Morphological Identification And Species Diversity Of The Earthworm Population (Clitellata: Opisthopora) From Malakand And Mardan District Khyber Pakhtunkhwa (KP), Pakistan

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ABSTRACT
Earthworms are the important soil engineers that recycle the nutrients, improve the soil health by aerating, and beak down the organic nutrients to increase the agricultural productivity. Earthworms are biological indicators, their healthy population indicate the soil health. Agricultural practices such as tilling, use of pesticides and other chemicals destroyed their habitats and population. The present study was conducted to investigate the species diversity of Earthworms in Mardan and Malakand districts, Khyber Pakhtunkhwa, Pakistan during the field study from March to September, 2025. The specimens were collected from the six different sites such as Sakha Kot, Chail Banda, Gujjar Ghari, Takht Bhai, Pir Abad, and Sarkai Bala. The collected samples were identified using standard taxonomic keys and preserved under the laboratory conditions. In this study, a total for three different species were identified such as *Amynthas morrisi*, *Amynthas hupeinsis* and *Ocnerodrilus occidentalis*. The morphological analysis showed some interspecific variations on segments of clitellum cover, location of male and female pores, shape of the prostomium, genital marks and in body weight and size. To find the species diversity, a total of 1460 samples were collected. In which the *Amynthas morrisi* (specimens; 505) was calculated more prominent and diverse as compared to *Ocnerodrilus occidentalis* (specimens; 500) and *Amynthas hupeinsis* (specimens; 455). The most diverse region in the selected sites were recorded as the Sakha Kot and Chail Banda, while the least diverse region were recorded Pir Abad and Gujjar Ghari. On the base species abundance, the species *A. morrisi* was more dominant and prevalent in Gujjar Ghari (49.16%), the species *A. hupeinsis* recorded more dominant and prevalent in Takht Bhai (50.40%) and Pir Abad (63%). While the species *O. occidentalis* was more prevalent and dominant in Sakha Kot (45%), Chail Banda (42.66%) and Sarkai Bala (37.27%). The present study provided a baseline for oligochaetologist to be utilized in

the further ecological and taxonomic studies of earthworms' population in Pakistan.

Introduction

Earthworms are soft-bodied segmented invertebrates belongs to class Clitellata, order Opisthopora, phylum Annelida, Animalia, most of the species are related to family Lumbricidae (Edwards & Bohlen, 1996). The earthworms are widely distributed across the world except permanently frozen region and extremely dry deserts. They are highly abundant in the moist soil in terrestrial habitats like forests, wetlands, and farmlands where they feed on dead organic matters, microorganisms and decomposed plant residues (Lavelle et al., 2006). Earthworm fauna is quite diverse in various geographical areas and their distribution of native species is heavily dependent on the environmental conditions such as the type of soil, temperature, climatic condition and the length of seasons (Lavelle & Spain, 2001).   Earthworms possesses elongated cylindrical body that divided in numerous segments known as metameres. The body length varies among the species that is ranged from 1mm up to 30cm. The body surface is covered by thin layer of cuticle to keep the surface moist to facilitate gaseous exchange through skin (Brusca et al., 2016). The anterior part of the body contains the mouth and the posterior end possess the anus. Earthworms have a specialized structure the clitellum that play a crucial role reproduction. Each metameres bears tiny bristle-like structures called setae that help to move through the soil surface. Earthworms are hermaphroditic and each organism has both male and female reproductive system (Sims & Gerard, 1999). However, for the reproduction the cross-fertilization of the individuals are very significant. The two earth worms align their bodies in opposite direction for fertilization and for the exchange of the sperms. The clitellum secretes a thick layer of mucus to make cocoon, where the sperm and eggs are deposited and fertilized (Domínguez & Edwards, 2011). The juveniles earthworms emerged from cocoon after few weeks depends upon suitable environmental condition. Earthworms show a critical role in sustaining soil health and ecosystem efficiency (Csuzdi et al 2015). Their feeding and foraging activities upturn the soil construction by increasing permeability and accretion. They ingest organic matter with soil to produce nutrient-rich casts that improve the soil fertility (Khan et al., 2024). Earthworms also stimulate microbial activity inside soil by breaking the organic materials and enabling their decomposition. This progression help to releases crucial nutrients such as potassium, phosphorus, and nitrogen that are very important for plant growth and development. (Edwards & Arancon, 2004; Ahmed et al., 2020).

The present study was conducted to investigate the species diversity and their abundance in district Mardan and Malakand from Khyber Pakhtunkhwa, Pakistan. The collected species were identified through morphological characters such as spermatheca, genital pores, setae, a prostomium and a clitellum, which are the taxonomic features important in identifying the species. Absence of that, the relative abundance and richness of species were quantified. This integrative approach gives a global evaluation of the earthworm species and abundancy in the chosen study zones and will serve as a great source of baseline data to be utilized in the further ecological and taxonomic studies.

Materials and methods

Study Area

The present stud was conducted in the selected districts Malakand and Mardan Khyber Pakhtunkhwa (KP) province of Pakistan. These districts situated at the Northwestern side of KP that represent the important ecological regions for studying soil biodiversity especially earthworms. Mardan District lies with geographic coordinates 34.12°–34.33° latitude and 71.97°–72.63° longitude. The district have a fertile plains and characterized by agricultural lands, irrigated crop fields, orchards, and rural settlements. The climate of the region is mostly subtropical with hot summers and mild winters, while the soils are mostly muddy and rich in organic matter, providing promising conditions for earthworm populations. Malakand District lies with geographic coordinate between 34.56°–34.90° latitude and 71.93°–72.53° longitude. In contrast, the Malakand district is mostly hilly and semi-mountainous terrain with mixed vegetation, cultivated land, and natural territories. The region experiences mild rainfall and relatively cooler temperatures compared to the plains, which support diverse soil organisms. The variation in altitude, soil types, vegetation cover, and land-use practices between these two districts creates suitable microhabitats for different species of earthworms. Therefore, these districts provide an ultimate study area for the exploration and collection of earthworm diversity, distribution, and ecological roles in different terrestrial habitats. The specimens were collected from the six different sites such as Sakha Kot, Chail Banda, Gujjar Ghari, Takht Bhai, Pir Abad, and Sarkai Bala (Fig. 1)
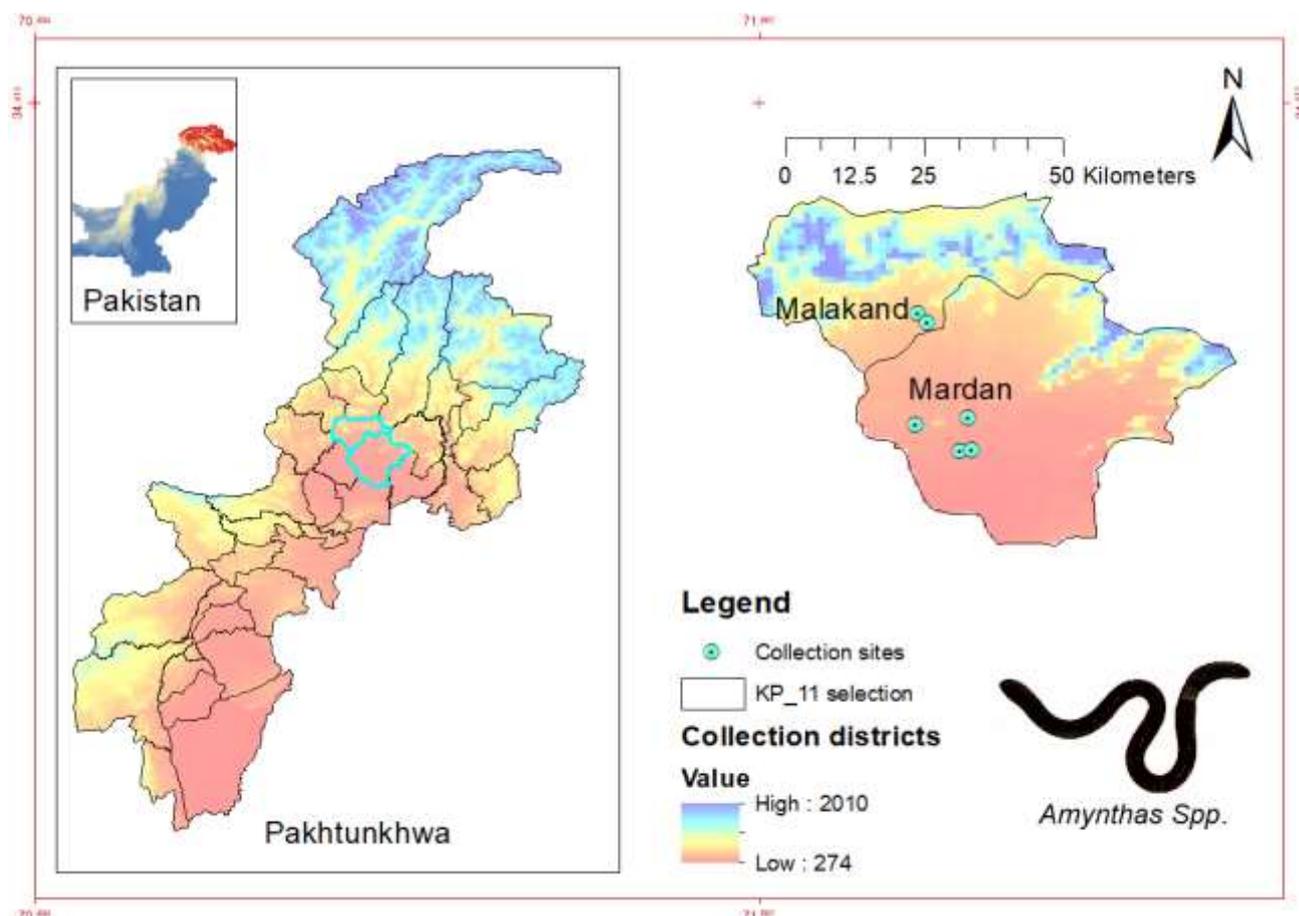

Figure 1: Arc GIS map showing collection sites along District Mardan and Malakand

Sample collection:
The earthworms' samples were collected by the excavation and manual hand-sorting method that was outlined by Anderson and Ingram (1993). Soil was dug out with the help of a shovel and spade, and earthworms were manually collected from the soil. To reduce the stress levels and to maintain the moisture level, the collected specimens were transferred into plastic buckets and partial filled with the same soil from where they were collected. The collected samples were brought to fisheries and aquaculture laboratory, Zoology department, Abdul Wali Khan University Mardan to be analyzed further.

Sample maintenance
The collected specimens were maintained a live in a bucket under regulated experimental oversight. The earthworms are extremely sensitive to loss of moisture, the hydration is constantly ensured to avoid desiccation. The sustenance provided to earthworms was actually meal worm *(Tenebrio molitor)* manure or frass. Frass is rich in nitrogen (N), potassium (K), phosphorus (P), and essential micronutrients such as copper (Cu) and zinc (Zn) (Houben et al., 2020). As frass is a rich source of nutrients additionally frass also absorb water so a balance of water and frass was maintained to sustain the earthworms in a thriving state. Due to proper care, the earthworms were nurtured even during the coldest season of the year in which the earthworm are typically dormant.

Sample identification
The fresh samples of earthworms were selected from the bucket and washed under the clean and cold water in a strainer and then preserved in 99.8 % ethanol for further analysis. The samples were washed again by clean water from ethanol and then transferred to microscopic slide and was moistened with distal water drops. The clitellum, clitellum covering segments, spermathecal pore, female pore, prostomium presence and shape, color pattern, and setae were observed under light microscope. Earthworm was observed from various perspectives to gather accurate information. The characteristics of each earthworm were documented, leading to the identification of three distinct species of earthworms (Fig. 2).

Figure 2: A) collection of earthworms, B) Morphological identification, and C) Morphological examination through microscope.

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Results and Discussion

The accurately documented information about earthworm characteristics enables to identify three species of earthworms; *A. morrisi, O. occidentalis* and *A. hupeiensis*. These species were identified through a standardized dichotomous key for earthworm identification that is; Earthworms of Solan, a constituent of Himalayan biodiversity.

Reddish worm (*Amynthas morrisi*)

The size of *A. morrisi* ranged between 10 to 15cm, color pattern is reddish brown and stripped. The weight is ranged between 0.37 to 1.05g. Setae present on the ventral side of the body, clitellum covering 3 segments. The segments above clitellum is approximately 13 and Paired spermathecal pores are present at segment 5, 6, 7. Male pore superficial discharge directly on to body surface. The prostomium present but round in shape.

Soil worm (*Ocnerodrilus occidentalis*)

The size of *O. occidentalis* ranged between 13 to 19cm and their color is similar to the color of soil. The body weight ranges between 0.40g to 1.14g. Setae present through out of the body and the number of setae on each segments is 8 that present in 4 pairs. The clitellum covering segments are 3 and the number of clitellum cover is 14 to 16. The genital marking present below clitellum and the Spermathecal pore at segment number 7 and 8. Female pore present on clitellum segment number 14 while the male pore present on segment number 18. The prostomium is present and have round in shape.

Green jumping worm (*Amynthas hupeinsis*)

The body size ranged from 9 to 11cm and body weight ranges from 0.28cm to 0.75cm. The body color is usually green and their color pattern is solid. The setae are absent, no any genital marking and male pore. The female pore present in clitellum and incomplete clitellum covering 1 or 2 segments. These worms are thin and fragile and spermethacal pore is present at segment 7/8 or 8/9. The prostomium is present and round have in shape (Figure. 3).
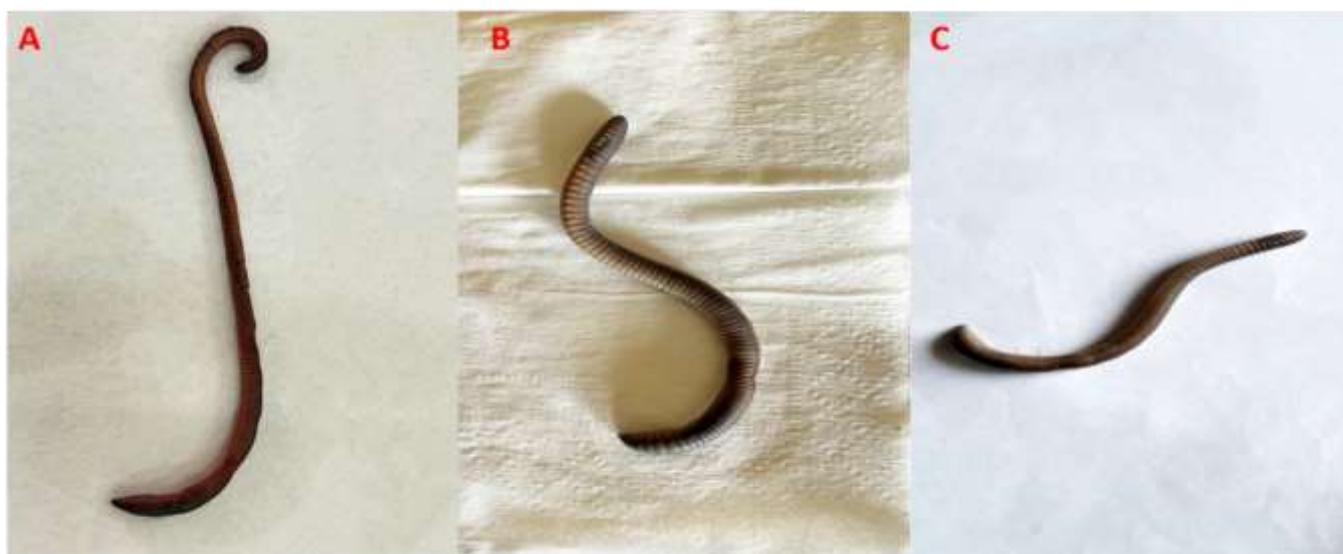

Figure 3. A) *Amynthas morrisi,* B) *Ocnerodrilus occidentalis*, C) *Amynthas hupeinsis*

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Species diversity of earthworms in selected districts

Earthworms were collected from six different areas of district Mardan and Malakand which include Sakha Kot, Takht Bhai, Gujjar Ghari, Pir Abad, Chail Banda and Sarkai Bala. The analysis of the collected samples ensured the presence of three different species in the selected areas that are *A. morrisi, A. hupeinsis and O. occidentalis*. From the selected area, a total of 1460 specimens were sampled in which 505 samples are of *A. morrisi*, 500 samples are *A. hupeinsis* and 455 sample of *O. occidentalis*. The *A. morrisi* species were calculated the more prominent and abundant species as compared to *A. hupeinsis* and *O. occidentalis* in district Malakand and Mardan Khyber Pakhtunkhwa, Pakistan (Table 1).

Table 1. The table shows the sampling area, number of specimens and name of species.

Sampling area	Specimens	*A. morrisi*	*A. hupeinsis*	*O. occidentalis*
Sakha Kot	300	90	75	135
Takht Bhai	250	84	126	40
Gujjar Ghari	240	118	32	90
Pir Abad	150	30	95	25
Chail Banda	300	125	47	128
Sarkai Bala	220	58	80	82
Total	1460	505	455	500

Relative abundance of earthworm's population

The relative abundance of the species were calculated by formula the "relative abundant index (**X**)". **N** indicate the total number of species while **n** indicate the total number of the individuals of selected species.

$$X = \frac{n}{N} \times 100$$

$$\text{Relative species abundance index} = \frac{\text{indivisual of X species}}{\text{total idivisul ofall species}} \times 100$$

The most diverse region in the selected sites were recorded as the Sakha Kot and Chail Banda, while the least diverse region were recorded Pir Abad and Gujjar Ghari. On the

base species abundance, the species *A. morrisi* was more dominant and prevalent in Gujjar Ghari (49.16%), the species *A. hupeinsis* recorded more dominant and prevalent in Takht Bhai (50.40%) and Pir Abad (63%). While the species *O. occidentalis* was more prevalent and dominant in Sakha Kot (45%), Chail Banda (42.66%) and Sarkai Banda (37.27%) (Table. 2).

Table. 2. The table shows the sampling area, specimens and relative abundance of the species

Sample area	Specimens	Relative abundance of *A. morrisi*	Relative abundance of *A. hupeinsis*	Relative abundance of *O. occidentalis*
Sakha Kot	300	30%	25%	45%
Takht Bhai	250	33.60%	50.40%	16%
Gujjar Ghari	240	49.16%	13.33%	37.50%
Pir Abad	150	20%	63%	16.66%
Chail Banda	300	41.66%	15.66%	42.66%
Sarkai Bala	220	26.36%	36.36%	37.27%

Conclusion:
The present study examined the earthworm morphology, species diversity and their abundance, in six different localities in the Mardan and Malakand district Khyber Pakhtunkhwa, Pakistan. In this study, three different earthworms were identified such as *A. morrisi, A. hupeiensis*, and *O. occidentalis*. All the identified species were consistently found in the selected sites in subsurface of soils. The most diverse region in the selected sites were recorded as the Sakha Kot and Chail Banda, while the least diverse region were recorded Pir Abad and Gujjar Ghari. On the base species abundance, the species *A. morrisi* was more dominant and prevalent in Gujjar Ghari (49.16%), the species *A. hupeinsis* recorded more dominant and prevalent in Takht Bhai (50.40%) and Pir Abad (63%). While the species *O. occidentalis* was more prevalent and dominant in Sakha Kot (45%), Chail Banda (42.66%) and Sarkai Bala (37.27%).

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