

## Original article

# Taxonomic status of the earthworm species *Metaphire saigonensis* (Omodeo, 1956) (Clitellata: Megascolecidae)

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### Abstract

The species *M. saigonensis* (Omodeo, 1956) was re-accessed based on a comparison between its original description and different variations of *M. bahli* and *M. peguana*, to have a comprehensive review about taxonomic position of *M. saigonensis* through previous studies, and on collected samples. A total of 65 specimens of *M. bahli* and 69 specimens of *M. peguana* collected from various parts in southern Vietnam were used to examine. As a result, *M. bahli* has two different morphs (with and without concave male region). *M. peguana* has two subspecies, but rather stable in morphology. *M. saigonensis* is totally different from *M. peguana* in closer ventral distance between male pores, spermathecal pores, but it is very similar to the not concaved male region morph of *M. bahli*. Therefore, it is concluded that *M. saigonensis* is a junior synonym of *M. bahli*.

## INTRODUCTION

The taxonomic position and the relationship between *Metaphire saigonensis* (Omodeo, 1956) and its close congeners have been confused and controversial. Omodeo (1956) described the new species, *Pheretima saigonensis* (= *Metaphire saigonensis*) based on samples collected from Sai Gon (= Ho Chi Minh city), Vietnam. He distinguished the new species from *M. peguana* (Rosa, 1890) by the closer distances between male pores, spermathecal pores, the absence of septum 10/11, and the difference of spermathecal shape.

After Omodeo's original description, there were many different point of views. Gates (1972) considered *M. saigonensis* as a synonym of *M. peguana* due to the differences mentioned in Omodeo (1956). He reported that all differences were quantitative, less characterized, and mostly related to the individual variations. Also, he proved that septum 10/11 always absent in *M. peguana* as well as in the type of *M. saigonensis*. Sims & Easton (1972) treated *M. saigonensis* as a valid species, and listed it within the *M. peguana* species group. Later, Thai (1983) stated that *M. saigonensis* is only a variant of *M. bahli* with the eversion of genital markings, and he also

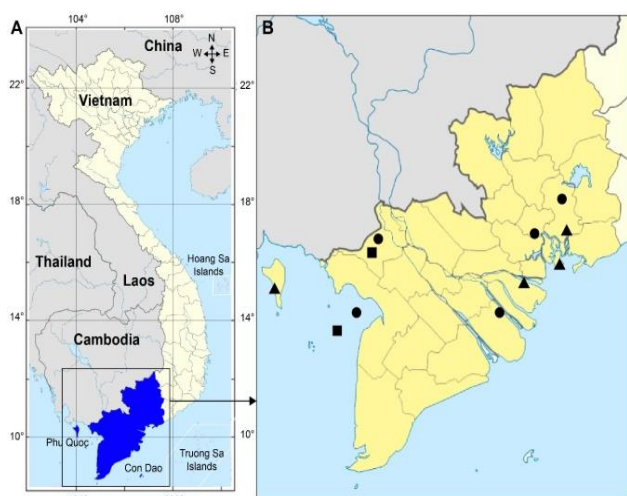
showed the similarities of the gaps of male pores and spermathecal pores between two species. Blakemore (2002) agreed with Gates (1972) at first, but later, in 2016, he restored *M. saigonensis* to be a valid species. Nguyen *et al.* (2016) also listed it as a valid species without more explanations.

During the examination of different populations of *M. bahli* and *M. peguana* collected in Vietnam, the taxonomic status of *M. saigonensis* is re-accessed based on a comparison between its original description and different variations of *M. bahli* and *M. peguana*.

## MATERIALS AND METHODS

Earthworm specimens have been collected from various parts of southern Vietnam from 2017-2020. A total of 134 earthworm individuals were collected and examined, including 65 specimens of *M. bahli* from five provinces and cities, An Giang (Nhon Mountain), Kien Giang (Hon Tre Island), Tra Vinh (Chau Thanh District), Dong Nai (Vinh Cuu District), and Ho Chi Minh City (Binh Chanh District); 48 individuals of *M. peguana* collected from Kien Giang (Phu Quoc Island), Ben Tre (Binh Dai District), Dong Nai (Long Thanh

District), and Ho Chi Minh City (Can Gio District); 21 samples of *M. peguana liaisonensis* from Kien Giang (Lai Son Island) and An Giang (Cam Mountain) (Table 1).



**Fig 1.** Collection sites of three earthworm species (modified from Google Map). A. Vietnam map, B. The South of Vietnam (●. *Metaphire bahli*, ▲. *Metaphire peguana peguana*, ■. *Metaphire peguana liaisonensis*).

Morphological characters were observed externally and dissected from the dorsal to examine internally, under the stereo Microscope Motic DM143-FBGG-C. The measurements were performed based on the micrometric slide Optika M-005 calibrated on the software Motic Image Plus version 2.0. The distances between male pores and spermathecal pore (estimated from the center of the pore) counted in the number of setae of the nearby segment (xvii) were also taken into count.

Earthworm important morphological features were captured and grouped by the help of Adobe Photoshop CS6 and Adobe Illustrator CS6. The obtained data were processed by Microsoft Excel 2016 and Minitab 19.

**RESULT AND DISCUSSION**

**Variations of *Metaphire bahli* populations**

The species *Metaphire bahli* collected from Vietnam has two different morphological forms distinguished by the shape of the male region (concave or not concave). The concave male region is very typically morphological form of *M. bahli* in Vietnam. This morph has been very commonly found, and the concave male region is a stable character, ventral locations of male pores and spermathecal pores. The ventral distance between male pores and spermathecal pores are slightly variable from the population in Ho Chi Minh city (male pores: 0.22±0.01; spermathecal pores: 0.15±0.02) to An Giang population (male pores: 0.20±0.02; spermathecal pores: 0.13±0.01) and Tra Vinh population (male pores: 0.20±0.02; spermathecal pores: 0.14±0.02).

**Table 2:** Comparison between two morphs of *Metaphire bahli*

No.	Characters	Morph 1 with concave male region <sup>(1)</sup> (N=39)	Morph 2 without concave male region <sup>(2)</sup> (N=13)
1.	Length (mm)	111.50 ± 11.19	120.31 ± 11.18
2.	Diameter (mm)	4.07 ± 0.32	4.36 ± 0.29
3.	Number of segments	108.42 ± 9.19	103.46 ± 12.18
4.	Setae number between two male pores	3.22 ± 1.53	3.62 ± 1.85
5.	Location of spermathecal pores	Ventral side	Lateroventral side
6.	Ventral distance between spermathecal pores (per body circumference)	0.20 ± 0.02	0.28 ± 0.03
7.	Setae number between spermathecal pores (by setae number)	9.29 ± 1.96	11.92 ± 2.11
8.	Ventral distance between male pores (per body circumference)	0.14 ± 0.02	0.17 ± 0.02
9.	Ventral distance between male pores (by setae number)	9.66 ± 1.41	10.38 ± 1.94
10.	Location of intestinal caeca	xxvii-xxiv (n=21) xxvii-xxv (n=15) xxvii-xxiii (n=3)	xxvii-xxiv (n=8) xxvii-xxv (n=5)

(1): Populations in Ho Chi Minh (Binh Chanh), Tra Vinh (Chau Thanh), An Giang (Nhon Mountain); (2): Population in Kien Giang (Hon Tre Island); N: Individual in total; n: individual.

Compared to the morph with concave male region, the other morph (without concave male region) has been found only in Hon Tre island (Kien Giang Province). The morph is characterized by longer ventral distance between male pores (0.28±0.03) and spermathecal pores (0.17±0.02).

In addition, the intermediate form has been also recorded between two morphs. In the intermediate form, the male region is not concave as morph 1, but the shapes and arrangement of genital markings are similar to morph 1 (Fig. 1).

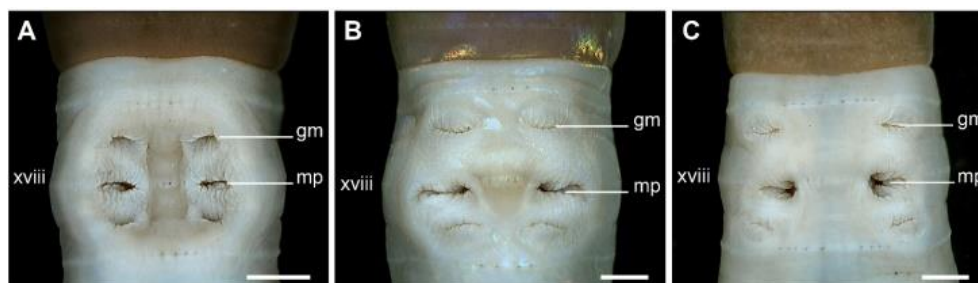
**Variations of *Metaphire peguana* populations**

The species *M. peguana* has been recorded in Vietnam with two subspecies, *M. peguana peguana* (Rosa, 1890) and *M. peguana liaisonensis* Nguyen & Nguyen, 2017. The subspecies, *M. peguana peguana*, has not much morphological variations except first dorsal pore in 13/14 (populations in coastal region, e.g. Can Gio – Ho Chi Minh city, Binh Dai – Ben Tre) or 12/13 (populations in islands or mainland).

**Table 1.** Total examined specimens of *M. bahli* and *M. peguana*

Species voucher	Number of mature individuals	Collected habitat	GPS coordinates		Elevations (m)	Localities	Collected date	Collector
			North	East				
<b><i>Metaphire bahli</i> (Gates, 1945)</b>								
CTU-EW.004.04	13	Acacia trees plantation	11°01'6.75	106°57'14.1	8	Vinh Cuu, Dong Nai Province	13/09/2012	Duong Chi Trong
CTU-EW.004.05	13	Natural forest	10°35'17.6	104°57'02.0	56	Nhon mountain, An Giang Province	07/11/2010	Nguyen Thanh Tung
CTU-EW.004.08	13	Mango garden	09°58'11.0	104°50'09.4	82	Hon Tre island, Kien Giang Province	16/10/2014	Trinh Thi Kim Binh
CTU-EW.004.34	13	Bushes	10°41'30.5	106°36'11.0	1	Binh Chanh, Ho Chi Minh city	24/09/2019	Nguyen Quoc Nam
CTU-EW.004.56	13	Bushes	09°58'01.0	106°21'18.1	2	Chau Thanh, Tra Vinh Province	13/10/2020	Lam Hai Dang
<b><i>Metaphire peguana peguana</i> (Rosa, 1890)</b>								
CTU-EW.009.04	15	Rubber trees plantation	10°41'01.3	107°01'02.1	8	Long Thanh, Dong Nai Province	21/10/2014	Le Van Nhan
CTU-EW.009.09	7	Natural forest	10°10'48.7	103°58'15.0	20	Phu Quoc island, Kien Giang Province	05/11/2016	Lam Hai Dang, Trinh Thi Kim Binh
CTU-EW.009.13	15	Mango garden	10°23'15.1	106°54'45.2	3	Can Gio, Ho Chi Minh city	23/09/2019	Nguyen Quoc Nam
CTU-EW.009.19	11	Sugarcane plantation	10°13'21.6	106°34'44.5	1	Binh Dai, Ben Tre Province	13/10/2020	Lam Hai Dang
<b><i>Metaphire peguana liaisonensis</i> Nguyen &amp; Nguyen, 2017</b>								
CTU-EW.087.01	8	Natural forest	09°48'10.5	104°36'51.3	32	Lai son island, Kien Giang Province	15/11/2013	Trinh Thi Kim Binh
CTU-EW.004.02	13	Natural forest (near border)	10°30'36.3	105°00'09.0	193	Cam mountain, An Giang Province	09/11/2010	Nguyen Thanh Tung

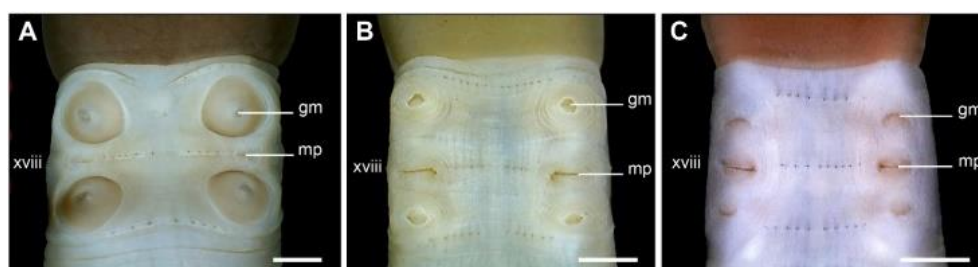
CTU = Can Tho University



**Fig 2.** Different shapes of the male region of *Metaphire bahli*. A. Morph 1 (concave), B (intermediate) and C (not concave). Morph 2. Scale bar 1 mm. (gm = genital marking, mp = male pore).

*Metaphire peguana liaisonensis* has been recorded in only two localities, Lai Son island (Kien Giang Province) and Cam mountain (= *Metaphire* sp.11 in Nguyen (2013)) (An Giang Province). There are slight differences between two populations. In Lai Son island, specimens have a longer size ( $94.13 \pm 13.53$  mm),

typical genital markings in 17/18 and 18/19, larger ventral distance between two spermathecal pores ( $0.46 \pm 0.07$ ), setae uniform in both ventral and dorsal sides, diverticulum directly attached to ampulla duct, and copulatory pouches elevated from body coelomic cavity.



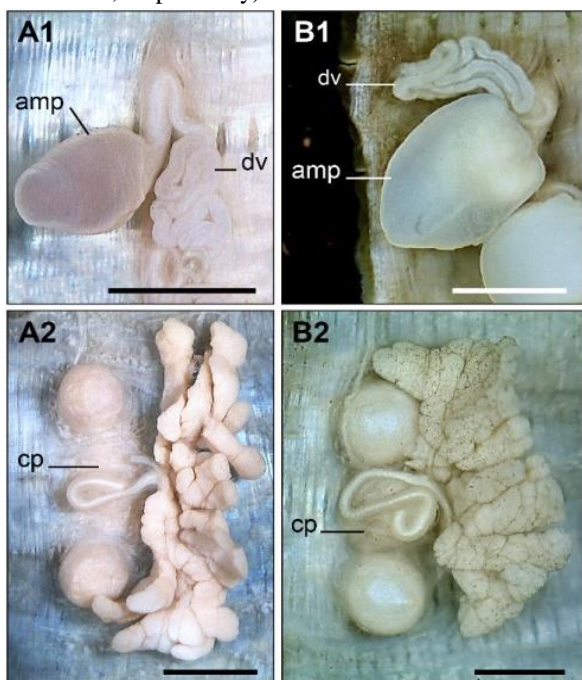
**Fig 3.** Male region of *Metaphire peguana peguana* (A), *Metaphire peguana liaisonensis* (B. Lai Son island population, C. Cam mountain population). (gm = genital marking, mp = male pore).



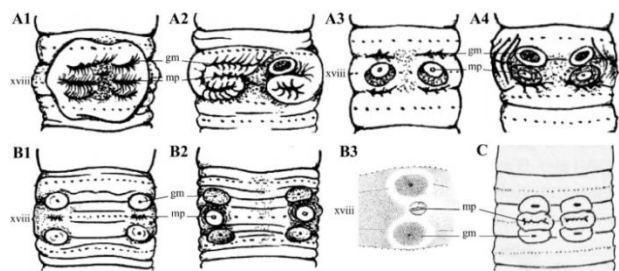
On the contrary, the population from Cam mountain has a shorter size ( $72.00 \pm 10.89$  mm), genital markings in xvii and xix, more setae on ventral side than on dorsal side, diverticulum directly attached to the base of ampulla, and copulatory pouches not elevated from body coelomic cavity.

**Synonymization of *Metaphire saigonensis***

Blakemore (2016) and Nguyen *et al.* (2016) revalidated *M. saigonensis* from the synonyms of *M. peguana*. According to them, two species can be distinguished by ventral distance between two male pores (*M. peguana* > *M. saigonensis*), characters of spermathecal ampulla (short, hardly visible ducts vs. longer, visible ducts). In our analysis, two of these species (*M. peguana* and *M. saigonensis*) are very clearly different in the distance between spermathecal pores and male pores (15-23 and 12-18 vs. 9-10 and 9-10 setae, respectively) and ventral distance between spermathecal pores and male pores (0.39-0.50 and 0.25-0.31 vs. < 0.25 and < 0.25, respectively).



**Fig 4.** Variation of spermatheca (A) and male region (B) of *Metaphire peguana laisonensis* found in Cam mountain (A1, A2) and in Lai Son island (B1, B2). (amp = ampulla, dv = diverticulum, cp = copulatory pouch)



**Fig 5.** Male region of *M. bahli* (A1-A4), *M. peguana peguana* (B1-B3), *M. saigonensis* (C). A1-A4 and B1-B2. after Thai (1983), B3. after Rosa (1890), C. after Omodeo (1956). (gm = genital marking, mp = male pore).

**Table 3.** Character comparison between *Metaphire saigonensis*, *M. bahli* (morph 2) and *M. peguana peguana*.

No	Characters	<i>M. saigonensis</i>	<i>M. bahli</i> (Morph 2)	<i>M. peguana peguana</i>
1.	Length (mm)	87-130	108-139	81-143
2.	Diameter (mm)	4-5	3.59-4.81	3.65-6.27
3.	Number of segments	108-117	85-115	60-157
4.	First dorsal pore	12/13	11/12 or 12/13	12/13 or 13/14
5.	aa/ab setal distance (viii)	1	1.04-3.40	1.02-3.30
6.	zz/zy setal distance (viii)	1	1.10-3.44	1.16-2.58
7.	Seta number between male pores (on xviii)		1-7	7-13
8.	Location of spermathecal pore on segment	Ventral*	Lateroventra 1	Lateroventra 1
9.	Ventral distance between spermathecal pores (by body circumference)	<0.25*	0.24-0.31	0.39-0.50
10.	Ventral distance between spermathecal pores (by seta number)	9-10 (at center point)	11-15	15-23
11.	Ventral distance between male pores (by body circumference)	<0.25*	0.15-0.21	0.25-0.31
12.	Ventral distance between male pores (by seta number)	9-10 (at center point)	7-14	12-18
13.	Location of genital markings	Ventral*	Ventral	Lateroventra 1
14.	Septum 10/11	Absent	Thin, present ventrally	Thin, present ventrally
15.	Spermathecal ducts	About 1/3 ampulla*	1/3 ampulla	Very short

\*Estimated from the original description of Omodeo (1956)

On the contrary, *M. saigonensis* is very similar to the morph 2 of *M. bahli* recorded in Kien Giang. Both species has similar ventral distance between male pores (9-10 and 7-14 setae) and between spermathecal pores (9-10 and 11-15 setae), spermathecal ducts ca. 1/3 ampulla, and diverticulum directly attached to the base of ampulla ducts. Furthermore, Thai (1983) recorded variations of genital markings in *M. bahli* in Vietnam. He reported that *M. bahli* has convex genital markings which is similar in *M. saigonensis* (Figure 2A1-A4). The convex genital markings have never been recorded in *M. peguana peguana* (Figure 2B1-B2).

The comparison between *M. saigonensis*, *M. bahli* and *M. peguana peguana* is presented in table 3. Based on the analysis, it can be concluded that *M. saigonensis* is a junior synonym of *M. bahli*.

**ACKNOWLEDGEMENT**

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