European Journal of Taxonomy 336: 1–14
https://doi.org/10.5852/ejt.2017.336
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2017 · Csuzdi C. *et al.*


Research article

urn:lsid:zoobank.org:pub:A61DAF10-1819-4C90-A64A-1F1DDDAC220D

# Three new species of *Kynotus* from the Central Highlands of Madagascar (Clitellata, Megadrili)

Csaba CSUZDI [1], Malalatiana RAZAFINDRAKOTO [2] & Yong HONG [3,*]

[1] Department of Zoology, Eszterházy Károly University, Eger, Hungary.
[2] Department of Animal Biology, University of Antananarivo, Madagascar and Laboratory of Radio-Isotopes, University of Antananarivo, Madagascar.
[3] Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Jeonju, Republic of Korea.

[*] Corresponding author: geoworm@hanmail.net
[1] Email: csuzdi.csaba@ektf.hu
[2] Email: malalasraz@yahoo.fr

[1] urn:lsid:zoobank.org:author:86F75D9D-740D-4D98-849B-B744C4438D76
[2] urn:lsid:zoobank.org:author:F21C5094-DE1F-4F7E-BF0A-C59C8AAF7D7A
[3] urn:lsid:zoobank.org:author:49F2D6FB-B6BA-438C-A452-ED4C109DB544

**Abstract.** The earthworm fauna of Madagascar is scarcely known. A recently launched exploration of the soil fauna ("Global Change and Soil Macrofauna Diversity in Madagascar") resulted in the discovery of six new earthworm species belonging to the Malagasy endemic family Kynotidae. The success of the collecting campaign carried out between 2008 and 2011 inspired a new exploration of the earthworm fauna across the Central Highland Region of the island in the spring of 2015. During this expedition, two new species of *Kynotus*, *K. ankisiranus* sp. nov. and *K. voimmanus* sp. nov., were discovered. Barcoding of the recently collected species of *Kynotus* revealed that the unpigmented worms referred previously to *K. alaotranus* Michaelsen, 1897 also represented a new, still undescribed species, *K. blancharti* sp. nov.

**Keywords.** Oligochaeta, Malagasy Region, endemism, Kynotidae, barcoding, COI.

Csuzdi C., Razafindrakoto M. & Hong Y. 2017. Three new species of *Kynotus* from the Central Highlands of Madagascar (Clitellata, Megadrili). *European Journal of Taxonomy* 336: 1–14. https://doi.org/10.5852/ejt.2017.336

## Introduction

Madagascar, with its territory of 592,800 km$^2$, is the fourth largest island in the world and, due to its tectonic history and environmental variability, is listed among the 25 megadiverse regions of the earth (Myres *et al.* 2000). This is why the island's fauna has always been the focus of research (Goodman & Benstead 2003). Interestingly though, data on the earthworm fauna of Madagascar is quite scarce; since the description of the first earthworm species *Geophagus darwini* (=*Kynotus darwini*) by Keller (1887) just a couple of papers dealing with the earthworm fauna of Madagascar (e.g., Michaelsen 1891, 1897, 1907, 1931; Rosa 1892) have been published. After the last paper by Michaelsen (1931), no new taxonomic works on the earthworm fauna of the island were published until a new project, entitled "Changement global et diversité de la macrofaune du sol à Madagascar" (Global Change and Soil Macrofauna Diversity in Madagascar), was launched in 2008. During this project, a comprehensive collecting campaign resulted in the discovery of several rare, as well as new, earthworm species (Razafindrakoto *et al.* 2010, 2011, 2016; Csuzdi *et al.* 2012).

These results inspired new collecting activities carried out by the latter authors, which resulted in the discovery of two further species new to science, belonging to the endemic family Kynotidae. Barcoding of the recently collected material of *Kynotus* revealed that the unpigmented specimens, previously relegated to the red-pigmented *K. alaotranus* Michaelsen, 1907 (Csuzdi *et al.* 2012), also represent a new species. Descriptions of the three new species of *Kynotus*, together with their barcodes, are herewith provided.

## Material and methods

### Study area

The new collecting sites are situated in the Central Highlands region of the island (Fig. 1).

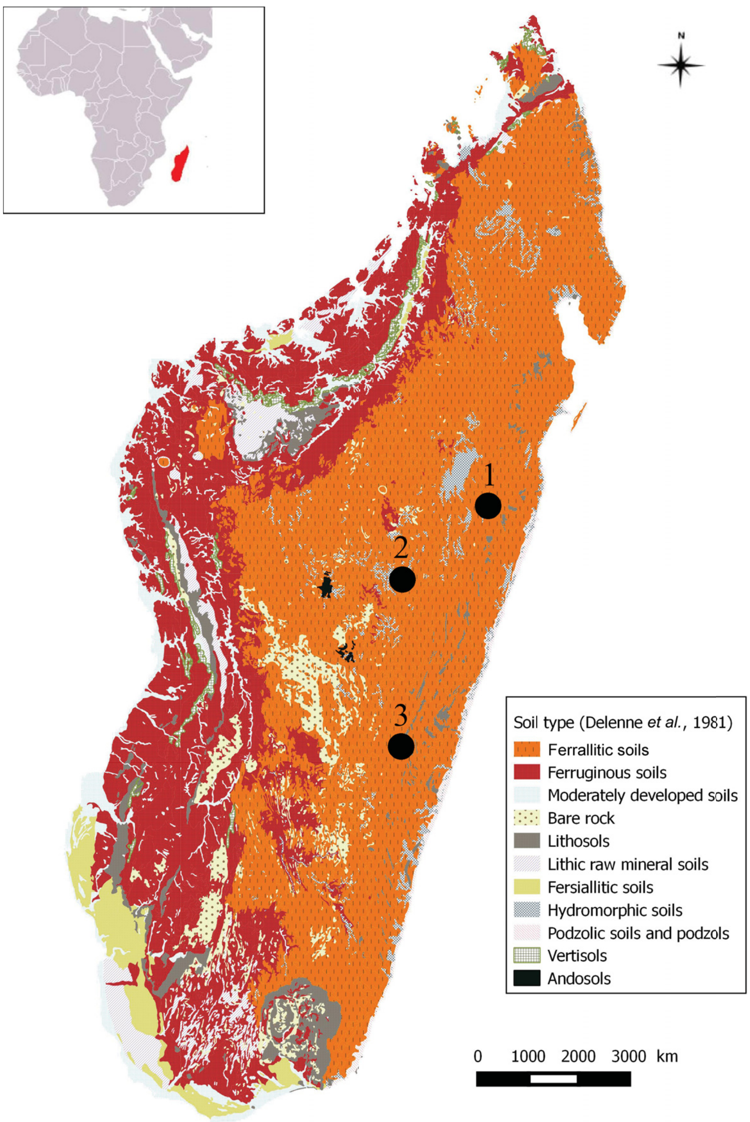


**Fig. 1.** Main soil types of Madagascar, with indication of the sampling localities. 1 = Moramanga District, Andasibe, Voimma Community Park; 2 = Antsirabe District, Sambaina; 3 = Ambalavao District, Mt Ankisira National Park.

### Methods

Earthworms were collected primarily by using the diluted formaldehyde method (Raw 1959) supplemented by digging and hand-sorting. The material collected was killed in 75% ethanol and fixed in 4% formaldehyde solution. Parallel material from each morpho-species was conserved in 96% ethanol for DNA studies.

### Deposition

The material collected is deposited in the Hungarian Natural History Museum (HNHM) and in the collection of Chonbuk National University, Korea (CHBNU).

### Molecular methods

A small piece of the postclitellate body wall was cut out from the paratype specimens for barcoding, as indicated in Table 1, and processed according to the methodology described in Szederjesi & Csuzdi (2015). Additional COI sequences of *Kynotus* were acquired from the BOLD database (Table 1).

COI sequences were aligned with ClustalW (Thompson *et al.* 1994), using the default settings. The final dataset was 671 bp long and contained no internal gaps. Maximum Likelihood analysis was carried out with Mega v. 6.06 (Tamura *et al.* 2013) using the best fitting substitution model GTR G + I and 1000 bootstrap replicates. Bayesian inference was performed with BEAST v. 1.8.2 software (Drummond *et al.* 2012) with the GTR G + I substitution model selected by MEGA using the Akaike Information Criterion. BEAST was run for 10 million generations, saving trees at every 1000$^{th}$ generation. The first 2000 trees were discarded "burn in" in TreAnnotator v. 1.8.2. The resulting tree was visualized with Fig Tree v. 1.4.2 (Rambaut 2014). Inter- and intraspecific genetic distances were calculated with MEGA using the K2P substitution model.

## Results

Class Clitellata Michaelsen, 1919
Subclass Oligochaeta Grube, 1850
Superorder Megadrili Benham, 1890
Order Opisthopora Michaelsen, 1929
Suborder Crassiclitellata Jamieson, 1988
Family Kynotidae Jamieson, 1971
Genus *Kynotus* Michaelsen, 1891

***Kynotus ankisiranus*** sp. nov.
urn:lsid:zoobank.org:act:DDAFA680-A9E8-4A8B-AD38-9FB52F7378F2
Figs 2, 5

### Diagnosis

Length 70–90 mm, diameter 3 mm. Colour grey with slight red pigmentation at the head on dorsum. Segments 1–3 simple, 4–10 biannulate. Setae appear from segment 2 and clearly visible, male pores ventral on 16. Clitellum circular on 18–27, ½28. Three rows of spermathecae of elongated oval-shape in 13/14, 14/15, 15/16. No septa notably thickened. Genital setal glands in 14, 15.

### Etymology

The species epithet refers to the type locality, Mt Ankisira National Park.

**Table 1.** Localities and COI Genbank accession numbers of the specimens of *Kynotus* analysed (EWSJC samples were obtained by Sam James).

| Species | Voucher no. | Locality | Accession no. |
|---|---|---|---|
| *K. ankisiranus* sp. nov. | HNHM/AF5659 | Madagascar, Ambalavao, Namoly, Mt Ankisira National Park | KX527886 |
| *K. alaotranus* 1 | HNHM AF/5528 | Madagascar, Vohimana Reserve, secondary *Pandanus* forest | KX372247 |
| *K. alaotranus* 2 | HNHM AF/5528 | Madagascar, Vohimana Reserve, secondary *Pandanus* forest | KX372248 |
| *K. blancharti* sp. nov. | HNHM/AF5539 | Madagascar, Antsirabe District, Sambaina | KX517831 |
| *K. blancharti* sp. nov. | HNHM/AF5539 | Madagascar, Antsirabe District, Sambaina | KX517832 |
| *K. michaelseni* 1 | HNHM AF/5530 | Madagascar, Sambaina, open bush-land | KX372243 |
| *K. michaelseni* 2 | HNHM AF/5530 | Madagascar, Sambaina, open bush-land | KX372244 |
| *K. minutus* 1 | HNHM AF/5235 | Madagascar, Vohimana Reserve, secondary *Pandanus* forest | KX372245 |
| *K. minutus* 2 | HNHM AF/5235 | Madagascar, Vohimana Reserve, secondary *Pandanus* forest | KX372246 |
| *K. proboscideus* | EWSJC848 | Madagascar, Mangalaza Ambatondrazaka, near villages | JN260829 |
| *K. proboscideus* | EWSJC849 | Madagascar, Mangalaza Ambatondrazaka, near villages | JN260830 |
| *K. proboscideus* | EWSJC850 | Madagascar, Mangalaza Ambatondrazaka, near villages | JN260831 |
| *K. proboscideus* | EWSJC851 | Madagascar, Mangalaza Ambatondrazaka, near villages | JN260832 |
| *K. proboscideus* | EWSJC852 | Madagascar, Mangalaza Ambatondrazaka, near villages | JN260833 |
| *K. proboscideus* | EWSJC855 | Madagascar, Vohitsoa Ambatondrazaka, along the road | JN260834 |
| *K. proboscideus* | HNHM AF/5512 | Madagascar, Vohitsoa Ambatondrazaka, Tsarahonenana | KX527885 |
| *K. sihanakus* | EWSJC924 | Madagascar, Bemanevika Bealanana, grassland | JN260884 |
| *K. sihanakus* | EWSJC925 | Madagascar, Bemanevika Bealanana, grassland | JN260885 |
| *K. sihanakus* | EWSJC927 | Madagascar, Bemanevika Bealanana, grassland | JN260886 |
| *K. sihanakus* | EWSJC928 | Madagascar, Bemanevika Bealanana, grassland | JN260887 |
| *K. sihanakus* | EWSJC929 | Madagascar, Bemanevika Bealanana, grassland | JN260888 |
| *K. sihanakus* | EWSJC930 | Madagascar, Ambatosoratra Ambatondrazaka, banana plantation | JN260889 |
| *K. sihanakus* | EWSJC931 | Madagascar, Ambatosoratra Ambatondrazaka, banana plantation | JN260890 |
| *K. sihanakus* | EWSJC939 | Madagascar, Ambatosoratra Ambatondrazaka, banana plantation | JN260895 |
| *K. voimmanus* sp. nov. | HNHM AF/5661 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX527887 |
| *K. voimmanus* sp. nov. | HNHM AF/5661 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517833 |
| *K. voimmanus* sp. nov. | HNHM AF/5661 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517834 |
| *K. voimmanus* sp. nov. | HNHM AF/5661 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517835 |
| *K. voimmanus* sp. nov. | HNHM AF/5662 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517836 |
| *K. voimmanus* sp. nov. | HNHM AF/5662 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517837 |
| *K. voimmanus* sp. nov. | HNHM AF/5662 | Madagascar, Moramanga, Andasibe, Voimma Community Park | KX517838 |

### Material examined

#### Holotype

MADAGASCAR: clitellate, Ambalavao District, Sendreisoa, Namoly, Mt Ankisira National Park, *Aristida* grassland at the edge of primary forest, 22°06′45.8″ S, 46°56′33.8″ E, 1500 m a.s.l., 24 Feb. 2015,Y. Hong and M. Razafindrakoto leg. (HNHM/AF5658).

#### Paratypes

MADAGASCAR: 2 clitellate, 3 aclitellate adults (HNHM/AF5659), 2 aclitellate (CHBNU MD-06-AL5), locality and date same as for holotype.

**Description**

Holotype 50 mm in length, diameter after clitellum 3 mm, segment number 98, tail missing. Paratypes 72–86 mm in length, 3 mm in diameter, segment number 170–179. Colour grey with red pigmentation at head on dorsum. Head zygolobous, segments 1–3 simple, 4–10 biannulate. Dorsal pores lacking. Setae small, *ab* and *cd* clearly observed from segment 2. Setal arrangement (holotype) after clitellum aa:ab:bc:cd:dd = 15:1.25:9.5:1:18, dd = 0.32 U (Fig. 2A). Clitellum annular on segments 18–27, ½ 28.

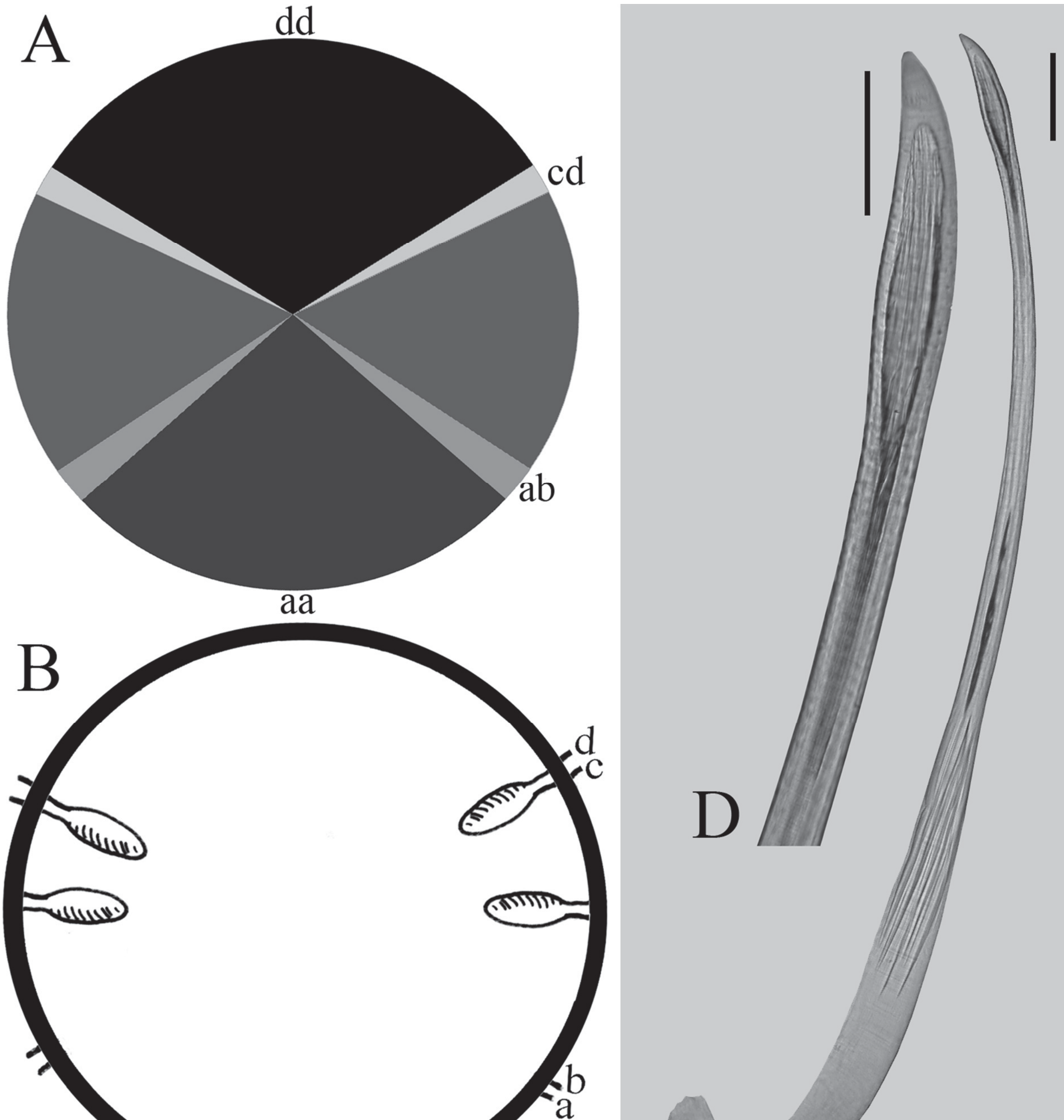


**Fig. 2.** *Kynotus ankisiranus* sp. nov. **A**. Setal arrangement, a, b, c, d representing setal lines. **B**. Spermathecae, schematic representation. **C**. Genital seta. **D**. Tip of the genital seta. Scale bars = 0.1 mm.

Male pores ventral on 16. Female pores not seen. Spermathecal pores in 13/14 (2), 14/15 (2), 15/16 (2) from *bc* to *cd*. Genital setal pores in variable position between *ab* and *cd* on 14, 15.

### Internal characters

Large muscular gizzard in 5. Septa all membranous. Calciferous glands, lamellae and typhlosolis lacking. Dorsal blood vessel simple throughout, last pair of hearts in 11. Excretory system holoic, vesiculate. Two pairs of testes and sperm funnels in 10, 11 enclosed in large peri-oesophageal testis sacs. Seminal vesicles lacking. Ovaries in 13. A pair of large, oval copulatory chambers occupying the ventral space of segments 16–17. Each copulatory chamber bearing an irregular prostate-like gland (pseudoprostate) reaching segment 21. Spermathecae elongated, oval-shaped, with short duct attached at 13/14(2), 14/15(2), 15/16(2) in variable position arranged from between *bc* to *cd* (Fig. 2B). Two pairs of genital setal glands present in segments 14, 15 above *ab* and around *cd* respectively. The genital setae are slightly curved, with spoon-shaped apex, length 1.6–1.7 mm, diameter at the middle 0.05 mm, ornamentation dense serrations (Fig. 2C–D).

### Remarks

The new species belongs to the *alaotranus* group (Table 2) characterized by biannulate segments between 4–10. With its smaller size and red pigmentation on the dorsum, *K. ankisiranus* sp. nov. most closely resembles *K. minutus* Csuzdi, Razafindrakoto & Blanchart, 2012, but differs from it by the longer clitellum (18–26 vs 18–27, ½ 28) and the number of genital setal glands (single pair in 15 vs two pairs in 14, 15). *K. amkisiranus* sp. nov. is clearly different from all the examined *alaotranus* group's species by its COI barcode as well (Table 3, Fig. 5).

## ***Kynotus blancharti*** sp. nov.

urn:lsid:zoobank.org:act:89CA5809-835D-4698-B6EA-2BDF9178F718

Figs 3, 5

*Kynotus alaotranus* Michaelsen, 1907 – Csuzdi *et al*. 2012: 37.

### Diagnosis

Length 80–90 mm, diameter 4–5 mm, segment number 180–195. Segments 1–3 simple, 4–10 biannulate. Colour light grey, pigmentation lacking. Setae appear from segment 2, clearly visible. Male pores ventral on 16. Clitellum circular on ½18–27. Three rows of spermathecae of oval shape, with long and thin duct in 13/14, 14/15, 15/16. Genital setal glands present in 14 and 15.

### Etymology

We dedicate this species to Eric Blanchart (L'Institut de Recherche pour le Développement (IRD), Antananarivo), who initiated the recent earthworm research in Madagascar.

### Material examined

#### Holotype

MADAGASCAR: clitellate, Antsirabe District, Sambaina, open bushland, 19°38′12″ S, 47°8′49″ E, 1700 m a.s.l., 21 Apr. 2008, Cs. Csuzdi, E. Blanchart and M. Razafindrakoto leg. (HNHM/AF5652).

#### Paratypes

MADAGASCAR: 3 clitellate ex., locality and date same as holotype (HNHM/AF5539).

**Table 2.** Distinguishing characters in the *K. alaotranus* species group.

| Species | Average size (length/width) (mm) | Pigment | Biannulate segments | First setal segment | Clitellum | Spermathecae (number) | Genital setal glands | Genital setae (mm) and apex shape |
|---|---|---|---|---|---|---|---|---|
| *K. ankisiranus* sp. nov. | 90/3 | slight red | 4–10 | 2 | 18–½ 28 | 13/14, 14/15, 15/16 (2) oval, short duct | 14, 15 | 1.7 spoon-shaped |
| *K. alaotranus* Michaelsen, 1907 | 120/6 | slight red | 4–10 | 3 | ½ 18–27 | 13/14, 14/15, 15/16 (2-4) oval, long duct | 14, 15 | 1.5 lanceolate |
| *K. blancharti* sp. nov. | 90/5 | lacking | 4–10 | 2 | ½ 18–27 | 13/14, 14/15, 15/16 (2-4) oval, long duct | 14, 15 | 1.9 spoon-shaped |
| *K. michaelseni* Rosa, 1892 | 130/8 | dark red | 4–10 | 3 | 19–25 | 13/14, 14/15 (1) finger-shaped | 13, 14 | 2.0 lanceolate |
| *K. minutus* Csuzdi *et al*., 2012 | 70/4 | dark red | 4–10 | 2 | 18–½ 27 | 13/14, 14/15, 15/16 (2-3) oval, short duct | 15 | 1.2 lanceolate |
| *K. parvus* Csuzdi *et al*., 2012 | 100/4 | slight red | 4–10 | 2 | 18–½ 27 | 13/14, 14/15, 15/16 (2-3) oval, short duct | 14, 15 | 0.9 lanceolate |
| *K. pittarellii* Cognetti, 1906 | 230/10 | lacking | 4–10 | 6 | 18–28 | 14/15, 15/16 (2-4) finger-shaped | 14, 16 | 3.6 lanceolate |
| *K. rosae* Cognetti, 1906 | 105/5 | slight red | 4–10 | 2 | 18–28 | 13/14, 14/15, 15/16, 16/17 (1-4) oval, short duct | 15 | 2.0 lanceolate |
| *K. sikorai* Michaelsen, 1901 | 205/10 | dark red | 4–10 | 9 | – | 13/14, 14/15, 15/16 (1-2) finger-shaped | 14, 15 | 3.5 lanceolate |
| *K. voimmanus* sp. nov. | 170/7 | slight red | 4–10 | 2 | 18–28 | 13/14, 14/15, 15/16 (2-3) oval, long duct | 15 | 2.3 lanceolate |

### Description

Holotype 90 mm in length, diameter after the clitellum 5 mm, segment number 194, tail missing. Paratypes 80–90 mm in length, 4–5 mm in diameter, segment number 180–195. Colour greyish, pigmentation lacking. Head zygolobous, segments 1–3 simple, 4–10 biannulate. Dorsal pores lacking. Setae small, *ab* and *cd* clearly observed from segment 2. Setal arrangement after clitellum aa:ab:bc:cd:dd = 22.5:1.5:13:1:20, dd = 0.27 U (Fig. 3A). Clitellum circular on ½18–27. Male pores ventral on 16.

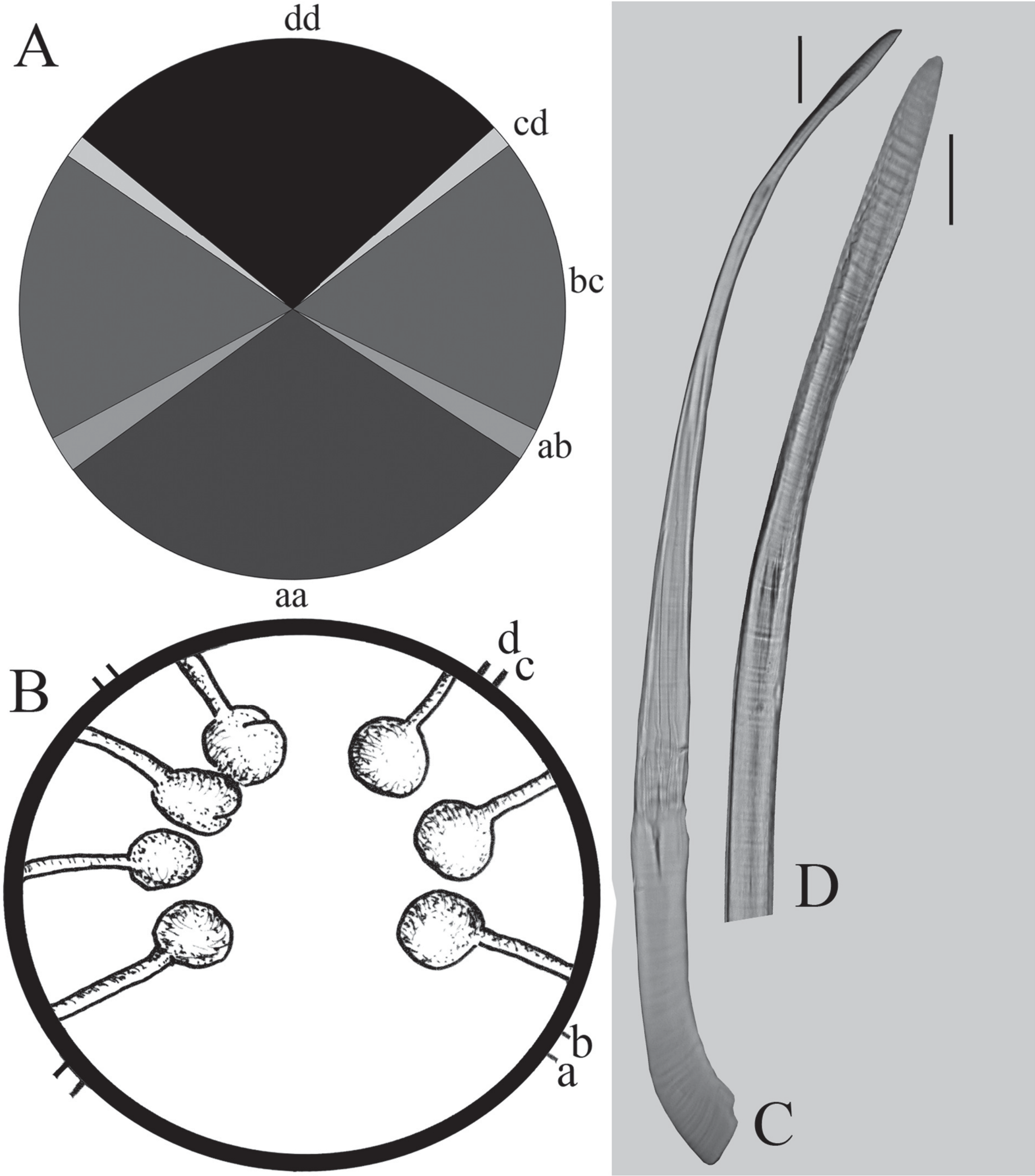


**Fig. 3.** *K. blancharti* sp. nov. **A**. Setal arrangement, a, b, c, d representing setal lines. **B**. Spermathecae (14/15) schematic representation. **C**. Genital seta. **D**. Tip of the genital seta. Scale bars = 0.1 mm.

**Table 3.** Average interspecific (below the diagonal) and intra specific (bold, in the diagonal) K2P genetic distances of the species of *Kynotus* Michaelsen, 1891 analysed.

| | *K. ank.* | *K. alao.* | *K. blan.* | *K. mich.* | *K. min.* | *K. prob.* | *K. sih.* | *K. voi.* |
|---|---|---|---|---|---|---|---|---|
| *K. ankisiranus* | – | | | | | | | |
| *K. alaotranus* | 0.206 | **0.0** | | | | | | |
| *K. blancharti* | 0.217 | 0.197 | **0.003** | | | | | |
| *K. michaelseni* | 0.264 | 0.255 | 0.256 | **0.003** | | | | |
| *K. minutus* | 0.237 | 0.221 | 0.224 | 0.252 | **0.0** | | | |
| *K. proboscideus* | 0.256 | 0.262 | 0.235 | 0.282 | 0.242 | **0.005** | | |
| *K. sihanakus* | 0.237 | 0.243 | 0.233 | 0.261 | 0.215 | 0.241 | **0.004** | |
| *K. voimmanus* | 0.215 | 0.143 | 0.219 | 0.265 | 0.262 | 0.264 | 0.245 | **0.001** |

Female pores not seen. Spermathecal pores in 13/14(2–3), 14/15(3–4), 15/16(2–3) irregularly arranged from *bc* to above *cd*. Genital setal pores in variable position between *ab* and *cd* on 14, 15. Nephridial pores between setal lines *ab* and *cd*.

### Internal characters

Large muscular gizzard in 5. Septa 7/8–9/10 moderately thickened, 5/6–6/7, 10/11 slightly strengthened. Calciferous glands, lamellae and typhlosolis lacking. Dorsal blood vessel simple throughout, last pair of hearts in 11. Excretory system holoic, vesiculate. Two pairs of testes and sperm funnels in 10, 11 enclosed in large peri-oesophageal testis sacs. Seminal vesicles lacking. Ovaries in 13. A pair of large, oval copulatory chambers occupying the ventral space of segments 16–18. Each copulatory chamber bearing an irregular prostate-like gland (pseudoprostate) reaching segment 26. Spermathecae oval-shaped, with long and thin duct attached at 13/14(2–3), 14/15(3–4), 15/16(2–3), arranged from *ab* to above *cd* (Fig. 3B). Two pairs of genital setal glands present in segment 14, 15 above *ab* and around *cd*. The genital setae are slightly curved, with elongated spoon-shaped apex, length 1.8–1.9 mm, diameter just under the apex 0.05 mm, ornamentation with dense short serrations (Fig. 3C–D).

### Remarks

*K. blancharti* sp. nov. was previously classified as an unpigmented *K. alaotranus* (Csuzdi *et al.* 2012). Besides the lack of red pigments, the penial setae are also somewhat different. Instead of being lanceolate as in *alaotranus,* in the new species the tip is flanked with slightly dentate rims making the seta more spoon-shaped. *K. blancharti* sp. nov. is clearly different from all the examined *alaotranus* group species by its COI barcode as well (Table 3, Fig. 5).

## ***Kynotus voimmanus*** sp. nov.

urn:lsid:zoobank.org:act:8998C949-547B-454E-86E5-50B2EF4DBCB0

Figs 4–5

### Diagnosis

Length 145–170 mm, diameter 6–7 mm. Colour greyish with slight reddish hints at the head on dorsum. Segments 1–3 simple, 4–10 biannulate. Setae appear from segment 2 and clearly visible; male pores ventral on 16. Clitellum circular on 18–½28, 28.Three rows of spermathecae of oval shape with long and thin duct in 13/14, 14/15, 15/16. Septa 6/7–8/9 strongly thickened. Genital setal glands in 15.

### Etymology

The species epithet refers to the type locality Voimma Community Park.

### Material examined

#### Holotype

MADAGASCAR: clitellate, Ambalavao District, Sendreisoa, Namoly, Mt Ankisira National Park, in primary forest, 18°55′38″ S, 48°24′50″ E, 900 m a.s.l., 2 Mar. 2015. Y. Hong and M. Razafindrakoto leg. (HNHM/AF5660).

#### Paratypes

MADAGASCAR: 1 clitellate, 9 aclitellate adults (HNHM/AF5661); 2 clitellate, 5 aclitellate adults (HNHM/AF5662); 3 aclitellate (CHBNU MD-10-AL4); 7 aclitellate (CHBNU MD-10-AL8); 9 aclitellate (CHBNU MD-10-C). Locality and date same as for holotype

### Description

Holotype clitellate, 100 mm in length, diameter after clitellum 7 mm, segment number 115, tail missing. Paratypes 145–170 mm in length, 6–7 mm in diameter, segment number 234–268. Colour greyish, with slight reddish pigmentation at head on dorsum. Head zygolobous, segments 1–3 simple, 4–10 biannulate.

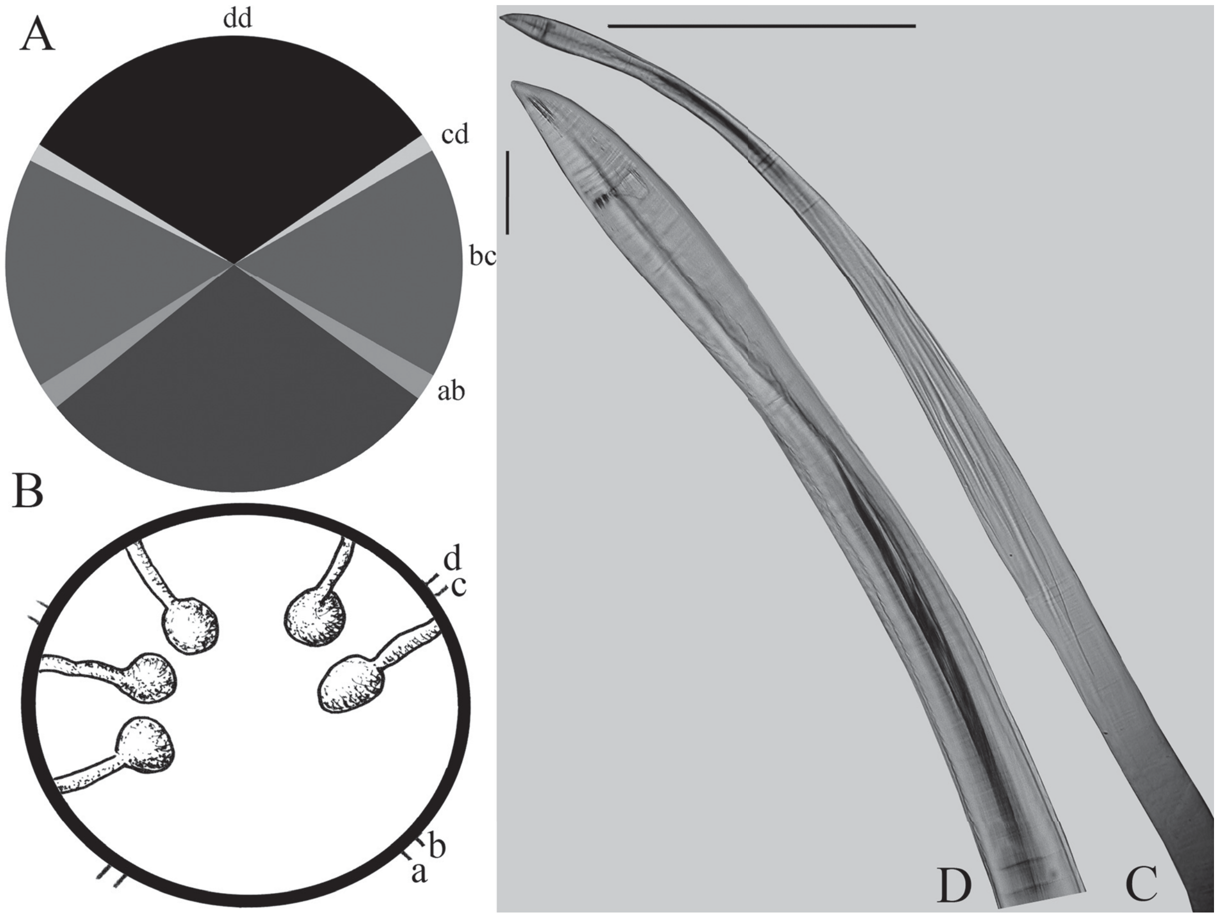


**Fig. 4.** *Kynotus voimmanus* sp. nov. **A**. Setal arrangement, a, b, c, d representing setal lines. **B**. Spermathecae (13/14) schematic representation. **C**. Genital seta. **D**. Tip of the genital seta. Scale bars: C = 0.6 mm; D = 0.1 mm.

Dorsal pores lacking. Setae small, *ab* and *cd* clearly observed from segment 2. Setal arrangement after clitellum aa:ab:bc:cd:dd = 23:1.5:13:1:25, dd = 0.32 U (Fig. 4A). Clitellum annular on segments 18–½ 28, 28. Male pores ventral on 16. Female pores not seen. Spermathecal pores in 13/14(2–3), 14/15(2–3), 15/16(2) irregularly arranged from *bc* to above *cd*. Genital setal pores in variable position between *ab* and *cd* on 15.

### Internal characters

Large muscular gizzard in 5. Septa 6/7–8/9 strongly thickened, 5/6, 9/10 less so. Calciferous glands, lamellae and typhlosolis lacking. Dorsal blood vessel simple throughout, last pair of hearts in 11. Excretory system holoic, vesiculate. Two pairs of testes and sperm funnels in 10, 11 enclosed in large peri-oesophageal testis sacs. Seminal vesicles lacking. Ovaries in 13. A pair of large, oval copulatory chambers occupying ventral space of segments 16–19. Each copulatory chamber bearing an irregular prostate-like gland (pseudoprostate) reaching segment 22. Spermathecae oval-shaped, with long and thin duct attached at 13/14(2–3), 14/15(2–3), 15/16(2) arranged from above *ab* to between *cd* and M (Fig. 4B). Single pair of genital setal glands present in segment 15 above *ab* and around *cd*. Genital setae slightly curved, with lanceolate apex, length 2.25–2.3 mm, diameter just under the apex 0.07 mm; ornamentation dense short serrations (Fig. 4C–D).

### Remarks

This new species, with biannulate segments between 4 and 10 belongs to the *alaotranus* group (Table 2). In the *alaotranus* group there are only two other species possessing a single pair of genital setal glands: *K. rosae* Cognetti, 1906 and *K. minutus* Csuzdi, Razafindrakoto & Blanchart, 2012. *K. voimmanus* sp. nov. differs from *K. rosae* by its smaller number of spermathecal segments (13/14, 14/15, 15/16, 16/17 vs 13/14, 14/15, 15/16) and also in the structure of spermathecae (*K. rosae* possesses oval spermathecae with very short ducts, *K. voimmanus* sp. nov. possesses oval spermathecae with thin and very long ducts, 2–3 times longer that the ampoule). The new species differs from *K. minutus* in its size and

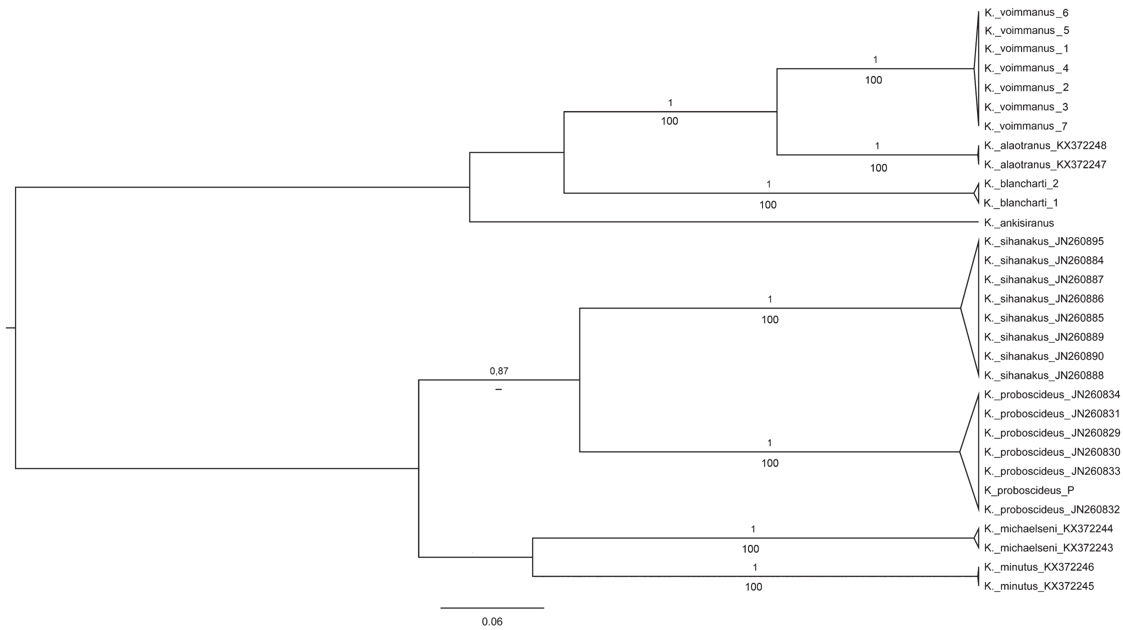


**Fig. 5.** Bayesian inference tree of some species of *Kynotus* Michaelsen, 1891 using COI sequences. Numbers above branches indicate Bayesian posterior probabilities, below branches ML bootstrap supports. Values lower than 80% are not shown.

coloration, and furthermore in the position of the clitellum (18–26 vs 18–½ 28), as well as the shape of the spermathecae (short vs long spermathecal duct). *K. voimmanus* sp. nov. is clearly different from all the examined *alaotranus* group species by its COI barcode as well (Table 3, Fig. 5).

## Discussion

Before launching the project "Changement global et diversité de la macrofaune du sol à Madagascar" (Global Change and Soil Macrofauna Diversity in Madagascar) in 2008, only 13 species were known from the Malagasy endemic earthworm family Kynotidae. Our activity in the recent years has resulted in the description of a further 9 *Kynotus* species new to science (Razafindrakito *et al.* 2011; Csuzdi *et al.* 2012; Razafindrakoto *et al.* 2017), increasing the number of valid species in the family to 22. It is worth noting that, due to difficulties accessing the rural sites in the rainy season (which is the most suitable for earthworm collections), larger regions of the country, especially in the western and southern part of the island, still remain unexplored. It is also important to highlight that several new species were discovered in highly disturbed habitats such as around Sambaina (*K. blancharti* sp. nov. in secondary bushland, with scattered *Eucalyptus* L'Hér.) or Antsirabe (*K. parvus* Csuzdi, Razafindrakoto & Blanchart, 2012 in secondary *Aristida* L. grassland). This indicates that not only the very few pristine regions should be sampled, but also disturbed areas, where native, still undescribed, species occur.

Our barcoding results clearly separated all the newly described species (Fig. 5), supporting the conclusion that this method is quite powerful in recognizing earthworm species, as demonstrated recently by Decaëns *et al.* (2016), Chang & James (2011) and Chang *et al*. (2009). The COI tree revealed that the *alaotranus* group's species (except *K. michaelseni*) form the second largest clade and show a clear south-north geographical distribution pattern. The most basal species of this clade, *K. ankisiranus* sp. nov., possesses the southernmost distribution and the terminal species pair *K. voimmanus* sp. nov. – *K. alaotranus* is distributed in the north (Figs 1, 5). However, it seems that COI alone is not suitable for inferring the phylogenetic relationships inside the genus. All deeper branches revealed showed no statistical support.

Kimura 2-parameter genetic distances (Kimura 1980) showed intraspecific and interspecific separation values ranging between 0%–0.5 % and 14.3%–28.2% respectively (Table 3). This data fits well with that observed by Decaëns *et al*. (2016) reporting 0%–5.25% (mean 1.27%) intra-MOTU divergence and (13.68%–31.01% (mean 23.33%) inter-MOTU divergence for earthworms collected in French Guiana.

## Acknowledgements

Our thanks are due to S.W. James (Iowa, USA) who provided his unpublished barcodes for several species of *Kynotus*. We are grateful to J. Philbert, J. Randriatsarafara, E.J. Razainjara, I.M. Mamoude, J.W. Maminiaina and M. Rakotonandrasana for their generous help during the fieldwork. Emma Sherlock (NHM, London) is thanked for polishing the English of the text. This work was supported by the Faune-M project of the Institut Français de la Biodiversité (IFB) and by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2015R1D1A2A01057305).

## References

Chang Ch.-H. & James S.W. 2011. A critique of earthworm molecular phylogenetics. *Pedobiologia* 54S: S3–S9. https://doi.org/10.1016/j.pedobi.2011.07.015

Chang Ch.-H., Rougerie R. & Chen J.-H. 2009. Identifying earthworms through DNA barcodes: Pitfalls and promise. *Pedobiologia* 52: 171–180. https://doi.org/10.1016/j.pedobi.2008.08.002

Csuzdi C., Razafindrakoto M. & Blanchart E. 2012. New and little known earthworm species from Central Madagascar (Oligochaeta: Kynotidae). *Zootaxa* 3578: 36–42.

Decaëns T., Porco D., James S.W., Brown G.G., Chassany V., Dubs F., Dupont L., Lapied E., Rougerie R., Rossi J.-P. & Roy V. 2016. DNA barcoding reveals diversity patterns of earthworm communities in remote tropical forests of French Guiana. *Soil Biology & Biochemistry* 92: 171–183. https://doi.org/10.1016/j.soilbio.2015.10.009

Drummond A.J., Suchard M.A., Xie D. & Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Goodman S.M. & Benstead J.P. 2003. *The Natural History of Madagascar.* University of Chicago Press, Chicago and London.

Keller C. 1887. *Reisebilder aus Ostafrika und Madagaskar*. C.F. Winter Press, Leipzig.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

Michaelsen W. 1891. Terricolen der Berliner Zoologischen Sammlung I. *Archiv für Naturgeschichte* 57: 207–228.

Michaelsen W. 1897. Die Terricolen des Madagassischen Inselgebiets. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 21: 217–252.

Michaelsen W. 1907. Oligochaeten von Madagaskar, den Comoren und anderen Inseln des westlichen Indischen Ozeans. *Reise in Ostafrika von A. Voeltzkow in den Jahren 1903–05, Wissenschaftliche Ergebnisse* 2: 41–50.

Michaelsen W. 1931. Ausländische opisthopore Oligochäten. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 61: 523–578.

Myers N., Mittelmeier R.A., Mittelmeier C.G., da Fonseca G.B.A. & Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. https://doi.org/10.1038/35002501

Rambaut A. 2014. *FigTree v.1.4.2: Tree Drawing Tool*. Available from http://tree.bio.ed.ac.uk/software/figtree [accessed 16 Jun. 2015].

Raw F. 1959. Estimating earthworm populations by using formalin. *Nature* 184: 1661–1662. https://doi.org/10.1038/1841661a0

Razafindrakoto M., Csuzdi C., Rakotofiringa S. & Blanchart E. 2010. New records of earthworms (Oligochaeta) from Madagascar. *Opuscula Zoologica (Budapest)* 41: 231–236.

Razafindrakoto M., Csuzdi C. & Blanchart E. 2011. New and little known giant earthworms from Madagascar (Oligochaeta: Kynotidae). *African Invertebrates* 52: 285–294. https://doi.org/10.5733/afin.052.0205

Razafindrakoto M., Csuzdi C., James S.W. & Blanchart E. 2017. New earthworms from Madagascar with key to the *Kynotus* species (Oligochaeta: Kynotidae). *Zoologischer Anzeiger* 268: 126–135. https://doi.org/10.1016/j.jcz.2016.08.001

Rosa D. 1892. *Kynotus michaelseni* n. sp. Contributo alla morfologia dei geoscolicidi. *Bollettino dei Musei di Zoologia ed Anatomia comparata della Reale Università di Torino* 7 (119): 1–9.

Szederjesi T. & Csuzdi C. 2015. A new earthworm species and new records from Albania with remarks on the molecular phylogenetic relationships of the *Eisenia* species of the Balkan Peninsula (Oligochaeta: Lumbricidae). *North-Western Journal of Zoology* 11 (1): 110–116.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson J.D., Higgins D.G. & Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. https://doi.org/10.1093/nar/22.22.4673

*Manuscript received: 7 July 2016*
*Manuscript accepted: 26 September 2016*
*Published on: 13 July 2017*
*Topic editor: Rudy Jocqué*
*Desk editor: Kristiaan Hoedemakers*

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Botanic Garden Meise, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Natural History Museum, London, United Kingdom; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain.