Notes on the earthworm species,
Metaphire anomala (Michaelsen, 1907) (Clitellata, Megascolecidae)
in Southern Vietnam, with descriptions of two new species

Tung T. NGUYEN 1,*, Dang H. LAM 2 & Anh D. NGUYEN 3

1,2 Department of Biology, School of Education, Can Tho University, Can Tho City, Vietnam.
3 Department of Soil Ecology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18, Hoangquocviet, Caugiay, Hanoi, Vietnam.
3 Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18, Hoangquocviet, Caugiay, Hanoi, Vietnam.

* Corresponding author: thanhtung@ctu.edu.vn
1 Email: lamhaidangct@gmail.com
3 Email: ducanh410@yahoo.com

Abstract. Integrative taxonomy was employed to exploit the differences between the known Metaphire anomala (Michaelsen, 1907) and other specimens collected in Vietnam. The results brought to light two new species, namely Metaphire iranomala sp. nov. and Metaphire decemtheca sp. nov. The former is easily recognised by having male pores on xix and four pairs of spermathecal pores on 5/6/7/8/9 while the latter is distinguished by having five pairs of spermathecal pores on 4/5/6/7/8/9. The K2P distances of the fragment of the cytochrome c oxidase subunit I (COI) gene are 13.1% between M. iranomala sp. nov. and M. anomala (Michaelsen, 1907) and 18% between M. decemtheca sp. nov. and Metaphire grandiverticulata Nguyen & Lam, 2017. The intraspecific divergences are 1.5%–10.6% for M. iranomala sp. nov. and 2.1%–11.4% for M. decemtheca sp. nov.

Keywords. Metaphire, taxonomy, biodiversity, COI genetic divergence, Vietnam.

Nguyen T.T., Lam D.H. & Nguyen A.D. 2021. Notes on the earthworm species, Metaphire anomala (Michaelsen, 1907) (Clitellata, Megascolecidae) in Southern Vietnam, with descriptions of two new species. European Journal of Taxonomy 746: 94–111. https://doi.org/10.5852/ejt.2021.746.1321

Introduction

Michaelsen (1907) described a new species, namely Pheretima anomala, from only eight specimens collected from the Botanical Gardens of Sibpur, Calcutta (India). The species is very specific without spermathecae and male pores on segment xx. Later, Gates (1925) commented on the species based on specimens collected from Rangoon (Myanmar). His specimens were slightly different from the
Gates (1925) also described a new species, *P. insolita*. The species contained six types (A, B, C, D, E, F) distinguishable in length, diameter, number of segments, location and numbers of papillae in the male region. *Pheretima insolita* was very similar to *P. anomala* in terms of traces of male pores on xx, but differs in having spermathecal pores on 5/6/7/8 and variations of papillae in the male region. Stephenson (1929) discussed all types of *P. insolita* and *P. anomala*. He commented that the original specimens (*P. anomala*) possibly acted as functional males while the functional females would be *P. insolita*. Stephenson (1929), therefore, suggested to unite all the forms of *P. anomala* and *P. insolita* as one species, *P. anomala*. Michaelsen (1907)’s specimens would constitute the form a typical.

*M. anomala* (Michaelsen, 1907) is widely distributed in India, Myanmar, Thailand, Laos, Yunnan (China) and Northern Vietnam (Michaelsen 1907; Gates 1925, 1972; Stephenson 1929, Nguyen et al. 2016; Yuan et al. 2019). However, *M. anomala* is highly variable due to parthenogenetic degeneration, and often specimens without spermathecae or without male pores are dominant (Gates 1925, 1972; Stephenson 1929). If present, the spermathecal pores are in 5/6/7/8 and/or male pores are in xx.

The species *Metaphire anomala* has previously been reported in Southern Vietnam as ‘so-called’ *Pheretima anomala* Michaelsen, 1907. It is widely distributed in Southern Vietnam: Ba Ria-Vung Tau (Con Lon Isl.), Dong Nai (Cat Tien), Kien Giang (Lai Son Isl., Phu Quoc Isl.), An Giang (Nhon Mts, Phu Cuong Mts, Ba Doi Mts, Cam Mts, To Mts), Dong Nai (Vinh Cuu, Xuan Loc, Dinh Quan, Trang Bom, Cam My, Long Thanh, Cat Tien NP), Tay Ninh (Ba Den Mts) (Thai et al. 2004; Nguyen 2014, Nguyen et al. 2016, 2017a, 2017b, 2019, 2020).

During our re-examination of previously and newly collected specimens housed at Can Tho University, we found that the specimens collected from Southern Vietnam were very different from their original description (Michaelsen 1907), Gates (1925, 1972) and recent re-description (Bantaogwon et al. 2011). An integrative approach combining morphological and molecular data was used to confirm the taxonomic status of the Vietnamese specimens. Two new species were discovered and named in this study.

**Material and methods**

Earthworms were manually searched and previously collected in Vietnam. After collection, specimens were cleaned with tap water, killed in formalin 2%, temporarily fixed in formalin 4% for 12 hours, then transferred to new formalin 4% for long-term preservation for morphological study. Specimens for molecular study were preserved in ethanol 90%. Specimens including holotypes and paratypes were deposited in Laboratory of Zoology, Department of Biology, CTU. Some were shared with the Department of Soil Ecology, Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

**Morphological examination**

Material was examined under a Motic Digital microscope (model: DMI43-FBGG-C) and dissected from the dorsal side for internal observation. Transverse body sections were processed using the classical method of haematoxylin and eosin. Selected segments were cleaned and dehydrated using graded ethanol concentrations. Segments were treated with paraffin, then cut using a microtome Sakura AccuSRM 200CW. The cut sections were stained using haematoxylin and eosin Y (Feldman & Wolfe 2014), then transferred onto glass slides.
Colour images were taken using a camera attached directly to the microscope. Line drawings and colour images were improved and grouped into plates using Photoshop CS6.

**DNA extraction, PCR reaction and phylogenetic analysis**

Total genomic DNA was extracted from the body walls of segments 30–35 using the DNAeasy Blood & Tissue Kit (QiagenTM). A 680bp fragment of the cytochrome c oxidase subunit I (COI) mitochondrial gene was amplified using the polymerase chain reaction (PCR) method with the universal primer set (HCO-2190/COI-E and LCO-1498/LCO-1498m) (Folmer et al. 1994). The PCR conditions for amplification were as follows: an initial denaturation at 95°C for 2 min followed by 36 cycles of 95°C for 20 s, 42°C for 45 s, and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were checked for potentially successful amplification using electrophoresis in 1% Agarose-TBE 1X. Successfully amplified PCR products were purified and sequenced at FishBase, Inc. (Malaysia) on an Applied Biosystems automatic sequencer (ABI3130 XL). Each successful sequence was manually checked using BioEdit ver. 7.1 (Hall 1999) and confirmed using BLASTN 2.6.0+ searches (Zhang et al. 2000). All confirmed sequences were aligned with MUSCLE (Edgar 2004). All nucleotide sequences have been deposited in GenBank (NCBI).

The dataset contained COI sequences from two new species and five other *Metaphire* sequences (*M. anomala, M. bahlil* [Gates, 1945], *M. grandiverticulata* Nguyen & Lam, 2017, *M. megascolioides* [Oto & Hatai, 1899] and *M. penguana* [Rosa, 1890]) (Table 1). *Polypheretima elongata* (Perrier, 1872) was selected as an outgroup, as *Polypheretima* is a sister genus of *Metaphire* (see Sims & Easton 1972). The genetic distance was calculated using the Kimura 2 parameter (K2P) model performed in MEGA ver. 7.0 with 1000 bootstrap replicates (Kumar et al. 2016).

ModelFinder performed in IQ-TREE ver. 1.6.12 was applied to find the best-fit maximum likelihood site substitution model (Kalyaanamoorthy et al. 2017). The substitution model, TIM2+F+I+G4, was selected using a likelihood ratio test with –lnL = 3509.852 and BIC score = 7513.414. A maximum likelihood bootstrap analysis was conducted using IQ-TREE ver. 2.1.1 with 1000 pseudoreplicates (Minh et al. 2020). A Bayesian inference (BI) tree was inferred using MrBayes ver. 3.2 (Ronquist et al. 2012) with 10 million generations, heating parameter of 0.06, burnin of 10% and sampling every 1000 generations.

**Abbreviations**

| Abbreviation | Definition                  |
|--------------|-----------------------------|
| ag           | accessory gland             |
| amp          | ampulla                     |
| B            | Bayesian Inference          |
| CTU          | Can Tho University          |
| dv           | diverticulum                |
| IEBR         | Institute of Ecology and Biological Resources |
| gm           | genital marking             |
| ML           | Maximum Likelihood          |
| mp           | male pore                   |
| ov           | ovary                       |
| os           | ovary sac                   |
| sp           | spermathecal pore           |
| sv           | seminal vesicle             |
| ts           | testis sac                  |
| No. | Species                        | Locality                        | Specimen voucher | Accession number |
|-----|--------------------------------|---------------------------------|------------------|------------------|
| 1   | Metaphire bahli (Gates, 1945)   | Ba Ria-Vung Tau Prov.           | CTU-EW.004.12    | MW076178         |
| 2   | Metaphire bahli (Gates, 1945)   | Binh Phuoc Prov.               | CTU-EW.004.23    | MW076179         |
| 3   | Metaphire bahli (Gates, 1945)   | Binh Duong Prov.               | CTU-EW.004.26    | MW076180         |
| 4   | Metaphire bahli (Gates, 1945)   | Ho Chi Minh city               | CTU-EW.004.41    | MW076181         |
| 5   | Metaphire bahli (Gates, 1945)   | Tay Ninh Prov.                 | CTU-EW.004.42    | MW076182         |
| 6   | Metaphire bahli (Gates, 1945)   | Dong Nai Prov.                 | CTU-EW.004.43    | MW076183         |
| 7   | Metaphire bahli (Gates, 1945)   | Ba Ria-Vung Tau Prov.          | CTU-EW.004.44    | MW076184         |
| 8   | Metaphire peguana (Rosa, 1890)  | Ba Ria-Vung Tau Prov.          | CTU-EW.009.06    | MW076185         |
| 9   | Metaphire peguana (Rosa, 1890)  | Binh Duong Prov.               | CTU-EW.009.07    | MW076186         |
| 10  | Metaphire peguana (Rosa, 1890)  | Ho Chi Minh city               | CTU-EW.009.13    | MW076188         |
| 11  | Metaphire peguana (Rosa, 1890)  | Dong Nai Prov.                 | CTU-EW.009.15    | MW076189         |
| 12  | Metaphire peguana (Rosa, 1890)  | Ba Ria-Vung Tau Prov.          | CTU-EW.009.16    | MW076190         |
| 13  | Metaphire iranomala sp. nov.   | Tay Ninh Prov.                 | CTU-EW.020.16    | MW076191         |
| 14  | Metaphire iranomala sp. nov.   | Chau Duc Distr., Ba Ria-Vung Tau Prov. | CTU-EW.020.19   | MW076193         |
| 15  | Metaphire iranomala sp. nov.   | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.020.20   | MW076194         |
| 16  | Metaphire iranomala sp. nov.   | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.020.20   | MW076195         |
| 17  | Metaphire iranomala sp. nov.   | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.020.20b  | MW076196         |
| 18  | Metaphire iranomala sp. nov.   | Vinh Cuu Distr., Dong Nai Prov. | CTU-EW.020.18    | MW076192         |
| 19  | Metaphire iranomala sp. nov.   | Kien Giang Prov.               | CTU-EW.020.23    | MW076196         |
| 20  | Metaphire grandiverticulata    | Ho Chi Minh city               | CTU-EW.089.4a    | MW076200         |
| 21  | Metaphire grandiverticulata    | Ho Chi Minh city               | CTU-EW.089.4a1   | MW076199         |
| 22  | Metaphire decemtheca sp. nov.  | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.183.p02  | MW076203         |
| 23  | Metaphire decemtheca sp. nov.  | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.183.03   | MW076202         |
| 24  | Metaphire decemtheca sp. nov.  | Con Son isl., Ba Ria-Vung Tau Prov. | CTU-EW.1183.06  | MW076201         |
| 25  | Polypheretima elongata         | Ho Chi Minh city               | CTU-EW.026.02    | MW076197         |
| 26  | Polypheretima elongata         | Ho Chi Minh city               | CTU-EW.026.02b   | MW076198         |
| 27  | Metaphire bahli (Gates, 1945)   | Prasankok et al. (2013)         |                  | KC404844         |
| 28  | Metaphire bahli (Gates, 1945)   | Blakemore (2016a)              |                  | KT626598         |
| 29  | Metaphire peguana (Rosa, 1890)  | Prasankok et al. (2013)         |                  | KC404831         |
| 30  | Metaphire peguana (Rosa, 1890)  | Jeraththikul et al. (2017)      |                  | KU565288         |
| 31  | Metaphire anomala (Michaelsen, 1907) | Bantaowong et al. (2011)   |                  | KC565251         |
| 32  | Metaphire anomala (Michaelsen, 1907) | Bantaowong et al. (2011)   |                  | KC565252         |
| 33  | Metaphire anomala (Michaelsen, 1907) | Bantaowong et al. (2011)   |                  | KC565253         |
| 34  | Metaphire anomala (Michaelsen, 1907) | Bantaowong et al. (2011)   |                  | KC565254         |
| 35  | Metaphire megascolidioides      | Blakemore (2016b)              |                  |                |
| 36  | Metaphire megascolidioides      | Minanuy et al. (2009)          |                  | AB482107         |

Table 1. Specimen vouchers and accession numbers deposited in GenBank

NGUYEN T.T. et al., Two new Metaphire species from Southern Vietnam
Results

Descriptions of new species

Class Clitellata Michaelsen, 1919
Subclass Oligochaeta Grube, 1850
Order Opisthopora Michaelsen, 1929
Suborder Crassiclitellata Jamieson, 1988
Family Megascolecidae Rosa, 1891
Genus Metaphire Sims & Easton, 1972

Metaphire iranomala sp. nov.  
urn:lsid:zoobank.org:act:2ECB8639-C770-4828-92D9-055E0778E422  
Figs 1–3, Tables 2–3

Pheretima anomala (non Pheretima anomalana Michaelsen, 1907) – Thai et al. 2004: 758. – Nguyen 2014: 108. – Nguyen et al. 2017a: 893, fig. 7; 2017b: 98, fig. 3; Nguyen et al. 2019: 120, fig. 3. Metaphire anomala – Nguyen et al. 2016: 50.

Diagnosis

Medium-sized worm, length 157–228 mm, diameter 6.5–8.0 mm, segments 85–145. Prostomium epilobous. Clitellum annular, within xiv–xvi. First dorsal pore in 12/13. Four pairs of spermathecal pores on ventrolateral intersegments 5/6/7/8/9. Male pores on the setal ring of segment xix; copulatory pouches present. Genital markings absent. Holandric. Testis sacs connected. Intestinal caeca simple. Septa 8/9/10 absent.

Etymology

The epithet ‘iranomala’ is formed by the prefix ‘ir’ and ‘anomala’ to emphasise the wrong name ‘anomala’ recorded in Vietnam.

Material examined

Holotype

VIETNAM • mature spec.; Ba Ria-Vung Tau Province, Con Dao National Park; 8°42′12″N, 106°35′41″E; 120 m a.s.l.; 19 Oct. 2019; Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; natural forest; CTU-EW.020.h01.

Paratypes

VIETNAM • 9 matures; same collection data as for holotype; CTU-EW.020.p02.

Other material

VIETNAM – Ba Ria-Vung Tau Province • 16 matures, same collection data as for holotype; CTU-EW.020.03 • 10 matures; Con Son Island, 8°41′59″N, 106°36′54″E; 10 m a.s.l.; 18 Oct. 2019; Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; residential gardens; CTU-EW.020.04 • 33 matures; Con Son Island, 8°39′53″N, 106°34′00″E; 20 m a.s.l.; 19 Oct. 2019; Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; natural forest; CTU-EW.020.05. – Kien Giang Province • 1 mature; Da Dung mountain, 10°25′07″N, 104°28′46″E; Nov. 2010; Nguyen Thanh Tung leg.; natural forest, CTU-EWDNA.020.23 • 49 matures; La Son Island, 09°48′01″N, 104°39′18″E; Nov. 2013; Trinh Thi Kim Binh leg.; natural forest; CTU-EW.020.11. – An Giang Province • 57 matures; Cam mountain, 10°30′36″N, 105°00′09″E; Nov. 2010; Nguyen Thanh Tung leg.; natural forest; CTU-EW.020.12 • 2 matures; Cam mountain, 10°30′36″N, 105°00′09″E; Nov. 2010; Nguyen Thanh Tung leg.; natural
NGUYEN T.T. et al., Two new Metaphire species from Southern Vietnam

Description
Body cylindrical, large-medium size, length 157–228 mm, diameter 6.5–8.0 mm, segments 85–145, weight 3.0–8.4 gr. Dorsum slightly dark grey, ventrum paler. Prostomium epilobous. First dorsal pore in 12/13. Perichaetine, setae at pre-clitellar segments stouter and sparser than that at post-clitellar segments, setal numbers: 55–62 in viii, 65–71 in xxx, 10–16 between male pores on xix. Setal distance: aa > ab, zz > zy. Clitellum annular, within xiv–xvi, darkish brown, without dorsal pores and setae. Female pore single, mid-ventral in xiv.

Four pairs of spermathecal pores on ventrolateral intersegments 5/6/7/8/9. Ventral distance between spermathecal pores ca 0.27–0.3 body circumference. Male pores on copulatory pouches in xix; ventral distance between male pores ca 0.2–0.3 body circumference. Genital markings absent in both spermathecal and male pores region.

Septa 5/6/7/8 thickened, 8/9/10 absent, 10/11/12 thin. Oesophageal gizzard between 7/8 and 10/11. Intestine origin at xv, caeca simple, short within xxvii–xxiv. Last hearts in xiii. Pharyngeal micronephridia developed in 5/6/7. Lymph glands absent. Typhlosole simple, lamelliform.

Spermathecae paired in vi–ix. Spermathecal ampulla large, mango-shaped, duct about a quarter of ampulla length. Diverticula attached to the base of ampulla ducts; distal part strongly coiled, swollen into coiled sinusoidal seminal chambers. Spermathecal ducts without nephridia. Accessory glands absent in the spermathecal region.

Holandric. Testis sacs in x and xi, connected ventrally. Seminal vesicles well developed in xi and xii. Ovaries on septum 12/13 posteriorly. Prostate glands, deeply lobuled, paired in xvii–xxi; prostatic ducts C-shaped. Accessory glands massed, covered the copulatory pouches.

DNA barcode
COI barcode data (partial) is for the paratypes uploaded to GenBank under the accession numbers MW076191, MW076192, MW076193, MW076194, MW076195, and MW076196. The new species shares the identity of 88.13% and 88.33% with Metaphire anomala (KU262251, KUS65252, KUS65253, KUS65254).

Habitat
The species was found in leaf-litters or in the top-soil layer, especially in moist places (near streams) or in rocky holes with organic matter. Metaphire iranomala sp. nov. has a soft body, violet light skin when alive. Its moving behavior is similar to a caterpillar locomotion. The species was commonly located in hilly/mountainous areas, but occasionally found in deltas.

Variations
Metaphire iranomala sp. nov. has two slightly different morphological types. The first type is more likely to be distributed in islands or in coastal provinces in Vietnam; the other type is found in mainland provinces.
Fig. 1. *Metaphire iranomala* sp. nov., holotype (CTU-EW020 h01). A1, A2. Ventral view of male pores region. B. Lateral view of spermathecal pores region. C1, C2. Right spermathecae. D1, D2. Intestinal caecum. E. Testis sacs and seminal vesicles. F1, F2. Right prostatic gland. G. Copulatory pouch transverse body section. Scale bars = 1 mm.
Fig. 2. *Metaphire decemtheca* sp. nov., holotype (CTU-EW183.h01). A1, A2. Ventral view of male pore region. B1, B2. Right spermathecae. C1, C2. Intestinal caecum. D. Ventral view of spermathecal pore region. E1, E2. Lateral view of spermathecal pores region. F1, F2. Left prostatic gland. G. Testis sacs and seminal vesicles. H. Male pore region transverse body section. Scale bars = 1 mm.
Table 2. Comparison between *M. iranomala* sp. nov. and *M. anomala* (Michaelsen, 1907) from Thailand, Myanmar and India (its original description).

| Characteristics                  | Vietnam $^1$ | Thailand $^2$ | Myanmar $^3$ | Original description (India) $^4$ |
|----------------------------------|--------------|---------------|--------------|----------------------------------|
| Length                           | 157–228      | 134–154       | 116–200      | 80–90                            |
| Diameter                         | 6.5–8.0      | 4.6–6.7       | 3.0–7.0      | 5.0–5.5                          |
| Segment                          | 85–145       | 116–126       | 119–130      | 130                              |
| Spermathecal pores               | four pairs in 5/6/7/8/9 | 3 pairs in 5/6/7/8 | absent or 5/6/7/8 | absent |
| Pre-clitellar setae              | 46–53 (viii) | 84–96         | 90–96 (viii) | 70 (v)                           |
| Post-clitellar setae             | 62–71 (xxx)  | 58–67         | 61–70 (xx)  | 74 (xxv)                         |
| Male pores                       | xix          | xx            | xx           | xx                               |
| Ventral distance between male pores | 0.2–0.3     | 0.33          | –            | 0.17                             |
| Genital markings                 | absent       | 3–6 pairs in xvii–xix, xxi–xxiii or absent | present in xvii–xix and xxi–xxiv | 4 pairs in xviii–xix, xxi–xxii |
| Septum 8/9                       | absent       | absent        | present      | present                          |
| Testis sacs                      | connected    | separated     | separated    | separated                        |

$^1$ Freshly collected specimens; $^2$ Bantaowong *et al.* (2011); $^3$ Gates (1925, 1972) and Stephenson (1929); $^4$ Michaelsen (1907).

Table 3. Comparison between *M. iranomala* sp. nov., *M. megascolidioides* (Cognetti, 1908) and *M. isselii* (Goto & Hatai, 1899) sharing location of male pores not in segment xix.

| Characteristics                  | *M. iranomala* | *M. megascolidioides* $^1$ | *M. isselii* $^2$ |
|----------------------------------|----------------|----------------------------|-----------------|
| Length                           | 157–228        | 240                        | 155–180         |
| Diameter                         | 6.5–8.0        | 15                         | 10              |
| Segment                          | 85–145         | 118                        | 94–115          |
| Spermathecal pore                | lateroventral, 5/6/7/8/9 | lateroventral, 4/5/6/7/8/9 | ventral, 6/7/8/9 |
| Setal number between two male pores | 10–16         | 9                          | 0               |
| Ventral distance between two male pores | 0.2–0.3   | ?                          | < 0.25          |
| Genital markings in male region  | absent         | 3 pairs in xvii, xviii and xx | 2 pairs in 18/19 and 19/20 |
| Septum 8/9                       | absent         | absent                     | thin            |
| Intestinal caeca                 | simple         | manicate                   | simple          |
| Intestinal origin                | xv             | xv                         | xvi             |

$^1$ Goto & Hatai (1899) and Blakemore (2016b); $^2$ Cognetti (1908).

There are not many differences between the two types except the ventral distance between the male pores (0.2–0.22 vs 0.25–0.3). However, the COI genetic distance also distinguishes two types (see below).

**Remarks**

The new species has been previously identified as *Metaphire anomala*. It is widely distributed in Southern Vietnam (Thai *et al.* 2004, Nguyen 2014, Nguyen *et al.* 2017a, 2017b, 2019, 2020). However,
this species is very different from both the original description (Michaelsen 1907), and re-description of *M. anomala* from Myanmar (Gates 1925, 1972), Thailand (Bantaovong *et al.* 2011) in the position of male pores, number and position of spermathecal pores, genital markings in male and spermathecal regions and body size. These differences are summarised in Table 2.

A few *Metaphire* species have been known to exhibit male pores not in segment xviii. Only *M. anomala* has male pores on xx, whereas two other species, *M. isselii* Cognetti, 1908 and *M. megascolidioides* Goto & Hatai, 1899, have male pores on xix. The new species is similar to these two species by having male pores on xix, a first dorsal pore in 12/13 and the absence of genital markings in the spermathecal region. However, the new earthworm species is clearly distinguished by body size, the number and position of spermathecal pores, the morphology of its male region, the status of septum 8/9, and they type of intestinal caeca. The differences are summarised in Table 3.

**Metaphire decemtheca** sp. nov.  
urn:lsid:zoobank.org:act:F717F325-F04E-409A-93B1-0A3997E11E9E  
Figs 2–3

**Diagnosis**

Medium-sized worm. Prostomium epilobous. First dorsal pore in 12/13. Clitellum annular, within xiv–xvi. Five pairs of spermathecal pores on ventrolateral intersegments 4/5/6/7/8/9. Male pores on the setal ring of xviii; copulatory pouches present. A pair of genital markings in xviii. Holandric. Testis sacs connected. Intestinal caeca simple. Septum 8/9 absent or very thin, 9/10 absent.

**Etymology**

The epithet ‘*decemtheca*’ emphasises the number of spermathecae of the new species.

**Material examined**

**Holotype**  
VIETNAM • mature; Ba Ria-Vung Tau Province, Con Son Island, 8°42′17″N, 106°35′28″E; 50 m a.s.l.; 19 Oct. 2019; Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; natural forest; CTU-EW.183.h01.

**Paratypes**  
VIETNAM • 9 matures; same collection data as for holotype; CTU-EW.183.p02.

**Other material**  
VIETNAM • Ba Ria-Vung Tau Province • 38 matures, same collection data as for holotype; CTU-EW.183.03 • 23 matures, Con Son Island, 8°43′50″N, 106°37′31″E; 10 m a.s.l.; 18 Oct. 2019, Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; natural forest; CTU-EW.183.04 • 55 matures, Con Son Island, 8°43′59″N, 106°37′21″E; 10 m a.s.l.; 18 Oct. 2019, Nguyen Thanh Tung and Nguyen Thi Bao Ngoc leg.; residential gardens; CTU-EW.183.05.

**Description**

Body cylindrical, medium-sized worms. Length 64–185 mm, diameter ca 3.0–6.0 mm, segments 79–124, weight 1.1–3.9 gr. Dorsum slightly dark grey with reddish browns stripes or absent, ventrum paler. Prostomium epilobous. First dorsal pore in 12/13. Perichaetine, setae at pre-clitellar segments stouter and sparser than that at post-clitella segments; setal numbers: 38–51 in viii, 46–57 in xix, 8–13 between two male pores on xviii. Setal distance: aa > ab, zz = zy. Clitellum annular (3/4xiv–3/4xviii), slightly brown, without dorsal pores and setae. Female pore single, mid-ventral in xiv.
Five pairs of spermathecal pores on ventrolateral intersegments 4/5/6/7/8/9. Ventral distance between spermathecal pores ca 0.38–0.45 body circumference. Genital markings absent in spermathecal region. Male pores on line with setal ring in xviii; copulatory pouches present; ventral distance between male pores is 0.38 body circumference. One pair of flat ellipsoidal genital markings in xviii located next to male pores ventrally.

Septa 5/6/7/8 thick, 8/9 absent or membrane (if present), 9/10 absent, 10/11/12/13 thin. Oesophageal gizzard between 7/8 and 10/11. Intestine origin at xv; caeca simple, somewhat rudimentary lobulated dorsally, within xxvii–xxv. Last hearts in xiii. Pharyngeal micronephridia developed in 5/6/7. Lymph glands sac-like, present from 27/28. Typhlosole simple, lamelliform.

Spermathecal paired in v–ix. Spermathecal ampulla oval-shaped, ducts stout, short, about one-third of ampulla length. Diverticula shorter than ampulla, strongly and constrainedly waved, attached to the base of ampulla.

Fig. 3. The distribution of the two new species in Southern Vietnam.
ducts; seminal chamber small oval-shaped. Spermathecal ducts without nephridia. Accessory glands absent in the spermathecal region.

Holandric. Testis sacs in x and xi, connected ventrally. Seminal vesicles developed in xi and xii. Ovaries on septum 12/13 posteriorly. Ovisacs developed on 12/13, poorly developed on 13/14. Prostate glands deeply lobulated within xvi-xx; duct S-shaped, enlarged basally. Accessory glands sessile massed corresponding to external genital markings.

DNA barcode

COI barcode data (partial) is for the paratypes uploaded to GenBank under the accession numbers MW076201, MW076202 and MW076203. The new species shares the identity of 85.8% and 86.1% with *Amynthas* sp. (KT252973, KT205464).

Habitat

The species was found in the leaf litter of soil layer at a depth of 0–10 cm, near streams, and scattered on Con Son Island.

Variations

There are two morphological types found on Con Son Island. The first type was found in natural forests, with the presence of reddish-brown stripes, a bigger size (l = 133–170 mm, d = 5.0–6.5 mm), the first dorsal pore in 13/14 and ventral distance between spermathecal pores ca 0.38–0.4 body circumference. The second type found in residential gardens and forest edges exhibited no stripes, a smaller size (l = 64–122 mm, d = 3.0–3.4 mm), the first dorsal pore in 12/13 and ventral distance between spermathecal pores ca 0.43–0.45 body circumference. The COI genetic distance also showed the variations of 2.2–11.3%.

Remarks

A few *Metaphire* species have been described with five pairs of spermathecal pores, including *M. megascolidioides* and *M. fordi* Michaelsen, 1934. The new species is very similar to *M. megascolidioides* with its first dorsal pore in 12/13 and an absence of genital markings in the spermathecal region. However, *M. decemtheca* sp. nov. is distinguished by having male pores on xviii, a pair of genital markings in xviii, separate intestinal caeca and a small size (l = 64–185 mm, d = 3.0–6.0 mm) whereas *M. megascolidioides* has male pores on xix, three pairs of genital markings in xvii, xviii and xx, marinate intestinal caeca and a larger size (l = 240 mm, d = 15 mm).

The new species is also distinguished from *M. fordi* by having a larger size (l = 64–185 mm, d = 3.0–6.0 mm vs l = 50–64 mm, d = 1–1.5 mm) and the first dorsal pore in 12/13 (vs 11/12). Additionally, *M. decemtheca* sp. nov. has no genital markings in the spermathecal region, but does have one pair of large, round genital markings located next to the openings of copulatory pouches in xviii. By contrast, *M. fordi* has small circular papillae paired in both spermathecal and male regions.

In terms of the morphology of the male region, the new species is very similar to *M. grandiverticulata* Nguyen & Lam, 2017 by having a pair of large, round genital markings located next to the openings of its copulatory pouches and an absence of genital markings in the spermathecal region. However, *M. decemtheca* sp. nov. has five pairs of spermathecal pores on 4/5/6/7/8/9, a larger size (l = 64–185 mm, d = 3.0–6.0 mm), connected testis sacs, oval-shaped spermathecal ampulla, stout ducts, strongly and constrainedly waved diverticula and a small oval-shaped seminal chamber. Meanwhile, *M. grandiverticulata* has four pairs of spermathecal pores on 5/6/7/8/9, a smaller size (l = 69–92 mm, d = 2.3–2.7 mm), separated testis sacs, a small heart-shaped spermathecal ampulla, extremely short muscular ducts, stout and long unwaved diverticula and a bullet-shaped seminal chamber.
Table 4. The K2P intra- and interspecific genetic distances between Metaphire species using the 609 bp COI fragment. The intraspecific distances are in bold.

|                   | M. bahli | M. peguana | M. iranomala | M. grandiverticulata | M. decemtheca | M. anomala | M. megascolidioides | Po. elongata |
|-------------------|----------|------------|--------------|----------------------|----------------|------------|---------------------|-------------|
|                   | 0.008 ± 0.002 | 0.166 ± 0.017 | 0.168 ± 0.017 | 0.062 ± 0.007 | 0.023 ± 0.005 | 0.179 ± 0.017 | 0.199 ± 0.018 | 0.175 ± 0.016 |
|                   | 0.209 ± 0.019 | 0.235 ± 0.022 | 0.206 ± 0.019 | 0.183 ± 0.017 | 0.175 ± 0.014 | 0.202 ± 0.020 | 0.173 ± 0.017 | 0.194 ± 0.019 |
|                   | 0.224 ± 0.021 | 0.191 ± 0.018 | 0.181 ± 0.017 | 0.194 ± 0.020 | 0.166 ± 0.016 | 0.177 ± 0.018 | 0.187 ± 0.018 | 0.202 ± 0.019 |
|                   | 0.175 ± 0.018 | 0.180 ± 0.018 | 0.181 ± 0.017 | 0.194 ± 0.020 | 0.166 ± 0.016 | 0.177 ± 0.018 | 0.187 ± 0.018 | 0.202 ± 0.019 |

Molecular analysis

Statement of DNA dataset

The COI dataset comprises a 609 bp fragment from 36 sequences of 8 earthworm species including an outgroup species, Polypheretima elongata (Table 1). The nucleotide frequencies of A, T, G and C were 29.7%, 28.7%, 18.6% and 23.0%, respectively. The GC content was 41.6%. The combined dataset contained 210 (34.5%) parsimony informative and 216 (35.5%) variable sites.

Genetic distance

The average K2P distance is 16.0% between earthworm morphospecies. The K2P distance between species varies from 13.0% (Metaphire iranomala sp. nov. and M. anomala) to 23.5% (M. peguana and M. grandiverticulata). The intraspecific distance varies from 0.2% (Polypheretima elongata, M. megascolidioides) to 8.0% (M. decemtheca sp. nov.). The new species, M. decemtheca sp. nov., has the K2P divergence of 17.7% with M. megascolidioides and of 18.3% with M. grandiverticulata (Table 4).

The species Metaphire iranomala sp. nov. has genetic variations of 0.2% to 9.4%. The maximum distance between two types is 9.4%, but it is considered to be less than the average distance of 16%. Therefore, it is suggested that two morphological types still reflect one species. Similarly, the species M. decemtheca sp. nov. also has genetic divergences of 2.2% to 11.3% corresponding to morphological variations.

The K2P species divergences were reported, but were different for earthworm groups, for example, 13-15% for Allolobophora Eisen, 1874 (King et al. 2008), 13.2% for Eisenia fetidlandrei complex (Rombke et al. 2016), more than 14% in Octolasion lacteum Orley, 1885, Lumbricus rubellus Hoffmeister, 1843 (Klarica et al. 2012), more than 18.7% in pterostigmoid species (Chang et al. 2008), 19.8% in the genus Lumbricus Linnaeus, 1758 (James et al. 2010), 8.9-22.9% in the genus Pheretima Kinberg, 1867, 14.2-21.9% in the genus Amynthas Kinberg, 1867, 7.5-18.0% in the genus Pithemera Sim & Easton, 1972 and 11.7-21.0% in the genus Polypheretima Michaelsen, 1934 (Aspe & James 2018). Recently, Jeraththitikul et al. (2017) also calculated the average species distance of 20% for Amynthas and Metaphire species in Thailand. Our calculation of 16% only applies to the Metaphire species.

Phylogenetic relationship

A phylogenetic tree was reconstructed for a 609 bp dataset using the Likelihood ML and Inference BI analysis (Fig. 4). Two new species are clearly separated from their congeners. Metaphire iranomala sp. nov.
is a sister clade of *M. anomala* with strong supports of bootstrap and BI values (90% and 1.00 bpp, respectively). Two subclades of *M. iranomala* sp. nov. are also well supported with bootstrap and BI values (89% and 0.92 bpp, respectively).

*M. decemtheca* sp. nov. is related to *M. grandiverticulata* and *M. megascolidioides* with moderate bootstrap support and strong BI values of 75% and 0.95 bpp. Within the clade of *M. decemtheca* sp. nov., two morphological variations also represent two lineages: CTU-EW183.06 and CTU-EW183.p02 + CTU-EW183.03. The relationship is well supported with bootstrap of 96% and BI value of 1.00 bpp.

**Discussion**

Nguyen et al. (2016) provided the comprehensive checklist of 212 earthworm species in Vietnam. Earthworms have been well surveyed in most areas in Vietnam, but not in highly mountainous or remote areas. For example, earthworms on islands still have been poorly recognised, except some data in Nam Du (Michaelsen 1934), Con Son (Thai et al. 2004), Lai Son, Hon Tre, Nam Du and Phu Quoc (Nguyen et al. 2017a, 2020). Several species were also recently described from Lai Son Island and Phu Quoc Island (Nguyen et al. 2017a, 2020). It is thus suggested that more intensive surveys in islands of Vietnam would bring more new taxa to discovery.

Nguyen et al. (2016) also suggested some species should be revised or rechecked to confirm their taxonomic status because those species, which were recorded in Vietnam, differentiated from original

---

**Fig. 4.** The phylogenetic diagram inferred from the 594 bp dataset using the Maximum Likelihood (ML) and Bayesian Inference (BI) analysis. The values at node show the bootstrap and BI. The information in parentheses are locations and number of spermathecal pores.
descriptions, e.g., *Metaphire multitheca* (Chen, 1938), *M. anomala* (Michaelsen, 1907). These records of *M. multitheca* and *M. anomala* in Vietnam have been corrected and found to be new species, *M. erroneous* Nguyen & Nguyen, 2015 and *M. iranomala* sp. nov.

The high intraspecific divergences in *Metaphire iranomala* sp. nov. and *M. decemtheca* sp. nov. may suggest more Vietnamese species containing these cryptic forms, such as *M. houletti* (Perrier, 1872) (as discussed in Nguyen et al. 2018). The cryptic speciation events have also been reported in many earthworm species using either mitochondrial data (King et al. 2008, James et al. 2010, Novo et al. 2010) or nuclear data (Rougerie et al. 2009). The COI barcode was also applied to discriminate earthworm species and to suggest cryptic forms (Jeratthitikul et al. 2017).

Acknowledgements

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.05-2018.04. This study is funded in part by the CTU Improvement Project VN14-P6, supported by a Japanese ODA loan. The English was checked and polished by Ms Kelly Morgan (TRACE Wildlife Forensics Network).

References

Aspe N.M. & James S.W. 2018. Molecular phylogeny and biogeographic distribution of pheretimoid earthworms (Clitellata: Megascolecidae) of the Philippine archipelago. *European Journal of Soil Biology* 85: 89–97. https://doi.org/10.1016/j.ejsobi.2018.02.001

Bantaowong U., Chanabun R., Tongkerd P., Sutcharit C., James S.W. & Panha S. 2011. A new species of the terrestrial earthworm of the genus *Metaphire* Sims and Easton, 1972 from Thailand with redescription of some species. *Tropical Natural History* 11 (1): 55–69.

Blakemore R.J. 2016a. Darwin’s earthworms (Annelida, Oligochaeta, Megadrilacea) with review of cosmopolitan *Metaphire* peguana-species group from Philippines. *Opuscula Zoologica* 47 (1): 9–30. https://doi.org/10.18348/opzool.2016.1.9

Blakemore R.J. 2016b. Eco-taxonomic profile and mtDNA barcode of *Metaphire* megascolioides (Goto & Hatai, 1899) – a megadrile earthworm from Japan, plus miscellaneous taxonomic mending. *VermEcology Occasional Papers* 1: 1–16.

Chang C.-H., Lin S.-M. & Chen J.-H. 2008. Molecular systematics and phylogeography of the gigantic earthworms of the *Metaphire formosae* species group (Clitellata, Megascolecidae). *Molecular Phylogenetics and Evolution* 49: 958–968. https://doi.org/10.1016/j.ympev.2008.08.025

Chen Y. 1938. Oligochaeta from Hainan, Kwangtung. Contributions from the Biological Laboratory of the Science Society of China. *Zoological Series* 12 (10): 375–427.

Cognetti M.L. 1908. Contributo alla conoscenza della drilofauna papuasica. *Bollettino dei musei di zoologia ed anatomia comparata della R. Università di Torino* 23 (584): 1–4.

Edgar R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 113. https://doi.org/10.1186/1471-2105-5-113

Feldman A.T. & Wolfe D. 2014. Tissue processing and hematoxylin and eosin staining. In: Day C. (ed.) *Histopathology. Methods in Molecular Biology (Methods and Protocols)* 1180: 31–43. Humana Press, New York.

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
Gates G.E. 1925. Some notes on *Pheretima anomola*, Mich. and a related species new to India and Burma. *Annals and Magazine of Natural History* 15 (9): 538–550. https://doi.org/10.1080/00222932508633244

Gates G.E. 1972. Burmese earthworms: an introduction to the systematics and biology of Megadrile oligochaetes with special reference to Southeast Asia. *Transactions of the American Philosophical Society* 62: 1–326. https://doi.org/10.2307/1006214

Goto S. & Hatai S. 1899. New or imperfectly known species of earthworm. *Annotationes Zoologicae Japonenses* 3 (2): 13–24.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

James S.W., Porco D., Decaens T., Richard B., Rougerie R. & Ersaeus C. 2010. DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellate): resurrection of *L. herculeus* (Savigny, 1826). *PLoS One* 5: e15629. https://doi.org/10.1371/journal.pone.0015629

Jeraththikul E., Bantaowong U. & Penha S. 2017. DNA barcoding of the Thai species of terrestrial earthworms in the genera *Amynthas* and *Metaphire* (Haplotaxida: Megascolecidae). *European Journal of Soil Biology* 81: 39–47. https://doi.org/10.1016/j.ejsobi.2017.06.004

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. https://doi.org/10.1038/nmeth.4285

King R.A., Tibble A.L. & Symondson W.O.C. 2008. Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology* 17: 4684–4698. https://doi.org/10.1111/j.1365-294X.2008.03931.x

Klareika J., Kloss-Brandstätter A., Traugott M. & Juen A. 2012. Comparing four mitochondrial genes in earthworms – implications for identification, phylogenetics, and discovery of cryptic species. *Soil Biology and Biochemistry* 45: 23–30. https://doi.org/10.1016/j.soilbio.2011.09.018

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: Molecular Evolution Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Michaelsen W. 1907. Neue Oligochäten von Vorder-Indien, Ceylon, Burma und den Andaman-Inseln. *Mitteilungen aus dem naturhistorischen Museum in Hamburg* 24: 143–193.

Minamiya Y., Yokoyama J. & Fukuda T. 2009. A phylogeographic study of the Japanese earthworm, *Metaphire sieboldi* (Horst, 1833) (Oligochaeta: Megascolecidae): inferences from mitochondrial DNA sequences. *European Journal of Soil Biology* 45 (5/6): 423–430. https://doi.org/10.1016/j.ejsobi.2009.06.004

Minh B.Q., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M.D., von Haeseler A. & Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular and Biological Evolution* 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nguyen A.D. & Nguyen T.T. 2015. Notes on *Metaphire multitheca* (Chen, 1938) (Oligochaeta, Megascolecidae) recorded from Vietnam, with descriptions of two new species. *ZooKeys* 506: 127–136. https://doi.org/10.3897/zookeys.506.9550

Nguyen Q.N., Nguyen V.T., Duong C.T., Le V.N. & Nguyen T.T. 2019. Species diversity of earthworms in Dong Nai Province, Vietnam. *Tap Chi Sinh Hoc* 41 (2): 117–129.
Nguyen T.T. 2014. Checklist and some remarks on faunistic characteristics of earthworms in the Mekong Delta, Vietnam. *Journal of Science, Cantho University, Section A: Science, Technology and Environment* 32: 106–119.

Nguyen T.T & Lam H.D. 2017. Three new earthworm species of the genus *Metaphire* Sims & Easton, 1972 (Oligochaeta, Megascolecidae) from Dong Nai Province, Vietnam. *Tap Chi Sinh Hoc* 39 (4): 406–415.

Nguyen T.T., Nguyen A.D., Tran T.B. & Blakemore R.J. 2016. A comprehensive checklist of earthworm species and subspecies from Vietnam (Annelida: Clitellata: Oligochaeta: Almidae, Eudrilidae, Glossoscolecidae, Lumbricidae, Megascolecidae, Moniligastridae, Ooncrodrilidae, Octochaetidae). *Zootaxa* 4140: 1–92. https://doi.org/10.11646/zootaxa.4140.1.1

Nguyen T.T., Trinh K.B.T., Nguyen H.L.T. & Nguyen A.D. 2017a. Earthworms (Annelida: Oligochaeta) from islands of Kien Hai District, Kien Giang Province, Vietnam, with descriptions of two new species and one subspecies. *Journal of Natural History* 51 (15–16): 883–915. https://doi.org/10.1080/00222933.2017.1294213

Nguyen T.T., Nguyen P.H., Truong T.A. & Nguyen Q.N. 2017b. Diversity and distribution of earthworms in Ba Ria, Vung Tau Province. *Journal of Science, Can Tho University* 53 (A): 96–107.

Nguyen T.T., Nguyen Q.N & Nguyen A.D. 2018. First record of the earthworm genus *Pheretima* Kinberg, 1867 sensu stricto in Vietnam, with description of a new species (Annelida: Clitellata: Megascolecidae). *Zootaxa* 4496 (1): 251–258. https://doi.org/10.11646/zootaxa.4496.1.20

Nguyen T.T., Lam D.H., Trinh B.T.K. & Nguyen A.D. 2020. The megascolecid earthworms (Annelida, Oligochaeta, Megascolecidae) in the Phu Quoc island, Vietnam, with descriptions of three new species. *ZooKeys* 932: 1–25. https://doi.org/10.3897/zookeys.932.50314

Novo M., Almodovar A., Fernandez R., Trigo D. & Díaz-Cosín D.J. 2010. Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. *Molecular Phylogenetics and Evolution* 56: 507–512. https://doi.org/10.1016/j.ympev.2010.04.010

Prasankok P., Bantaowong U., James S.W. & Panha S. 2013. Low heterogeneity in populations of the terrestrial earthworm, *Metaphire peguana* (Rosa, 1890), in Thailand, as revealed by analysis of mitochondrial DNA CO1 sequences and nuclear allozymes. *Biochemical Systematics and Ecology* 51: 8–15. https://doi.org/10.1016/j.bse.2013.07.001

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Rougerie R., Decaens T., Deharveng L., Porcol D., James S.W, Chang C.-H., Richard B., Potapov M., Subarjono Y. & Hebert P.D.N. 2009. DNA barcodes for soil animal taxonomy. *Pesquisa Agropecuária Brasileira* 44: 789–801. https://doi.org/10.1590/S0100-204X2009000800002

Sims R.W. & Easton E.G. 1972. A numerical revision of the earthworm genus *Pheretima* auct. (Megascolecidae: Oligochaeta) with the recognition of new genera and an appendix on the earthworms collected by the Royal Society North Borneo Expedition. *Biological Journal of the Linnean Society* 4: 169–268. https://doi.org/10.1111/j.1095-8312.1972.tb00694.x
NGUYEN T.T. et al., Two new Metaphire species from Southern Vietnam

Stephenson J. 1929. The Oligochaeta of the Indawgyi lake (upper Burma). Records of the Indian Museum 31: 225–239.

Thai T.B., Huynh T.K.H. & Nguyen D.A. 2004. Remarks of earthworms on the islands in southern of Vietnam. Proceedings of the national Workshop on the basic Issues in Life Science. Hanoi Science and Technics Publishing House: 757–760.

Yuan Z., Dong Y., Jiang J., Jiang J., Zhao Q. & Qiu J. 2019. Three new species of earthworms (Oligochaeta: Megascolecidae) from Yunnan Province, China. Zootaxa 4664 (3): 390–400. https://doi.org/10.11646/zootaxa.4664.3.6

Zhang Z., Schwartz S., Wagner L. & Miller W. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7 (1–2): 203–214. https://doi.org/10.1089/10665270050081478

Manuscript received: 6 October 2020
Manuscript accepted: 22 December 2020
Published on: 21 April 2021
Topic editor: Rudy C.A.M. Jocqué
Desk editor: Charlotte Gérard

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; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; 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; Zoological Research Museum Alexander Koenig, Bonn, Germany; National Museum, Prague, Czech Republic.