they may also affect the plant-parasitic dynamics in the soil. The present result showed that EC, temperature, and phosphorus in mustard during the growing season affect *Z. punctata* population dynamics. Therefore, the species' ecological role is suggested to be more studied using various soil physicochemical properties and concerning plant-parasitic nematodes, aiming for crop yield to increase.

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**Authors' contributions** The study was designed by HS and AKC. Interpretation of the data was done by HS and ES. Manuscript writing was done by HS. Tables and reference settings were done by LB and HS. Errors and grammatical mistakes in the manuscript were removed and edited by AKC and ES. Then, the final manuscript was read and approved by all authors.

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**Data availability** The data and material of this manuscript are available from the corresponding author on reasonable request.

## Declarations

**Ethical approval** This article does not contain any studies with human participants or animals.

**Competing interests** The authors declare that they have no competing interests.

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