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# Reply to Narayanan et al. Comment on "Lone et al. Phylogenetic Relationships in Earthworm *Megascolex* Species (*Oligochaeta: Megascolecidae*) with Addition of Two New Species. *Diversity* 2022, *14*, 1006"

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We acknowledge and value the feedback provided by Narayanan et al. on the published manuscript [1]. The authors concede to the existence of spelling issues in certain sections and have released the Errata. The authors emphasise that these errors were not deliberately included in the text. Nevertheless, it should be stressed that most of the concerns were subjective and scientifically unjustified. Particularly, the phenotypic plasticity and overlapping of taxonomic characteristics were emphasised at different times by Narayanan et al. The study, which primarily focused on the phylogenetic relationship, holds no significance in juxtaposition. Nevertheless, the classical description is crucial for undertaking taxonomy studies. It is irrelevant to the principles of evolution in general, as variations are more common in most terrestrial species, particularly in soil-dwelling animals including earthworms [2–5].

Specifically, Narayanan et al. pointed out the statement made by us [6] pertaining to the initial DNA-based molecular analysis conducted on the *Megascolex* species and stated that it "is a wrong statement, as already the DNA barcodes of *M. konkanensis konkanensis* Fedarb, 1898 [7] and *M. cochinensis* Stephenson, 1915 [8] have been made available in the GenBank database (accession numbers: OM714798.1, OM100709.1 respectively)". On that statement, we specified that accession numbers OM714798.1 and OM100709.1 had been submitted to Genbank but had not been published at that time. Therefore, only published sequences were regarded as pertinent submissions of sequences and considered in the study.

Another concern highlighted by Narayanan et al. was for *Megascolex auriculata* Aiyer, 1929, [9] where they mentioned that Stephenson [10] specified that in the case of *Megascolex* "setae, at least in the middle and hinder parts of the body, are numerous (more than eight) in each segment", in reference to the perichaetine setal arrangement. Similarly, "if a specimen shows an increase in the number of setae in any part of the body, it is a *Megascolex*". In the original description of *M. auriculata* by Aiyer [9], it was clearly stated, about the lumbricine arrangement at the anterior portion and from the middle, that it changes according to the perichaetine condition. We mentioned that the phenomenon of setae lumbricine losing their posterior orientation suggests a decrease in the arrangement of lumbricine as the number of setae grows. The precise terminology of perichaetine was not cited, corresponding to the earlier works by Stephenson [10] and Aiyer [9]. Further, Narayanan et al. pointed out that in the original description and figure of *M. auriculata* by Aiyer [9], the spermathecal ampulla was long and thickly club- shaped, the basal portion was more than half as wide as the distal half, and the spermathecal duct was very short with unidiverticulate, ental

diverticulum. Similarly, in *M. cochinensis cochinensis*, the clitellum extended over segments ½13–1/317; however, as per Stephenson [10], it should be in segments 14–2/317 (=3⅔). The description of *M. filiciseta* is based on three syntypes. In the original description, it was clearly stated that the origin of the intestines in *M. filiciseta* is in segment 15, whereas Lone et al. [6] stated that it is in segment 16. Along the same lines, the *Megascolex konkanensis konkanensis* Fedarb [7] specimens of Lone et al. had a diameter of 4–5 mm, whereas it should only have a diameter of 2–3 mm [7,10]. The anterior portion of this species is truncated, their prostomium is epilobus, and their tongue is narrow and small, but we had mentioned that the prostomium is epilobus and with two short longitudinal grooves on the dorsal surface of the first segment. The presence of the first dorsal pore should be in the intersegmental furrow of 4/5 instead of 5/6. Clitellum is in segments 14–16 or 14–½17 (=3 or 3½ segments) [7,10], while it should be in 14–17 (4 segments); furthermore, septa 5/6–11/12 should not be moderately thickened, but it should be thickened. Further, Narayanan et al. motioned that *M. polytheca polytheca* Stephenson, 1915, by Lone et al. [6] provided the dimensions of the specimen as a length of 105–140 mm, diameter of 3 mm, segments 159–194, and that it was shorter than the dimensions provided by Stephenson [10]. Similarly, Narayanan et al. stated that, in *M. triangularis*, the first dorsal pore starting from the intersegmental furrow should be from 4/5 instead of 6/7. Moreover, septa 5/6–11/12 are thickened, but they should be 4/5–6/7 thin, while 7/8 should be slightly thickened, 8/9–11/12 are somewhat strengthened, and 12/13/14 are slightly thickened [11]. The prostatic duct must be relatively long, thin, shiny, and of the same diameter throughout; however, in our specimens, the prostatic duct was not visible. In *Megascolex papparensis* Lone, Thakur, Tiwari, James & Yadav, 2022, the description of the *M. papparensis* was based on three specimens (one holotype and two paratypes) and the range was not mentioned. In addition, Narayanan et al. highlighted that the authors did not provide diagnostic characters. However, we used a similar approach to describe the novel species as reported by the earlier authors [12,13]. We acknowledge that Narayanan et al. observed phenotypic plasticity in all these cases. However, we should also highlight that taxonomic obstacles pose a significant challenge in earthworm taxonomy. Several instances of substantial plasticity have been reported in classical taxonomy, which have opened up new tools of taxonomy, including molecular methods, to counteract the overlapping of taxonomic characteristics. The conventional classification system depends on morphological traits, which may be inadequate because of the significant level of similarity across the different species of earthworms. Several species demonstrate convergent evolution, a phenomenon in which unrelated species acquire nearly identical characteristics, thereby complicating their differentiation only by physical appearance. The parameters observed in the study are subjective and expressed on the basis of observation. We introduced the concept of morpho species as a conjecture, positing its potential existence in *Megascolex* species.

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**Data Availability Statement:** The molecular data used in the study are available on DS-IEWMG on Diversity studies in earthworms of India using DNA Barcode approach on Barcode of Life Data System (BOLD).

**Conflicts of Interest:** The authors declare no conflict of interest.

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