

# First record of genus *Bionychiurus* Pomorski 1996 (Collembola: Onychiuridae) from India.

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## Original Article

**Keywords:** Chaetotaxy, Post antennal organ, Pseudocelli, Barcoding, Grasslands.

**Posted Date:** February 2nd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-156881/v1>

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**Version of Record:** A version of this preprint was published at *Biologia* on August 18th, 2021. See the published version at <https://doi.org/10.1007/s11756-021-00839-1>.

## Abstract

The new species of *Bionychiurus* Pomorski 1996 is described from India for the first time. The new species, *Bionychiurus tamilensis* sp.nov., possess a dorsal pso formula as 32/133/33343 and a ventral pso formula as 11/011/11121. The dorsal pso formula the new species shows similarity to *Bionychiurus changbaiensis*, *Bionychiurus normalis*, and *Bionychiurus oblongatus* reported from China and Korea. Status of the new species was confirmed molecularly by sequencing the mitochondrial COI region. The new species is designated as *Bionychiurus tamilensis* sp nov. An identification key to the genus *Bionychiurus* is provided.

## Introduction

The genus *Bionychiurus* (family Onychiuridae) was established by Pomoroski (1996) based on morphological characters of the 1st larval instar of *Onychiurus normalis*. Weiner (1996) similarly established a new genus *Bangallophorus* based on *O.normalis*. Pomorski (1998) synonymized *Bangallophorus* with *Bionychiurus*. Based on the characters described by Pomorski (1998), Sun & Wu (2012) described the diagnostic characters of the genus as follows: posterior cephalic pseudocelli present, well-developed post antenna with compound vesicles, chaetae d0 on the head present, and the furca reduced to cuticular pocket with 2 + 2 setulae. The distal whorl of setae on tibiotarsi has eleven pointed setae. Only six species are included under the genus *Bionychiurus* (Bellinger et al. 1996–2020). The representatives of the genus were recorded from Europe (*B.normalis* and *B. orghidani*), South Korea (*B. oblongatus* and *B. yongyeonensis*), and China (*B.quinglongensis* and *B.changbaiensis*) (Gisin 1949; Yoshi 1966; Lee and Park 1986; Sun and Wu 2012; Sun and Wu 2014 ). We report the first Indian record of *Bionychiurus* from the Nilgiri Hills in the Nilgiri Biosphere Reserve, a World Heritage Site (UNESCO). In India, the Nilgiri Hill is a mountain Peak (~ 2500m a.s.l) in the Western Ghats Mountain Chain, is the hot spot of biodiversity (Raman et al. 2020). The present study targeted to describe a collembolan genus that is previously not reported from India.

## Materials And Methods

Specimens of the new species were collected in the grassland of Udthagamandalam (Ootacamund), the Nilgiris, Tamilnadu, India. Material was obtained by extraction in Tullgren's funnel and preserved in absolute ethanol. The specimens for morphological investigation were sorted using Olympus stereomicroscope, and mounted in Hoyer's medium. Morphological characters were studied using NLCD–307B Lawrence and Mayo microscope. We followed Massoud (1967), Deharveng (1983), Yoshii (1996), Weiner (1996), Fjellberg (1999), D'Haese (2003), and Bellinger et al. (1996–2020) for the description up to genus. The descriptions of Sun and Wu (2012; 2014) were followed for the identification of new species.

DNA was isolated from the three different complete specimens retrieved from the mount after identification and preserved in 99.9% alcohol. The DNA extraction was done using Qiagen DNA easy Blood and Tissue kit (Catalogue No: 69504) with 48hr initial digestion in ATL buffer (QIAGEN) and proteinase K. The 658 base pair mitochondrial cytochrome oxidase I (COI), referred to as the barcoding region described by Hebert et al. (2003) was amplified for molecular identification. The primers designed by Greenslade et al. (2011) were used for amplification of the COI region.

Forward primer: 5'WYTCDACWAAYCRYAARGAYATYGG-3'

Reverse primer: 5'-TANACYTCNGGRTGNCCRAARAATCA-3'

The PCR assay was carried out using ABI step one Reverse Transcription PCR (RT-PCR) in a 20µl assay using 10X Standard *Taq* Reaction Buffer mMdNTPs, 10 µM Forward Primer, ten µM Reverse Primer, *Taq* DNA Polymerase, and Nuclease-free Millipore water. The assay concentration is finally 1U 2X *Taq* (*Taq* DNA polymerase, PCR buffer with 3Mm MgCl<sub>2</sub> and 400 µM of each dNTP), 1.0 mM MgCl<sub>2</sub>, 1x BSA, 1 µl each of the primers and 5 µl DNA template. Thermocycling conditions were as follows: initial denaturation at 95°C for 60 seconds, followed by 35 cycles of denaturation at 94°C for 30s, 50°C for 30s, and 72°C for 30 sec. The final extension was 72°C for 10 minutes, followed by the hold at 4°C. PCR products were purified using the Qiagen PCR purification kit (Cat No. 28104). The purified products were sequenced by the Sanger method after following the snapchil protocol, bidirectionally in the ABI 3730 Genetic Analyser. We deposited the sequences in NCBI Genbank with Accession Number (*Will be provided*). The sequence were searched for similarities in the BLAST and BoLD platforms. The available published sequences of collembola were downloaded from NCBI for phylogenetic analysis. The maximum likelihood tree was constructed using the downloaded sequences and the query sequence in R studio using the phangorn 2.5.5 package (Schliep 2011, Schliep et al. 2011). The maximum likelihood was estimated following Felsenstein (1981) with 1000 bootstraps. The branch length was optimized in 'GTR+I+G' evolutionary model based on the Bayesian Index Criterion (BIC) values. The phylogenetic trees were visualized in FigTree v1.4.3 software.

### Abbreviations used in descriptions

Ant - antennal segments, Th - thoracic segments, Abd - abdominal segments, PAO - post antennal organ, psx - parapseudocellus, ms - microsensillum, pso - pseudocellus, AllIO – sensory organ of Ant III segment.

The pseudocellular formula: anterior head, posterior head / Th.I, Th.II, Th.III/ Abd.I, Abd.II, Abd.III, Abd.IV, Abd.V (eg: 32/133/33343).

### Description of species

*Bionychiurus tamilensis* sp. nov

**Etymology.** The name of the new species is *Bionychiurus tamilensis* sp. nov., denoting the state of Tamil Nadu in India from where the specimen was collected.

*Type material.* The specimens were obtained from the samples collected between 14 - 28 August 2018 by A.M. Thunnisa and N. Sumithra. The holotype is male, and the seven paratypes include both males and females. The specimens were extracted from the soil samples collected from the grasslands of Old Ooty (11°41'N, 76°70'E), Garden mund (11°41'N, 76°69'E), and Government Arts College campus (11°41'N, 76°71'E), Nilgiri Hills, Tamil Nadu, India. We deposited the holotype and paratypes at the 'Apterygota Division' of Zoological Survey of India, Kolkata, (Acc. No 2027/H14) and the 'Collembola repository' of Molecular Biodiversity Lab, GAC, Ooty.

*Diagnosis.* Pso formula dorsally: 32/133/33343 and ventrally: 11/011/11121; Subcoxa 1 of leg I–III with 1,1,1 pso and 1,1,1 psx respectively, not visible in head and body; AllIO with five guard chaetae and five papillae, two small sensory rods, and two sensory clubs; PAO with 22–24 compound vesicle; labial type AC; Abd. IV tergum with unpaired m0 and p0. Abd. V and VI with unpaired m0; furca are reduced to cuticular fold with four small dental hairs.

*Description.* Colour white in alcohol. Body length (excluding antennae and spines) 1.2–1.5 mm ( Refer supplementary material S1 for detailed morphometric analysis)

Dorsal pso formula:32/133/33343 (Fig 1a), ventral pso formula–11/011/11121. Subcoxa 1 of leg I–III with 1,1,1 pso and 1,1,1 psx, respectively. Psx not visible on the head and body. Antennae shorter than the head; length up to 0.1–0.2 mm. AllIO with five guard chaetae and five papillae, two small sensory rods, and two sensory clubs (Fig 1c). Basolateral ms placed above the second proximal row of chaetae. Ant I with 6-8 chaetae, 12–14 chaetae in Ant II, 16–18 chaetae in Ant III, and 25–30 chaetae in Ant.IV dorsally with 25-30 chaetae and ventrally with 40–45 chaetae (Fig 1b).

PAO with 22–24 compound vesicles, arranged in two rows (Fig 1d). Dorsal cephalic chaetae d0 present on the head (Fig 1a). Mandible with four apical teeth, and Maxilla with three teeth and six lamellae. Labial type AC, Labium with six proximal, four basomedian (E, F, G, f), and five basolateral chaetae (b, c, d, e, e'). The labial papillae A to E with 1, 4, 0, 3, and 2 guard chaetae, respectively (Fig 1e). 4+4 postlabial chaetae along the ventral groove (Fig 2a).

Body Chaetotaxy.. Macro, meso, and micro chaetae are well differentiated. S- chaetae poorly marked. Th. II and Th.III with tiny and blunt lateral ms. Th.I tergum with 9–11 chaetae. Thorax II - III with 4+4 axial chaetae, Abd I–III with 3+3 axial chaetae. Abd. IV tergum with unpaired m0 and p0. Abd. V and VI with unpaired m0. Th.I–III ventrally with 0+0, 2+2, 2+2 chaetae. Abd VI with two curved anal spines (Fig 1a).

Appendages. Subcoxae 1 of leg I, II, III with 5,6,6 chaetae, subcoxae 2 with 1, 6, 6 chaetae, respectively. Femora with 15,18,18 chaetae, and tibiotarsi of leg I, II, and III with 22 (3, 8, 11), 22 (3, 8, 11), and 21 (2, 8, 11) chaetae, respectively. Unguis without denticle, unguiculus pointed, and without inner basal lamella (Fig 2b).

Ventral tube with 7–9 + 7–9 chaetae and 1+1 basal chaetae (Fig 2c), MVO absent, furca reduced to cuticular fold with four small dental chaetae (Fig 2d). Female genital plate with 25–30 chaetae (Fig 2e) and male genital plate with 45–50 chaetae (Fig 2f). Anal valve with several acuminate chaetae; each lateral valve with a0, 2a1 and 2a2; upper valve with chaetae a0, 2a1, 2b1, 2b2, c0, 2c1, 2c2 (Fig 2g). The detailed photographic descriptions are provided as supplementary material S2. Comparison with other reported species is given in table 1.

*Barcoding.* The BLAST analysis indicates the species have the nearest similarity with the *Allonychiurus kimi* (Similarity: 85.53%, Accession no: MT975431.1; Query cover: 98% and E value: 0.0). No reports of the COI region of the *Bionychiurus* genera are available in NCBI or BoLD. The maximum likelihood analysis showed the new species is a separate distant outshoot of the Onychiuridae cluster, confirming the entity. The outgroups used for the present study are the *Isotoma*, *Sminthurus*, and *Entomobrya*. The details of various accessions used for the phylogenetic analysis and the ML tree are given as supplementary material S3.

## Discussion

*B. tamilensis* sp.nov., shows most similarity with the species of *B. changbaiensis* as having the same dorsal pso formula (32/133/33343), nearly same number of vesicles in PAO (22-26 in *changbaiensis* and 22-24 in the new species), number of pso on subcoxa 1 of legs I-III (1/1/1) and chaetae on subcoxa 1 of legs I-III (5/6/6). But these two species are separated by the characters of ventral pso formula (*B.tamilensis* sp.nov. -11/000/01120 and *B. changbaiensis*-11/011/11121), ventral psx formula (00/000/100000 in *B. changbaiensis* and 00/000/000000 in *B.tamilensis* sp.nov.), chaetotaxy of ventral tube (0+0/8+8/0+0 in *B. changbaiensis* and 0+0/7-9+7-9/1+1 in *B.tamilensis* sp.nov.) and Chaetae on lateral anal valve (a0, 2a1 in *B. changbaiensis* and a0, 2a1, 2a2 in the new species). *B.normalis* and *B.oblongatus* also share the same dorsal pso formula with the new species. The number of vesicles in PAO shows variations with other two related species (18-22 in *B.normalis* and 18 in *B.oblongatus*). Refer the table 1 for detailed comparison of morphological characters..

### Key to genera *Bionychiurus* (Modified after Sun and Wu 2012)

1. a) Dorsal pso formula 22/133/33333; South Korea.....  
.....*yongyeonensis* (Yosii, 1966)
- b)Dorsal pso formula 32/1(0)33/33343 . . . . . 2
2. a) PAO with more than 22 vesicles;  
5/6/6 chaetae on subcoxae I .....3
1. b) PAO with lesser than 22 vesicles; subcoxa 1 with different number of chaetae .....4

2. a) Ventral pso formula 11/000/01120; Ventral psx formula 00/000/100000; China .....  
 ..... *changbaiensis* (Sun & Wu, 2012)
1. b) Ventral pso formula 11/011/11121; Ventral psx formula 00/000/000000; The Nilgiris, India .....  
 ..... *tamilensis* sp nov.
4. a) Subcoxa 1 of legs I–III with 2, 2, 2 pso respectively; Switzerland, Poland (in green houses) .....  
 ..... *normalis* (Gisin, 1949) sensu Pomorski, 1998
1. b) Subcoxa 1 of legs I–III with 1, 1, 1 pso respectively ..... 5
2. a) Dorsal pso formula 32/033/33343; PAO with 14-17 vesicles; Chaetae on  
 subcoxa 1 of legs I–III 5/7/6; China ..... *qinglongensis* (Sun & Wu, 2014).
1. b) Dorsal pso formula 32/133/33343; PAO with 18 vesicles; Chaetae on  
 subcoxa 1 of legs I–III 4/5/2; South Korea ..... *oblongatus* (Lee et Park, 1986)

## Declarations

### Acknowledgments

We express our sincere thanks to the University Grants Commission, New Delhi, India, for providing the fellowship and financial support as MANF JRF & SRF (F1-17.1/2014-15/MANF-2014-15-MUS-TAM-41353) to the first author.

### Declaration of Conflict of Interests

The authors do not have any financial and non-financial conflict of interests.

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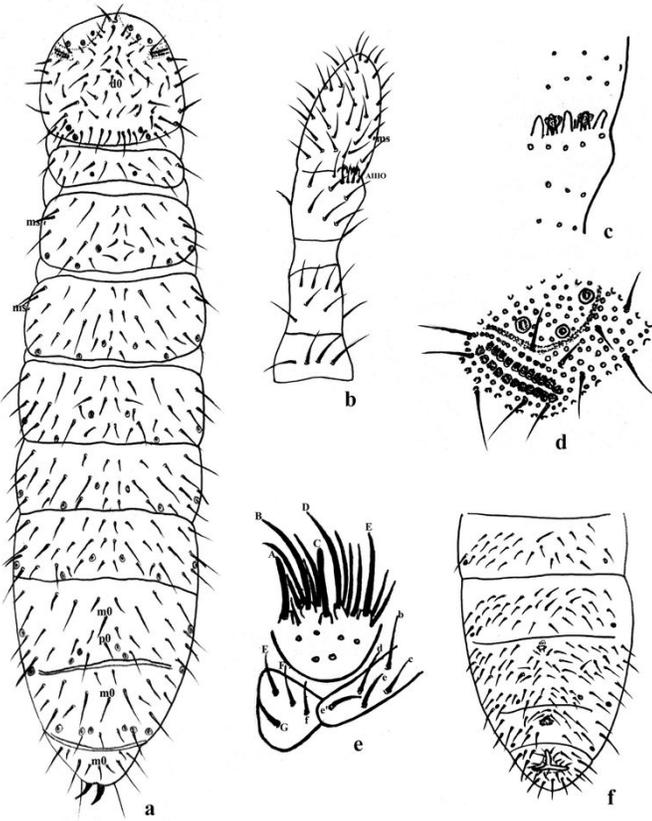
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## Table

Table 1: Comparison of morphological characters of all available *Bionychiurus* sp.

	<i>B.changbaiensis</i>	<i>B.normalis</i>	<i>B.oblongatus</i>	<i>B.yongyeonensis</i>	<i>B.qinglongensis</i>	<i>B.tamilensis</i> <i>sp.nov</i>
Distribution	China (Mountain, alt. 1763 m)	Switzerland; Poland (Lowland)	South Korea (Cave)	South Korea (Cave)	China (Mountain)	Nilgiri, India (Mountain Grassland)
Dorsal formula pso	32/133/33343	32/133/33343	32/133/33343	22/133/33333	32/033/33343	32/133/33343
Ventral formula pso	11/000/01120	11/000/01010	Unknown	00/000/011200	11/000/01(0)010	11/011/11121
Number of pso on subcoxa 1 of legs I-III	1/1/1	2/2/2	1/1/1	1/1/1	1/1/1	1/1/1
Ventral psx formula	00/000/100000	00/000/101000	Unknown	Unknown	00/000/100001	00/000/000000
Number of vesicles in PAO	22-26	18-22	18	30	14-17	22-24
Number of chaetae on Th.I tergum	9-10+9-10	8+8	9+9	7+7	9-12	9-11
Chaetae on subcoxa 1 of legs I-III	5/6/6	3/4/4	4/5/2	Unknown	5/7/6	5/6/6
Dorsal axial chaetae on Abd. IV	m0 (or absent) and p0	p0	m0 and p0	m0	m0	m0 and p0
Dorsal axial chaetae on Abd.V	m0	m0	m0	a0 and p0	m0	m0
Chaetae on ventral tube	0+0/8+8/0+0	0+0/6-9+6-9/0+0	0+0/8-9+8-9/0+0	1+1/10+10/0+0	0+0/6-9+6-9/1+1	0+0/7-9+7-9/1+1
Chaetae on lateral anal valve	a0, 2a1	a0, 2a1	a0, 2a1, 2a2	a0, 2a1	a0, 2a1, 2a2	a0, 2a1, 2a2

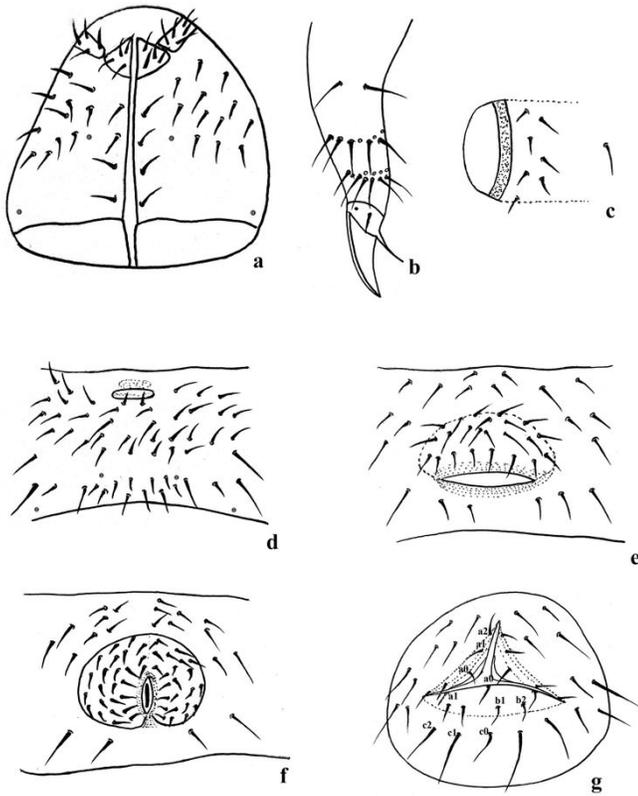
## Figures



**Fig.1** *Bionychiururs tamilensis* sp. nov. a. dorsal chaetotaxy b. antennae c. Ant.III sensory organ d. PAO e. labium f. general view of Abd II-IV

**Figure 1**

*Bionychiururs tamilensis* sp. nov. a. dorsal chaetotaxy b. antennae c. Ant.III sensory organ d. PAO e. labium f. general view of Abd II-IV.



**Fig.2** *Bionychiurus tamilensis* sp.nov a. dorsal side of head b. distal part of leg III c.ventral tube d. Abd IV sternum e. female genital plate f.male genital plate g. anal valve

## Figure 2

*Bionychiurus tamilensis* sp.nov a. dorsal side of head b. distal part of leg III c.ventral tube d. Abd IV sternum e. female genital plate f.male genital plate g. anal valve

## Supplementary Files

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