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Diversity and New Records of Earthworms in Arunachal Pradesh, Northeast India

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Abstract

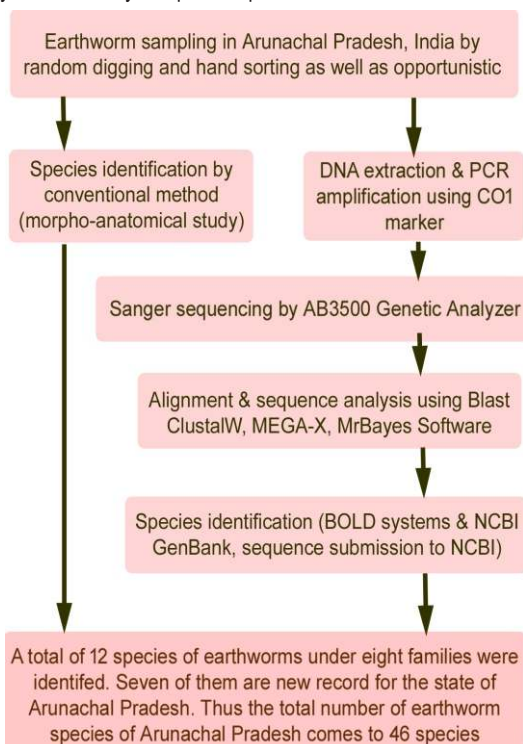
Aim: Arunachal Pradesh, one of the best tropical rain forest areas within Eastern Himalayan biodiversity hotspot is expected to have a rich earthworm diversity. Therefore, the present study was undertaken to document the diversity of earthworms in Arunachal Pradesh from field collection and existing literature.

Methodology: Earthworm specimens were collected by random sampling method by digging and hand sorting method from different habitat in Arunachal Pradesh, northeast India. Conventional methods of identification based on morpho-anatomical characters was performed. Molecular characterization, the genomic DNA was extracted followed by PCR amplification by mtDNA CO1 gene. The PCR product obtained was subjected to the Sanger sequencing by AB3500 Genetic Analyzers (ThermoFisher).

Results: Twelve earthworm taxa were identified using conventional and molecular methods of identification under eight families. Out of which, four families were previously recorded and two families viz., Rhinodrilidae and Eudrilidae are new to the existing record. Out of the 12 earthworm species, seven species are new record from the state, such as *Amyntas corticis*, *A. gracilis*, *Bimastos parvus*, *Dichogaster modigliani*, *Eudrilus eugeniae*, *Octochaetona beatrix* and *Pontoscolex corethrurus*. Among the identified species, *A. gracilis* was the most widely distributed species followed by *A. corticis* and *Drawida nepalensis*. With these seven new additions, the valid species of earthworms of Arunachal Pradesh comes to 46 species. The CO1 gene sequence of newly recorded species from Arunachal Pradesh was submitted to NCBI GenBank.

Interpretation: Our findings enriched the diversity of earthworm species in Arunachal Pradesh by contributing two new families i.e. Rhinodrilidae and Eudrilidae and seven new species record such as *Amyntas corticis*, *A. gracilis*, *Bimastos parvus*, *Dichogaster modigliani*, *Eudrilus eugeniae*, *Octochaetona beatrix* and *Pontoscolex corethrurus*. Thus, the total earthworm diversity in Arunachal Pradesh has become 46, the second-highest record of all north-eastern Indian states with a high potential of the new addition.

Key words: Diversity, Earthworm, Molecular characterization, Species



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Introduction

The contribution of earthworms to the physical and biochemical functions of the soil profile is significant and they are commonly considered as “friends of farmers” and “ecological engineers”. They form the major components of soil invertebrate fauna and constitute about 80% of the total soil invertebrate biomass (Lavelle and Spain, 2001). Earthworms are not uniformly distributed (Singh *et al.*, 2016), their numbers usually differ relating to the kind of surface soil (Curry, 1998) and ecological aspects especially edaphic factors like moisture, temperature conditions (Kaleemurrahman and Ismail, 1981; Lalthanzara and Ramanujam, 2014), soil nutrients and rainfall patterns (Fragoso and Lavelle, 1995) and land-use systems (Chauhan *et al.*, 2015).

Earthworm diversity and density studies in India have been initiated since the time of Michaelsen (1907), Stephenson (1914, 1923), Gates (1940), Julka (1976), and then many Indian scientists contributed the earthworm diversity data. Julka *et al.* (2009) reported the presence of 590 species of earthworms in India. However, Blakemore reported 425 species and subspecies of earthworms belonging to 67 genera and 10 families have been identified in India (Blakemore, 2010). The work of earthworm diversity in North Eastern India have also been reported from Meghalaya (Mishra and Ramakrishnan, 1988; Halder, 1999; Kharkongor, 2018), Manipur (Haokip and Singh, 2012), Mizoram (Ramanujam *et al.*, 2004), Assam (Rajkhowa *et al.*, 2015), Tripura (Chaudhuri *et al.*, 2012) and a few diversity survey records from Sikkim (Soota and Halder, 1981, Subedi *et al.*, 2018a,b,c). Thyug and kakati (2018) did the first ever earthworm study in Nagaland. Recently, the conventional method of earthworm identification has been supplemented with the molecular method of identification. This new method has been used to solve the taxonomic dilemma from any life stages of earthworms by using the *mtDNA* CO1 region as a standard DNA tag (Hebert *et al.*, 2003; Huang *et al.*, 2007; Richard *et al.*, 2010). The molecular techniques allowed rapid identification of earthworms (Yadav *et al.*, 2017). A combination of the two approaches is now considered to provide a powerful tool for earthworm identification (Huang *et al.*, 2007).

Regarding the earthworm diversity study of Arunachal Pradesh, Julka made previous studies twice. Julka (1976) reported 12 different species such as *Drawida tihunensis* (Julka), *Drawida nepalensis* (Michaelsen), *Drawida kempi* (Stephenson), *Desmogaster ferina* (Gates), *Tonoscolex michaelseni* (Julka), *Tonoscolex oneilli* (Stephenson), *Pheretima/Amyntas diffringens* (Baird), *Perionyx excavatus* (Perrier), *Perionyx modestus* (Stephenson), *Argilophilus* (Plutellus) *mishmiensis* (Julka), *Dichogaster bolaii* (Michaelsen) and *Dichogaster saliens* (Beddard) during his Daphabum expedition. In 1981, Julka again reported 27 species from his Subansiri expedition in Arunachal Pradesh and he described 12 new species (Julka, 1981). Halder *et al.* (2007) reported 10 species from their studies on unnamed collections of the Zoological Survey of India, Kolkata. Out of these 10 species, four species *viz.*, *Perionyx variegatus* (Michaelsen),

Perionyx turaensis (Stephenson), *Eutyphoeus gammiei* (Beddard) and *E. incommodus* (Beddard) are a new record for Arunachal Pradesh. Thus, the total number of earthworm species in Arunachal Pradesh comes to 36, which is one of the richest earthworm species diversity in northeast India. Arunachal Pradesh is located within the eastern part of the Himalayan Biodiversity Hotspot, covered by thick and dense tropical rain forests and a variety of habitats, thus expecting a myriad of different life forms including earthworms (Paul *et al.*, 2005). It has been almost four decades since the last survey. Therefore, an attempt has been made to investigate the species diversity of earthworms in Arunachal Pradesh.

Materials and Methods

Study area: Arunachal Pradesh is the biggest among seven sister states of Northeast India, situated at 26.28°N and 29.30°N latitude and 91.20°E and 97.30°E longitude. According to Paul *et al.* (2005), the forest occupies one-third of the habitable area of the Himalayan biodiversity hotspot. It joins the border with Nagaland and Assam to the south and share international borders with Myanmar in the east, Bhutan in the westward and is separated by McMahon Line from China in the north. The climate of Arunachal Pradesh varies with elevation and receives 2000 mm to 5000 mm of annual rainfall. Soils vary considerably with the terrain and are mainly acidic in mountainous areas. The major soil types are inceptisols, entisols and utisols and the river valleys are rich in alluvial soils (Choudhury, 1996; Norbu, 2008). The topsoil texture is sandy clay loam to clay loam and is acidic with the pH ranges from 3.1 – 5.7 (Poddar *et al.*, 1999). The present study covered 21 different locations from five districts. The study was carried out in May 2018 at an altitude ranging from 138 m to 1754 m above sea level. The study sites are presented in Fig. 1 and Table 1.

Collection of Earthworms and Morphological identification:

Earthworm sampling was done by random sampling through digging and hand sorting method as well as opportunistic sampling from house garden, agroforestry, paddy field, reserved forest, roadside, side drains, river banks and any other sites where earthworms were available. Habitat details and specimen details were noted down. The specimens were further segregated by hand sorting and cleansed with water, narcotized in 30% ethanol, and then fixed in 100% ethanol. Tissues were taken from the posterior part and preserved in 90% ethanol for further molecular characterization. The specimens were stored in 5% formalin for further morpho-anatomical identification. The conventional identification from the morphological and anatomical study of earthworms was performed at the Research and Instrumentation Centre, Pachhunga University College, Aizawl based on published literature (Stephenson, 1923; Gates, 1972; Julka, 1976, 1981, 1988 and Halder *et al.*, 2007) under a stereo-zoom microscope (Optika SZN 8 with Optikam Pro 8 LT). All the specimens were deposited in the PUC Zoological museum, Aizawl, Mizoram and the catalogue numbers are given at the end of the material examined.

DNA extraction and Sanger sequencing: The molecular identification of earthworms was done using the *cytochrome c oxidase subunit 1 (CO1)* gene. Mitochondrial DNA was isolated from tissues of the posterior section by using the Cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980). A base pair region of the mitochondrial *cytochrome c oxidase subunit 1 (CO1)* gene was subjected for PCR amplification using LCO1490 5' - GGTCACAAATCATAAAGATATTGG-3' and reverse primer: HCO2198 5' - TAGAATTAGAAGATCAACCAG-3' primers (Folmer et al., 1994). The PCR reaction was carried out in 25 µl volumes which consisted of 0.8 µl (80 ng) DNA template, 12.5 µl PCR Master Mix (Takara Tlontekh), 11 µl nuclease-free water and 10 pmol (1 µl) of each of the primer. PCR cycling consisted of an initial denaturation step at 94 °C for 4 minutes, followed by 35 cycles for 1 minute at 94 °C, 1 minute at 49 °C and 1 minute at 72 °C. And the last extension step was done at 72 °C for 10 minutes. The amplified products were quantified in 1.5% agarose gel electrophoresis stained with Invitrogen SYBR Safe, with 100 bp DNA ladder as a marker. The PCR products were then purified with QIAquick PCR Purification Kit (Qiagen), then sequenced by 3500 Genetic Analyser 8ch RUO, model no. 620010 (ThermoFisher Scientific). The derived DNA sequences were aligned, edited and analyzed using BLAST (Altschul et al., 1990) and Bioedit sequence alignment editor (Hall, 1999).

Results and Discussion

Systematic accounts

Amyntas gracilis (Kinberg, 1867)

Material examined: 25 adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Boleng, Pagin, Pasoghat, Kheel, Longte Tapu, Pakke Kesang, Seppa, Old ziro, Yazuly, Rajiv Gandhi University. Altitude 142-1754 m asl. PUCZMA/VI/1017g,h,i. Fig. 2(a).

External characters: Clitellum annular, 14-16 segments, length 60-160 mm, no. of segments 69-97. Prostomium epilobous. Spermathecal pores three pairs in 5/6-7/8. 1st dorsal pore at 10/11 or on 11/12 in few specimens. Single mid-ventral female pore at 14. Male pores are small circular pair at 18, lateral to one or more pairs of post-setal small genital markings (small discs).

Internal characters: Septa are thickened in 6/7/8, aseptum in 8/9/10, slightly thickened in 10/11-13/14. Intestine begins from 15 and gizzard in 8-10. Simple intestinal caeca in 27, extending to 24 or 25 towards the anterior. Three pairs of spermathecae in 6-8. Paired ovaries at 13. Two pairs of testicular sacs in 10 and 11 joined ventrally. Paired seminal vesicles in 11 and 12 having a dorsal lobe. A pair of prostate glands in 18, extending between 16-22 segments with the straight prostatic duct.

Amyntas corticis (Kinberg, 1867)

Material examined: 22 adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Itanagar Biological Park, Ganga Lake, Pagin,

Boleng, Kheel, Pakke Kesang, Lapu Rieng. Altitude 154-1754 m asl. PUCZMA/VI/1008r,s,t. Figure 2(b).

External characters: Annular clitellum in 14-16, length 95-185 mm, no. of segments 93-119. Epilobous prostomium. 1st dorsal pore at 11/12 or 12/13 in few specimens. Spermathecal pores - four pairs in 5/6-8/9. Genital papillae in 6-9, paired near spermathecal pores, pre-setal and/or post-setal in a median or in line with spermathecal pores. Single female pore at 14, male pores paired in 18.

Internal characters: Septa 5/6 - 7/8, thickened in 10/11 - 13/14 aseptum on 8/9/10. Gizzard in 8-10. Intestine begins from 15 or 16 in some. Paired intestinal caeca in 27 which is long and slender, extending up to 23 or 24 towards the anterior end. Four pairs of spermathecae in 6-9. Ovaries present in 13. Two pairs of testis sacs in 10 and 11 joined ventrally. A pair of large seminal vesicles in 11 and 12, dorsal lobe present. Well-developed prostate glands, paired in 18, spans 16-20, divided into 3/4 lobes.

Octochaetona beatrix (Beddard, 1902)

Material examined: 11 adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Boleng, Rajiv Gandhi University. Altitude 215-399 m asl. PUCZMA/VI/1024b,d,e. Fig. 2(c).

External characters: Annular clitellum 13-18 segment with a parallel seminal groove on the ventral side of segment 17-19, length 60-101 mm, no. of segments 120-164. Setae lumbricine. Prostomium epilobous. 1st dorsal pore 12/13 on intersegmental furrow. Spermathecal pore paired minute in the middle of segments 8 and 9. Male pore minute, paired on either side of the midventral line in depressed male fields on the seminal grooves at 18. Prostatic pores 2 pairs at either end of concave seminal groove on 17 and 19. Female pores paired, minute, presetal in position at the line of spermathecae at 14.

Internal characters: 5/6-7/8 aseptum. Gizzard between 4-9 segments. Paired Calciferous gland at segment 15/16. Intestine originates at 17. Typhlosole, inter-digited ventrally directed, bifid extends from 25 to 55. Last pair of hearts in 13. Testes metandric and male funnel paired, free in 10, seminal vesicles paired in 9 and 11. Prostate tubular, paired in 17 and 18. Penile setae present. Spermatheca unidiverticulate, shorter than the ampulla.

Eudrilus eugeniae (Kinberg, 1867)

Material examined: 15 adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Boleng, Rajiv Gandhi University. Altitude 215-222 m asl. PUCZMA/VI/1027k,l. Fig. 2(e).

External characters: Clitellum on segments 14-18. Length 90-185 mm. No. of Segments 211-215. Prostomium epilobous, setae lumbricine. Dorsal pores absent. Paired female pores, large transverse slits, near body sides on 14. Minute, paired male pores, at tips of penes, able to be drawn in into copulatory

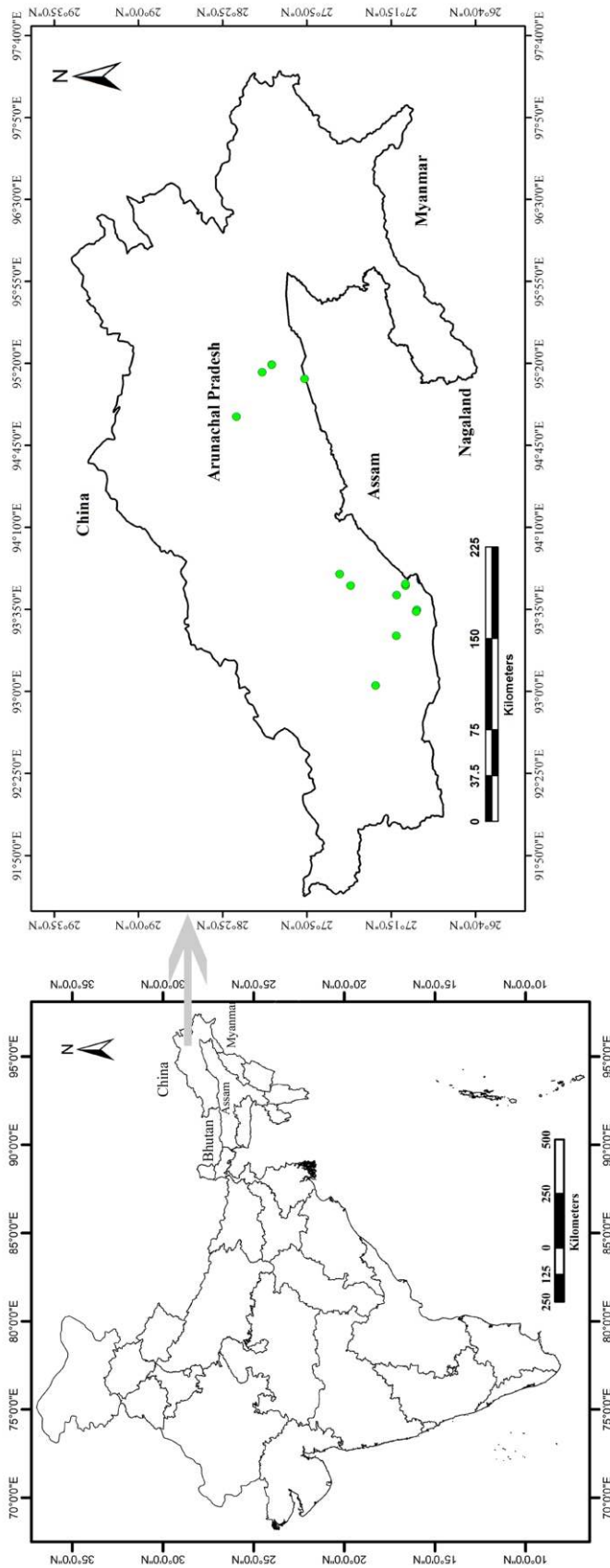


Fig. 1: Map showing different locations of sampling sites in Arunachal Pradesh, India.



Fig. 2: New record of earthworms of Arunachal Pradesh. (a) *Amynthus gracilis*, (b) *Amynthus corticis*, (c) *Octochaetona beatrix*, (d) *Dichogaster modigliani*, (e) *Eudrilus eugeniae*, (f) *Pontoscolex corethrurus* and (g) *Bimastos parvus*.

chambers, copulatory aperture large transverse slits on 17, in front of intersegmental furrow 17/18.

Internal characters: Septa present from 4/5, 6/7/8, and 14/15 thickened. Intestinal origin closes on 14/15. Weak muscular gizzard in 5. Typhlosole and caeca not found. Supra-intestinal glands are small, paired, post setal in 8-42 consecutive segments 62-132. Ovisac attached to muscular spermathecae in 14, that extends into a long-convoluted gland. Prostates long, duct short and slender extending from 18-22.

***Pontoscolex corethrurus* (Müller, 1857)**

Material examined: Twenty one adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Ngukir, Kimsing, Itanagar Biological Park, Ganga Lake, Boleng, Pasighat, Seppa, Doimukh, Rajiv Gandhi University. Altitude 141-426 m asl. PUCZM A/VI/1019r,s. Figure 2 (f).

External characters: Clitellum saddle-shaped, covering

segments 15-22. No. of segments 92-169, length 50-113 mm; diameter 2-4.5 mm. Dorsal pores absent. Female pore single, small transverse slit left to the mid-ventral line, in front of intersegmental furrow 14/15. Male pores and spermathecal pores not conspicuous. Peregrine, setae closely paired in the first two segments, AB and CD gradually wider until the irregular arrangement in the caudal region. No genital markings.

Internal characters: Septa, membranous, found from 5/6 intersegmental furrow, thickly muscularized in 6/7-9/10. Gizzard in between 6-10, Calciferous glands paired. Last pair of hearts in 11. Intestine begins at 14/15. Simple, lamelliform type of Typhlosole in 21-25. Intestinal caeca and suprainestinal glands are not found. Male funnels large, seminal vesicles one pair in 12 extending anteriorly up to 8-10. Spermatheca three pairs at 6/7-8/9, club-shaped with long, slender ducts.

***Bimastos parvus* (Eisen, 1874)**

Material examined: 3 adults, coll. Lalthanzara, Vabeiryureilai,

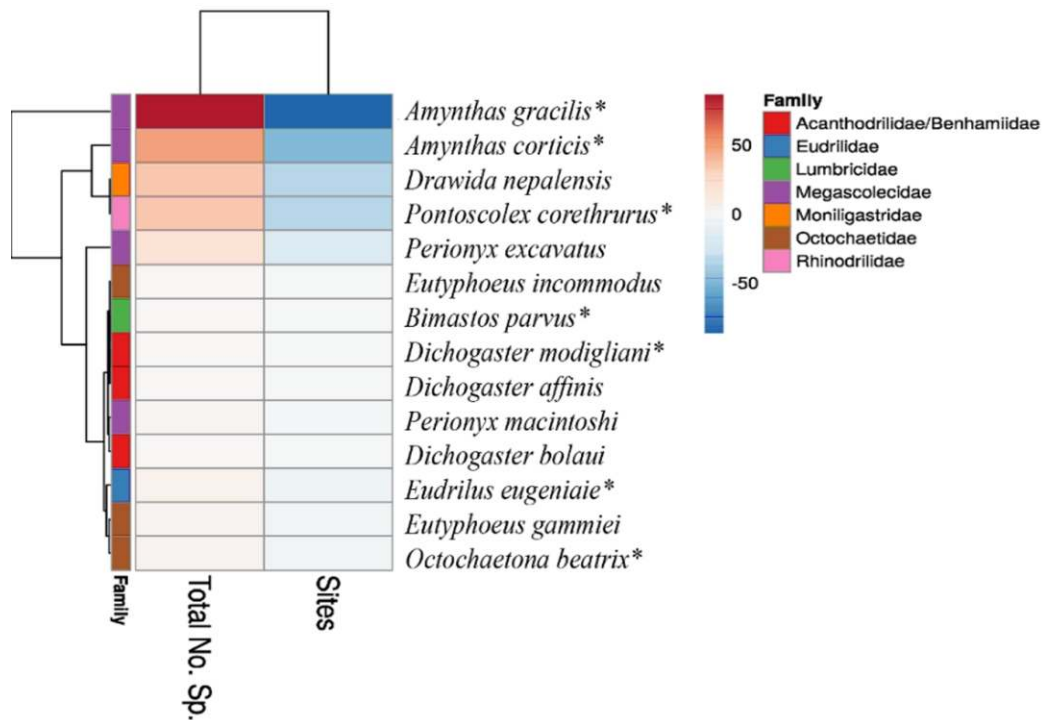


Fig. 3: Occurrence of earthworm species collected from different location in Arunachal Pradesh. *New record

Zothansanga, Riverbank muddy soil (27°31'54"N, 93°47'41"E). Altitude 1754 m asl. PUCZMA/VI/1003a,b. Fig. 2 (g).

External characters: Length 17-45 mm, No. of segment 84-123. Saddle-shaped clitellum in 24/25-30/31, Prostomium epilobic. Closely paired lumbricine type setae, AB slightly > CD. 1st dorsal pore 5/6. Spermathecal pore not found. Female pores paired small slits in 14. Paired male pores in 15, lateral slits with glandular areas adjacent to setae.

Internal characters: Septa reduced posterior to 16/17. Gizzard positioned in 16-18. Calciferous glands in 11-12. Intestine begins from 19. Hearts in 7-11. No spermathecae. Ovaries in 13. Two pairs of testes in 10 & 11. Seminal vesicles situated in 10-12. Typhlosole well-developed, lamelliform. Prostate glands not seen.

***Dichogaster modigliani* (Rosa, 1896)**

Material examined: 3 adults, coll. Lalthanzara, Vabeiryureilai, Zothansanga, Ngukir, Kimsing, Boleng, Loamy reddish soil (28°32'19"N, 94°95'17"E). Altitude 399 m asl. PUCZM A/VI/1020a2,b2,c2. Fig. 2(d).

External characters: Length 22-60 mm, No. of segment 74-118. Pro-epilobic prostomium. 1st dorsal pore 4/5. Clitellum annular 13-20. Female pores paired, pre-setal, slightly median or postero-median to a setae on 14. Two pairs of spermathecal pores at

7/8/9. Minute, paired male pores in seminal grooves linking prostatic pores in 18. Paired and minute prostatic pores at the ends of seminal grooves, on 17 and 19.

Internal characters: Gizzard positioned between septa 4/5 and 7/8. Last pair of hearts 12. Holandric, testes and male funnels surrounded in unpaired sacs, seminal vesicles absent or vestigial in 12. Spermathecae paired, in 8 and 9, each with a small; spheroidal to ellipsoidal shortly stalked diverticulum; barrel-shaped spermathecal duct.

A total of 550 earthworm specimens were collected during the survey. The morphological characteristics were considered for sorting of individual earthworm followed by an anatomical examination, and comparison with known CO1 gene sequences of NCBI GenBank and BOLD systems databank. We identified 12 valid species that belong to eight families such as Megascolecidae, Moniligastridae, Acanthodrilidae, Benhamiidae, Octochaetidae, Lumbricidae, Rhinodrilidae and Eudrilidae (Table 1). Recently, there was a re-erection of family Rhinodrilidae Benham, 1890 (James, 2012). So that *P. corethrurus* belongs to Rhinodrilidae, not to glossoscolecidae. Likewise, the genus *Dichogaster* has been separated from the family Acanthodrilidae to a recently upgraded family Benhamiidae from a subfamily Benhamiinae. The pioneering earthworm exploration work of Julka (1976) reported four families such as Moniligastridae, Megascolecidae, Octochaetidae and

Table 1: Earthworm species composition of Arunachal Pradesh

Species	Family	Habitat	Location	Altitude (m, above sea level)
<i>Amyntas gracilis</i> *		H,J,G,W,T	b,pa,pg,k,l,pk,s,po,oz,y,rgu	142-1754
<i>Amyntas corticis</i> *		H,J,G,L,S,W	ibp,gl,pa,b,pa,ru,k,pk,lr	154-1754
<i>Amyntas diffringens</i>		Julka, 1976		
<i>Perionyx excavatus</i>		G,S,W	lbp,pg,pa,b,ru,po,R,do	141-1754
<i>Perionyx macintoshi</i>		S	oz	1736
<i>Perionyx modestus</i>				
<i>Perionyx depressus</i>				
<i>Perionyx daflaensis</i>				
<i>Perionyx daminensis</i>				
<i>Perionyx foveatus</i>				
<i>Perionyx gravelyi</i>	Megascolecidae			
<i>Perionyx kaboensis</i>				
<i>Perionyx kempi</i>		Julka, 1976, 1982; Checklist of 505 earthworms from India, 2007, Halder et al., 2007; Tiwari et al., 2020		
<i>Perionyx vidakensis</i>				
<i>Perionyx turaensis</i>				
<i>Perionyx variegatus</i>				
<i>Tonoscolex oneilli</i>				
<i>Tonoscolex michaelseni</i>				
<i>Tonoscolex indicus</i>				
<i>Tonoscolex kabakensis</i>				
<i>Tonoscolex striatus</i>				
<i>Drawida kempi</i>				
<i>Drawida nepalensis</i>		H,J,G,L,S,W,T	lbp,gl,b,psa,ru,k,s,lr,y,do,rgu	117-1178
<i>Drawida tihuensis</i>				
<i>Drawida aruna</i>	Moniligastridae			
<i>Drawida beddardi</i>				
<i>Drawida constricta</i>				
<i>Drawida duttai</i>		Julka, 1976, 1982; Checklist of 505 earthworms from India, 2007		
<i>Desmogaster ferina</i>				
<i>Argilophilus mishmiensis</i>				
<i>Argilophilus bahli</i>				
<i>Argilophilus damiensis</i>	Acanthodrilidae			
<i>Argilophilus richikensis</i>				
<i>Argilophilus taksingensis</i>				
<i>Dichogaster bolau</i>		G,W	pg,b,do,rgu	141-222
<i>Dichogaster saliens</i>		Julka, 1976		
<i>Dichogaster modigliani</i> *	Benhamiidae	G	b	399
<i>Dichogaster affinis</i>		G	b	215
<i>Octochaetona beatrix</i> *	Octochaetidae	G	b,rgu	215-399
<i>Eutyphoeus gammiei</i>	Octochaetidae?	Halder et al., 2007; Tiwari et al., 2020		
<i>Eutyphoeus incommodus</i>				
<i>Eutyphoeus kempi</i>				
<i>Bimastos rubidus</i>				
<i>Bimastos parvus</i> *	Lumbricidae	G	r	1754
<i>Pontoscolex corethrurus</i> *	Rhinodrilidae*	H,G,L,S,W,T	ibp,gl,ru,b,pg,s,do,rgu	141-426
<i>Eudrilus eugeniae</i> *	Eudrilidae*	G	b,rgu	215-222

*New record of earthworm species and family from Arunachal Pradesh; Habitat: H=house garden, J= Jhum cultivation, G= road Grass, R= River banks, L= Lake banks, S= Side drain, W=Sewage disposal, T=Tree forest; Location: Pasighat (pg), Pagin (pa), Boleng (b), Kheel (k), Longte tapu (l), Pakke kesang (pk), Seppa (s), Potin (p), RL5754 (R), Old ziro (oz), Yazuly (y), Ragiv Gandhi University (rgu), Itanagar biological park(ibp), Ganga lake (gl), Lapu riang (lr) & Doimukh (do)

Acanthodrilidae. Again Julka (1981) reported moniligastridae, megascolecidae, lumbricidae and octochaetidae families. Recently Halder et al. (2007) reported species belonged to three families viz., moniligastridae, megascolecidae and octochaetidae. The present study revealed the presence of another two more families from Arunachal Pradesh, viz., Rhinodrilidae and Eudrilidae (table 1).

The present study added seven new records of earthworm for Arunachal Pradesh such as *Amyntas corticis* (Megascolecidae), *A. gracilis* (Megascolecidae), *Bimastos parvus* (Lumbricidae), *Dichogaster modigliani* (Benhamiidae), *Eudrilus eugeniae* (Eudrilidae), *Octochaetona beatrix* (Octochaetidae) and *Pontoscolex corethrurus* (Rhinodrilidae) (Table 1, Fig. 2a-g). *O. beatrix* and *E. eugeniae* are collected from roadside grass at Boleng and Rajiv Gandhi University campus at an altitude of 215 - 399 m asl. Kale and Karmagam (2010) recorded *D. modigliani* in red sandy mesohumic soil with 20-40% moisture, while Mubeen and Hatti (2018) record the species in highly alkaline Borewell in Karnataka, India. *B. parvus* is sampled at an elevation of 1754 m asl in the roadside grass areas, mix mud and cow dung moist soil, a place marked as RL5754 (27°31'54.19"N, 93°47'41.57"E). This is the highest collection site in the state of Arunachal Pradesh. *D. modigliani* is also recorded at only one site i.e., roadside grassy home garden with loamy reddish soil at Boleng (28°32'19"N, 94°95'17"E) at an altitude of 399 m asl. *P. corethrurus* is recorded from eight different sites of six different types of habitats at an altitudinal range of 141 – 426 m asl. Though sampled from eight different sites, all sites are lower altitude, indicating their habitat preference. However, recorded this species at 300 - 1270 m asl in the neighbouring state of Sikkim (Subedi et al., 2018a).

The two megascolecid worms viz., *A. corticis* and *A. morrisoni* are versatile in altitudinal distribution, they are widely distributed in various habitats in between 142–1754 m asl. Meanwhile, Cardoso et al. (2013) recorded *A. corticis* below 1000 m asl in Brazilian Atlantic Rainforest. Among the twelve identified species, it was observed that *A. gracilis* and *Drawida nepalensis* were the most frequently occurred species found in 11 different locations each, followed by *A. corticis* found in 9 locations, *Perionyx excavatus* and *Pontoscolex corethrurus* in 8 locations each (Table 1, Fig. 3). It was observed that *E. gammiei* and *O. beatrix* occupies the bottom in the heat map (Fig. 3) indicating their restricted spatial distribution status.

These new records of earthworm species could be due to various reasons. It might be due to more geographical area coverage in the present study. Areas that are not covered previously by Julka (1976, 1981) and Halder et al. (2007) were covered by us, as well as the season of sampling. The previous studies were confined to a specific area (Julka 1976, 1981). The present sampling is carried out in the onset of monsoon season; by this time the soil receives a lesser amount of water, the soil is not yet fully saturated to activate earthworms. This could be the reason for the lower species recorded in the present study as

compared to the previous study by Julka (1981) who recorded 27 species. However, in his first investigation, Julka (1976) also recorded 12 species only whose number of species is exactly similar to our result. With regard to earthworm diversity studies in the Northeast Indian States, Sikkim has been investigated by a number of researchers and holds the highest earthworm species diversity record with a total of 53 earthworm species under seven families (Soota and Halder, 1981; Subedi et al., 2018a,b,c; Tiwari et al., 2020). In Meghalaya, the most updated record is 32 species under 4 families (Kharkongor, 2018). In addition to this, recently Lone et al. (2020) reported four new species of *Kanchuria* earthworms from Meghalaya.

Tripura housed 38 species of earthworms (Chaudhuri et al., 2012). The state of Assam has a record of 17 species (Rajkhowa et al., 2015). Mizoram has a record of 12 species of earthworms (Ramanujam et al., 2004). Manipur is known to harbour seven species (Haokip and Singh, 2012). Nagaland has a recent record of three valid species (Thyugh and Kakati, 2018). Recently, the presence of *Perionyx foveatus*, *P. kaboensis* and *Eutyphoeus kempfi* in Arunachal Pradesh have been reported by Tiwari et al. (2020), which were a new record for the study site. Arunachal Pradesh being endowed with a variety of habitat, landscape, rivers and vegetations, is harbouring a good earthworm species diversity. With these new records of seven species, Arunachal Pradesh has become the second-highest earthworm species diversity record in northeast India.

The full compilation of 46 earthworm species of Arunachal Pradesh is presented in Table 1. In terms of abundance, the family Megascolecidae dominates the study site comprising 47.7% of the total species, followed by Moniligastridae (17.3%) and Acanthodrilidae (10.9%). Similar observation has been reported by Tiwari et al. (2020) from their diversity studies at north-eastern India. The genus *Perionyx* comprises the most diverse genera with 13 species followed by *Drawida* species represented by seven species. This result corroborates the record of Tiwari et al. (2020) who reported *Perionyx* earthworms as the most diverse genera with 32 different species in north-eastern region of India. The CO1 gene sequences obtained were analyzed using MEGA7 (Kumar et al., 2018), and the CO1 gene sequences of newly recorded species from Arunachal Pradesh were submitted to NCBI GenBank. Accession numbers are allotted as MH191377 (*A. gracilis*), MN179623 (*A. corticis*), MN175259 (*O. beatrix*), MN125034 (*E. eugeniae*), MN179624 (*P. corethrurus*), MN125035 (*B. parvus*) and MN179625 (*D. modigliani*).

Conclusion: Our findings enriched the diversity of earthworm species in Arunachal Pradesh by contributing seven new species records viz., *A. corticis*, *A. gracilis*, *B. parvus*, *D. modigliani*, *E. eugeniae*, *O. beatrix* and *P. corethrurus* belonging to six families. The two newly recorded families are Rhinodrilidae and Eudrilidae. With this new addition, the earthworm diversity in Arunachal Pradesh has become 46 species, the second-highest record of all north-eastern Indian states after Sikkim. Many more species of earthworms are perhaps, still not discovered.

Therefore, further investigation is suggested particularly at the vast protected areas covered with virgin forests.

Conflict of Interest: The authors declare that they have no competing interests.

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