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DESCRIPTION OF A NEW SPECIES OF *HETEROMETRUS* EHRENBERG, 1828 (SCORPIONES: SCORPIONIDAE) FROM THAILAND WITH REMARKS ABOUT THE UTILIZATION OF CYTOGENETIC DATA IN TAXONOMY OF THE GENUS

Jana Plíšková[1], František Kovařík[1, 2], Ondřej Košulič[3] and František Šťáhlavský[1,*]

[1]*Department of Zoology, Charles University, Viničná 7, CZ-128 44 Prague 2, Czech Republic*
[2]*P.O. Box 27, CZ-145 01 Prague 45, Czech Republic*
[3]*Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University, Zemědělská 3, CZ-613 00 Brno, Czech Republic*
[*]*Corresponding author: e-mail: stahlf@natur.cuni.cz*

Abstract.— A new species of the genus *Heterometrus* is described on the basis of a specimen recently collected in Thailand. *Heterometrus minotaurus* **sp. nov.** is morphologically closest to *H. longimanus*. The newly described species is well distinguished by its smaller overall size (83 mm) and shorter and less narrow metasoma with specific dorsolateral carinae on the fourth metasomal segment consisting of 9 or fewer granules. No females are known and so knowledge of sexual dimorphism in this species is currently lacking. In addition to the morphological characterization of *H. minotaurus* **sp. nov.**, we present here also a description of the male holotype's karyotype. The diploid set of *H. minotaurus* **sp. nov.** consists of 54 chromosomes with a predominance of metacentrics, which gradually decrease in size. The presence of two types of multivalent association observed in postpachytene and metaphase I is commented on. Current knowledge of karyotypes of *Heterometrus* species is briefly summarized and compared with our cytogenetic results. In conclusion, we discuss the possible usefulness of karyotype as another interspecific feature applicable in the taxonomy of this scorpion group.

Key words.— Arachnida, Scorpiones, karyotype, taxonomy, Southeast Asia

Introduction

Together with three related lineages (*Scorpio* Linnaeus, 1758; *Pandinus sensu lato* Thorell, 1876; and *Opistophtalmus* C. L. Koch, 1837), the genus *Heterometrus* Ehrenberg, 1828 forms the separate subfamily Scorpioninae within the family Scorpionidae. *Heterometrus* has a centre of diversity in Southeast Asia and its members inhabit various biotopes of tropical and subtropical forests of the Indian subcontinent, Southeast Asia, and the Indonesian archipelago (Prendini *et al.* 2003, Tahir and Prendini 2014). Similar to scorpions of the African sister genus *Pandinus*, representatives of *Heterometrus* are among the largest

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extant scorpions in the world (Prendini *et al.* 2003). Our knowledge of the taxonomy of *Heterometrus* is particularly based on several key studies, most of them are listed below. First, in a series of various publications Pocock described a considerable number of species and also established the basic criteria for their identification (see Pocock 1892, 1893, 1894a, 1896, 1897, 1899, 1900). Several decades later, Couzijn (1981) published a monographic revision of the genus *Heterometrus* with a key to all 21 known species. Subsequently, Tikader and Bastawade (1983) focused on all Indian *Heterometrus* species, giving their redescriptions and a determination key and also describing two new species. Recently, Kovařík (2004) published a new revision of the genus *Heterometrus*. On the basis of obtained knowledge, Kovařík (2004) synonymized several species and subspecies as well as subgenera established by Couzijn (1981). Taking into account Kovařík's morphological revision from 2004, there were 31 valid species recognized within the genus *Heterometrus*. Recently, various authors have described four additional species (Lourenço *et al.* 2005, Zhu and Yang 2007, Javed *et al.* 2010, Mirza *et al.* 2012), and thus the genus *Heterometrus* currently includes 35 species (Rein 2015). As Tahir and Prendini (2014) have noted, the total of recently recognized *Heterometrus* species might not reflect the potential real diversity of this genus. Across its entire area of distribution, numerous range-restricted or narrowly endemic species as well as species complexes are expected (Prendini *et al.* 2003).

Many taxonomic studies of various organisms today utilize standard morphological analysis in combination with molecular phylogenetic or cytogenetic approaches (e.g. Fet *et al.* 2014, Granjon and Dobigny 2003, Post *et al.* 2003). Cytogenetic data seem to represent additional suitable characteristics useful for taxonomic analysis and may also help to differentiate morphologically similar species into different groups of arachnids (e.g. Řezáč *et al.* 2008, Zaragoza and Šťáhlavský 2008). Cytogenetic data are currently known for 105 scorpion species (Schneider *et al.* 2015) and the genus *Heterometrus* is among the scorpion groups more frequently studied from a cytogenetic point of view. At present, information on karyotypes is available for 8 *Heterometrus* species (see Schneider *et al.* 2009, Sharma *et al.* 1962, Vítková *et al.* 2005). This group exhibits a wide range of diploid numbers of chromosomes (56–122). Interspecific differences in the number and morphology of chromosomes among related species suggest that a description of karyotype could be another useful specific characteristic applicable in *Heterometrus* taxonomy. For this reason, we describe the karyotype of *H. minotaurus* sp. nov. and discuss the utility of karyotypes as important characteristic features in scorpion taxonomy.

MATERIAL AND METHODS

Sampling. The specimen was found at night by UV detection in front of a massive stone at the border between a rubber plantation and a water stream. The area was sheltered by rubber trees and shrubs with high canopy coverage and humidity. No other scorpions were found sympatrically with *H. minotaurus* sp. nov. during a field trip to the studied area.

Morphological analysis. The specimen was studied under stereomicroscope and measured using an ocular micrometre. Measurements are given in millimetres. Nomenclature and measurements follow Stahnke (1970), Kovařík (2009), and Kovařík and Ojanguren Affilastro (2013). The sternum was analysed according to Soleglad and Fet (2003). Trichobothria were denominated and described according to Vachon (1974). Morphological terminology follows Stahnke (1970) and Kovařík (2009).

Karyotype analysis. For the karyotype analysis, we used the gonads of the *H. minotaurus* sp. nov. male holotype. Chromosome slides were made using the spreading technique described by Traut (1976). The gonads were dissected and hypotonized in 0.075 M KCl for 20 min and then fixed in a methanol: glacial acetic acid (3:1) solution for at least 20 min. The small parts of the testes were then dissociated in a drop of 60% acetic acid on a microscope slide, and the suspension was evaporated on a warm histological plate (45°C). The chromosomes were stained with 5% Giemsa solution in Sörensen phosphate buffer (pH = 6.8) for 30 min. Additionally, we used the C-banding technique according to the standard protocol of Sumner (1972). In this case, the chromosome slides were stained with DAPI and the photographs' colour was inverted in Adobe Photoshop CS4 11.0 for higher contrast. Chromosomes were observed with an Olympus AX70 Provis microscope and documented with an Olympus DP72 camera. For chromosome measurements, we used ImageJ 1.45r software (http://rsbweb.nih.gov/ij) with the Levan plugin (Sakamoto and Zacaro 2009). Relative diploid set length was calculated for each chromosome as a percentage of the diploid set based on 11 metaphases I. For the cytogenetic analysis, we used meiotic stages that may reveal unusual pairings. This approach has the ability to disclose specific rearrangements or hybrids (e.g. Gorlov and Tsurusaki 2000).

TAXONOMY

Family Scorpionidae Latreille, 1802

Subfamily Scorpioninae Latreille, 1802

Genus ***Heterometrus*** Ehrenberg, 1828

Heterometrus minotaurus Plíšková, Kovařík, Košulič et Šťáhlavský sp. nov.
(Figs 1–23)

Type locality. Thailand, Surat Thani province, Phanom district, 8°52'N, 98°36'E, 395 m a.s.l.

Type material. Thailand, Surat Thani province, Phanom district, trail along rubber plantation and water stream, 8°52'N, 98°36'E, 395 m a.s.l., 1 male holotype, 11.VII.2014, O. Košulič leg. Holotype specimen is deposited in the public collection of Faculty of Science, Charles University in Prague (CUP-S651).

Differential diagnosis. The described features distinguish *H. minotaurus* sp. nov. from all other species of the genus. *H. minotaurus* sp. nov. from Thailand seems to be closest to *H. longimanus* (Herbst, 1800) from Indonesia (Java, Kalimantan and Sumatra), Singapore, Malaysia (Kalimantan) and Philippines. In published keys to the *Heterometrus*, the two species are categorized under the *H. longimanus* (Kovařík, 2009: 47–48, couplet 28). Males of the two species can be unequivocally separated by: 1) total length 90–140 mm in *H. longimanus* and 83 mm in *H. minotaurus* sp. nov.; 2) longer and more narrow metasoma in

Figures 1–5. *Heterometrus minotaurus* sp. nov., male holotype: (1) dorsal view; (2) ventral view; (3–5) Metasoma and telson: (3) lateral view; (4) dorsal view; (5) ventral view. Scale bars = 10 mm.

H. longimanus (ratio length to width the first metasomal segment is 0.96–1.04 in *H. longimanus* and 0.78 in *H. minotaurus* sp. nov.); 3) more densely granulated carinae on metasoma in *H. longimanus* (dorsalolateral carinae of the fourth metasomal segment consists of 12 or more granules in *H. longimanus* and 9 or less granules in *H. minotaurus* sp. nov.).

Summary of description. Total length male holotype 83 mm, female unknown. Base colour uniformly black (Figs 1–2), telson reddish brown (Figs 3–5). Chelicerae yellow strongly reticulate, anterior part reddish black (Fig. 6). Pectines with 16/17 teeth in male (Fig. 7). Male with fingers, chela, femur and patella of pedipalp narrow and long, ratio chela length manus width 3.42 in male (Figs 8–14). Chela not lobiform in male, smooth, sparsely tuberculate with pronounced carination. Patella of pedipalp with pronounced internal tubercle. Carapace smooth, sparsely granulated. Metasomal segment I wider than long, ratio 0.78. Telson hirsute, elongate, vesicle approximately as long as aculeus.

Description. Total length male holotype 83 mm. Coloration (Figs 1–2) base uniformly black; telson, tarsomeres of legs and sternopectinal area reddish brown.

Chelicerae (Fig. 6): Chelicerae yellow strongly reticulate, anterior part reddish black. Fingers reddish brown to black. Dentition typical for the genus, teeth sharp. Tegument basally smooth and shiny without granulation.

Pedipalps (Figs 8–14): Trichobothriotaxie type C. Pedipalp femur with three trichobothria, of them only one on internal surface. Patella of pedipalp with 20 trichobothria (Figs 11–13), one internal, two dorsal, three ventral and 14 external from which em3 trichobothrium is accessory (Fig. 12 and figs 80–84 in Vachon, 1974: 918). Chela of pedipalp with standard 26 trichobothria (Figs 8–10). Male with fingers, chela, femur and patella of pedipalp narrow and long, ratio chela length manus width 3.42 in male. Chela not lobiform in male, smooth, sparsely tuberculate with pronounced partly incomplete carination. Patella smooth with seven obsolete carinae developed, with pronounced internal tubercle. Femur smooth, with four granulate carinae developed. Fingers long (ratio chela length movable finger length 1.78 in holotype male, curved and with straight rows of granules and internal and external denticles (Fig. 14).

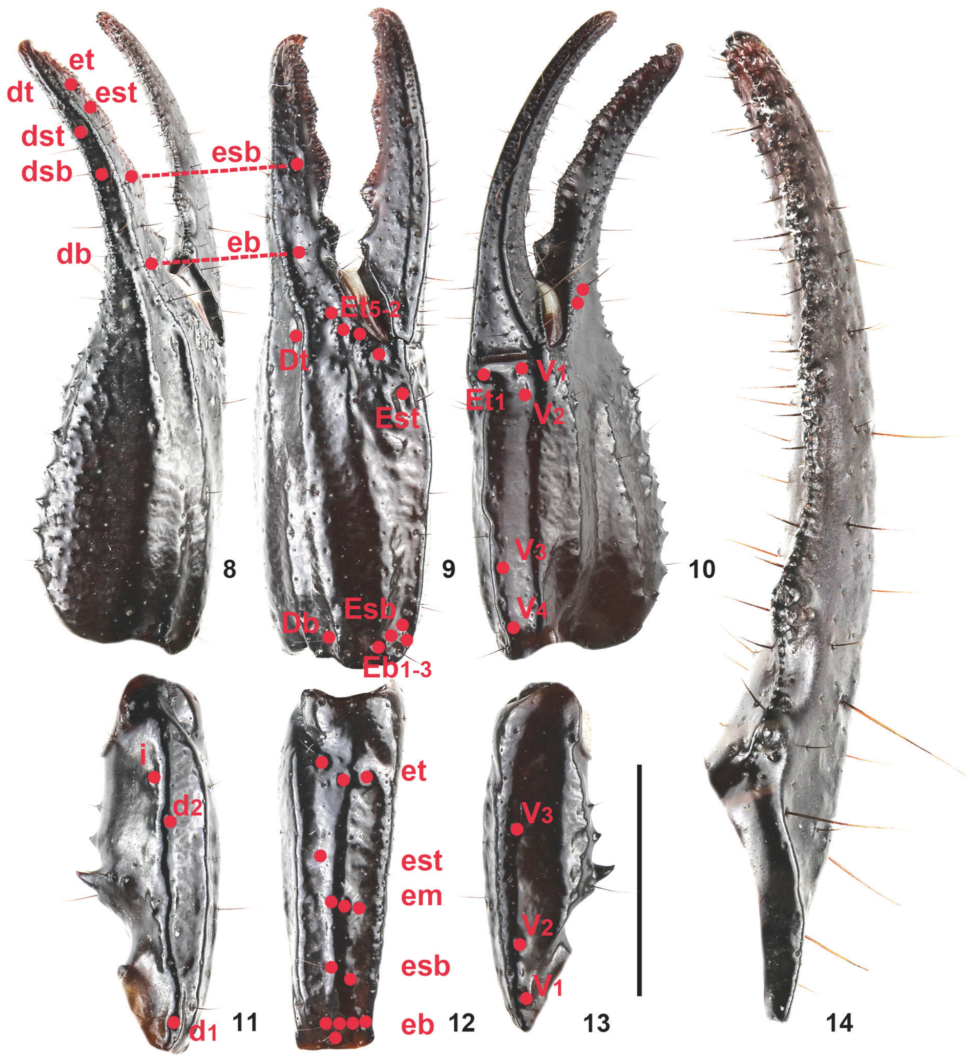
Figures 6–7. *Heterometrus minotaurus* sp. nov., male holotype: (6) chelicerae, carapace and tergites I–III; (7) sternopectinal region and sternites III–VII.

Carapace (Fig. 6): Slightly trapezoidal (narrower anteriorly) and slightly longer than wide; anterior margin strongly concave medially. Tegument smooth sparsely covered by large granules which do not form carinae. Median and posteriolateral furrows wide and deep, other vestigial to absent. Median eyes large and raised; three pairs of lateral eyes same-sized and aligned along each anterolateral corner.

Mesosoma (Figs 1–2, 6–7): Tergites smooth, lack carinae. Sternum (Fig. 7) standard for the genus: type 2 with six sides, posterior emargination and convex lateral lobes, vertical compression absent, apex width slightly narrower than posterior width. Pectines standard-sized for the genus (Fig. 7): short, wide and setose. Tooth count 17 in male. Pectines have 3 marginal lamellae and 4 middle lamellae. Sternites lack carinae, surfaces are smooth and sparsely setose, more setose is sternite III. Posterior margin of sternites IV–VI with smooth median patch.

Legs (Figs 15–18): Retrolateral pedal spurs absent. Lateroapical margins of tarsi produced into rounded lobes. Legs are smooth, without carinae and granules, and unevenly hirsute. Tarsomere I is hirsute. Tarsomere II has spiniform setae and several spines. Spiniform formula of tarsomere II is 4/6: 4/6: 4/6: 4/6.

Metasoma and telson (Figs 3–5): All segments with granulate complete carinae developed. Metasomal segment I wider than long, ratio 0.78. The first metasomal segment has a total of 10 carinae, the second through fourth segments have eight carinae, and the



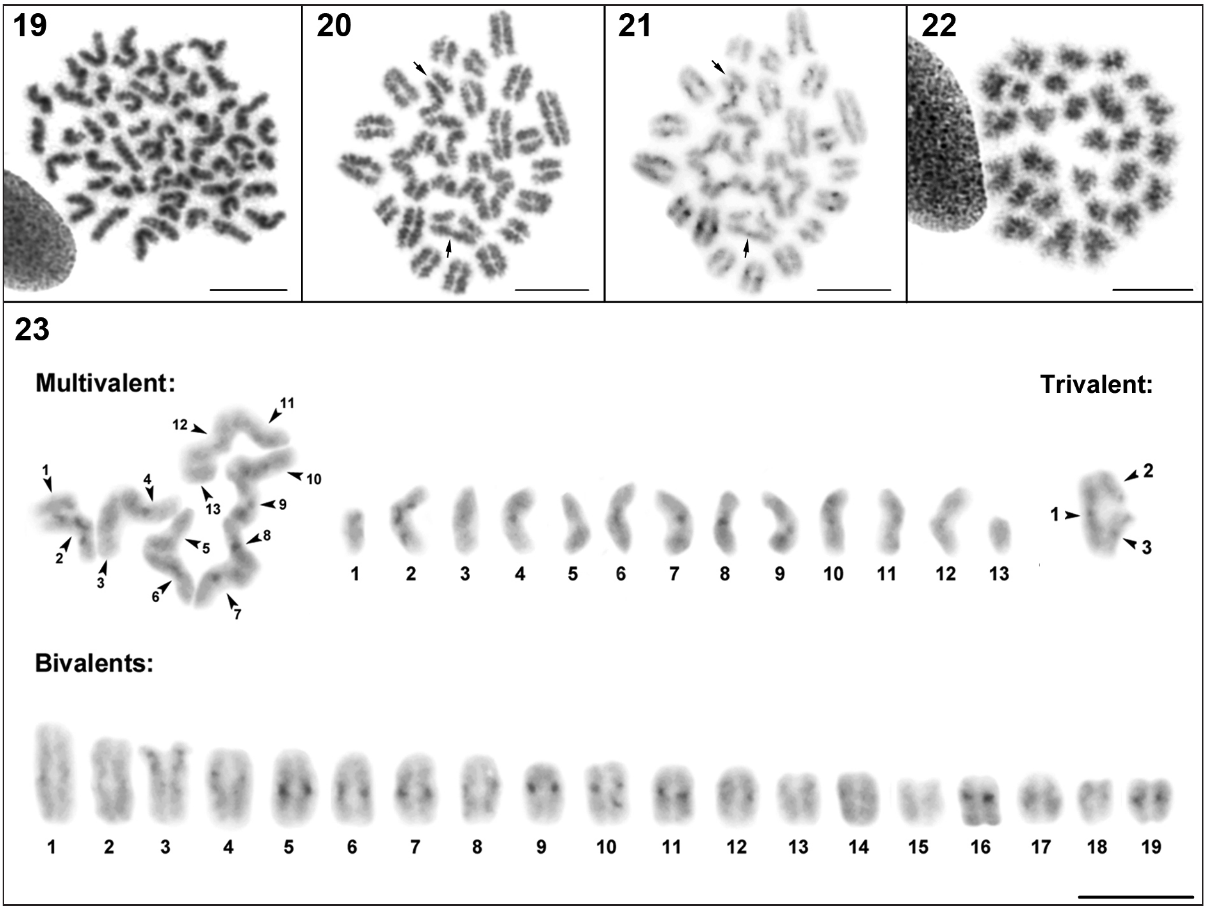
Figures 8–14. *Heterometrus minotaurus* sp. nov., male holotype: 8–10. Pedipalp chela: (8) dorsal view; (9) external view; (10) ventral view; 11–13. Pedipalp patella: (11) dorsal view; (12) external view; (13) ventral view; (14) pedipalp movable finger, dorsal view. The trichoborial pattern is indicated in Figs 8–13. Scale bar = 10 mm.

fifth segment has five to seven carinae. All metasomal segments are smooth, laterally as well as ventrally sparsely granulated by minute granules; dorsal surface entirely smooth without granules. Metasoma is sparsely hirsute by reddish setae. Telson more hirsute by long spiniform setae, smooth. Vesicle elongate ellipse. Aculeus curved, approximately as long as vesicle.

Measurements (in mm): Total length of male holotype 83; carapace length 14.1, width 13.8; metasoma and telson length 48.3; first metasomal segment length 5, width 6.4; second metasomal segment length 5.9, width 5.7; third metasomal segment length 6.5, width 5.35; fourth metasomal segment length 8.2, width 4.85; fifth metasomal segment length 11.4, width 4.5; telson length 11.3; telson width 4.4; telson depth 3.7; pedipalp femur length 15.1, width 5.1; pedipalp patella length 15.45, width 5.3; chela length 28.8; manus width 8.4; movable finger length 16.1.

Karyotype. Karyotype of *H. minotaurus* sp. nov. was established on the basis of meiotic and mitotic cells of male holotype. Specimen exhibits monocentric type of chromosomes that differ one from another by their morphology and size. The chromosome complement consists of 54 chromosomes (Fig. 19), which gradually decrease in size from 2.82% to 1.06% of the diploid set. In all postpachytene and metaphase I nuclei, we observed 19 homomorphic bivalents (Figs 20, 21) and two different types of multivalent association - one trivalent and one multivalent chain composed of 13 distinct chromosomes (Figs 20, 21). Despite the presence of unusual multivalents, all observed metaphases II had the same number of chromosomes (n=27) (Fig. 22). Chiasmata between homologous chromosomes during the first meiotic division were not observed. Cytological observation gave no evidence for existence of diplotene-diakinesis stage during prophase I. No positively or negatively heteropycnotic bodies were present during the meiotic division. We also did not detect presence of morphologically differentiated sex chromosomes in all analysed nuclei. Morphology of some chromosomes was not clearly visible in standard chromosome slides stained directly by Giemsa. For better visualization of centromere, which is formed by constitutive heterochromatin, and thus proper determination the morphology of individual chromosomes, we applied C-banding accompanied by DAPI staining. However, in some cases centromere was not clearly detected even after using C-banding technique. When Giemsa stained chromosomes had noticeable primary constrictions, C-bands corresponded with these areas. The karyotype of *H. minotaurus* sp. nov. is composed mainly by metacentric chromosomes (Fig. 23). Trivalent consists of one large metacentric and two small telocentric chromosomes, multivalent chain is

Figures 15–18. *Heterometrus minotaurus* sp. nov. male holotype, distal segments of legs, retrolateral view: (15) Leg I; (16) Leg II; (17) Leg III; (18) Leg IV. Scale bar = 5 mm.



Figures 19–23. *Heterometrus minotaurus* sp. nov., male holotype, chromosomes: (19) Mitotic metaphase (2n=54), Giemsa staining; (20) Postpachytene, Giemsa staining; (21) Postpachytene, C-banding, DAPI staining (inverted); (22) One sister metaphase II (n=27), Giemsa staining; (23) Karyotype based on postpachytene, DAPI staining (inverted). Arrows indicate multivalents, arrowheads show individual chromosomes in multivalents. Scale bar = 10 μm.

compiled of 10 metacentric, 1 submetacentric and 2 small telocentric chromosomes (multivalent chromosomes No. 1, 13). Nevertheless, position of centromeres in chromosomes associated in multivalent chain was not entirely evidential. In case of standard bivalents, 13 of them are metacentric, 3 submetacentric (pairs No. 4, 13, 18), 2 subtelocentric (pairs No. 15, 17) and 1 telocentric (pair No. 3).

Distribution. Thailand (known from the type locality only).

Etymology. This species is named according to the mystic creature from ancient Greek mythology. Minotaurus was a beast with the head of a bull on the man's body.

Discussion

In this article, we described *Heterometrus minotaurus* sp. nov. from Thailand. As with other scorpionid genera, males of the genus *Heterometrus* are important and essential for species identification and delimitation (Tahir and Prendini 2014). The *H. minotaurus* male holotype is clearly distinguished from the Indonesian *H. longimanus*, the morphologically closest species, by several features mentioned above. Unfortunately, no females of the described species are known, and so we cannot comment on aspects of potential sexual dimorphism. To date, the genus *Heterometrus* includes 36 species. However, Tahir and Prendini (2014) pointed out certain weak spots in some previous taxonomic works, e.g. the recent description of *H. atrascorpius* Mirza *et al.*, 2012 was based on only non-adult specimens. It is therefore possible that a detailed revision of this genus could reveal some taxa of dubious validity. Despite this assumption, the total number of currently recognized *Heterometrus* species might not reflect the real diversity of this endemic Asian genus. A reliable answer to the question of its true diversity requires further detailed taxonomic studies with wider sampling and ideally additional methodical approaches, e.g. molecular phylogeny or cytogenetics. New methodical approaches and interspecific markers not only could bring us deeper knowledge of species diversity and phylogeography but might also resolve some discrepancies in traditional *Heterometrus* taxonomy, which is based mainly on external morphology. It is highly advantageous to include new markers as a part of prospective descriptions of new species, where the type series is established and analysed. For this reason, we cytogenetically analysed the *H. minotaurus* sp. nov. male holotype, and also preserved muscular tissue in absolute ethanol for prospective DNA analysis. *H. minotaurus* sp. nov. exhibits 54 chromosomes with a typical monocentric nature. This type of chromosome with a localized centromere is typical for scorpions with the exception of the family Buthidae, which represents the only lineage of scorpions displaying chromosomes with a holocentric nature (Schneider *et al*. 2009). Previously published cytogenetic studies have shown that the genus *Heterometrus* exhibits a wide range of diploid numbers of chromosomes from 2n=56 (*H. spinifer* (Ehrenberg, 1828)) to 2n=112 (*H. gravimanus* (Pocock, 1894b)). Accordingly, *H. minotaurus* sp. nov. with 2n=54 represents a species with the lowest known number of diploid sets within its genus. With 8 species cytogenetically analysed so far, the genus *Heterometrus* forms one of the more frequently studied scorpion genera from a cytogenetic point of view (see Schneider *et al*. 2015). Interestingly, all analysed specimens (except for *H. spinifer*) have come from a geographically restricted area, the Indian peninsula, and have displayed great interspecific differences. However, previous information on specific karyotypes cannot be used currently for this group's taxonomy. The authors did not focus on precise determination and delimitation of the specimens analysed and also did not use karyology results for the taxonomy of particular taxa. Moreover, descriptions of karyotypes were published mainly during the 1960s. Since that time, the definition of some *Heterometrus* taxa has changed rapidly, especially during recent decades (e.g. Couzijn 1981, Kovařík 2004). We can illustrate this problem through the example of *H. longimanus*, the species morphologically closest to *H. minotarus* sp. nov. According to Srivastava and Agrawal (1961), the Indian population of this species exhibits 64 chromosomes. As far as we now know, however, *H. longimanus* is endemic to Indonesia and does not occur in India (see Kovařík 2009). Therefore, the obtained cytogenetic data on Indian populations belong to another as yet undefined species. This scenario could be applied similarly to other cytogenetically studied *Heterometrus* species from India.

As indicated above, differences in karyotypes are present among particular *Heterometrus* taxa and these specific markers have the potential to be usable in the taxonomy of this scorpion group. However, it is advisable to analyse more specimens or populations for appropriate and precise results. Moreover, this approach is also necessary to understand potential chromosomal polymorphism in the species analysed, e.g. whether the unusual chromosomal rearrangements detected (see Kovařík *et al*. 2015a, Kovařík *et al*. 2015b) are fixed in particular scorpion populations of concrete species. In the case of *H. minotaurus* sp. nov., unfortunately, we had only one live specimen available for cytogenetic analysis, and a great deal of additional material from the type location would be required to answer this question. This wide-sampling analysis might also help to clarify what role chromosomal rearrangements could play in causing multivalent associations in karyotype differentiation and by extension how they participate in the reproductive isolation of closely related species in the genus *Heterometrus*.

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