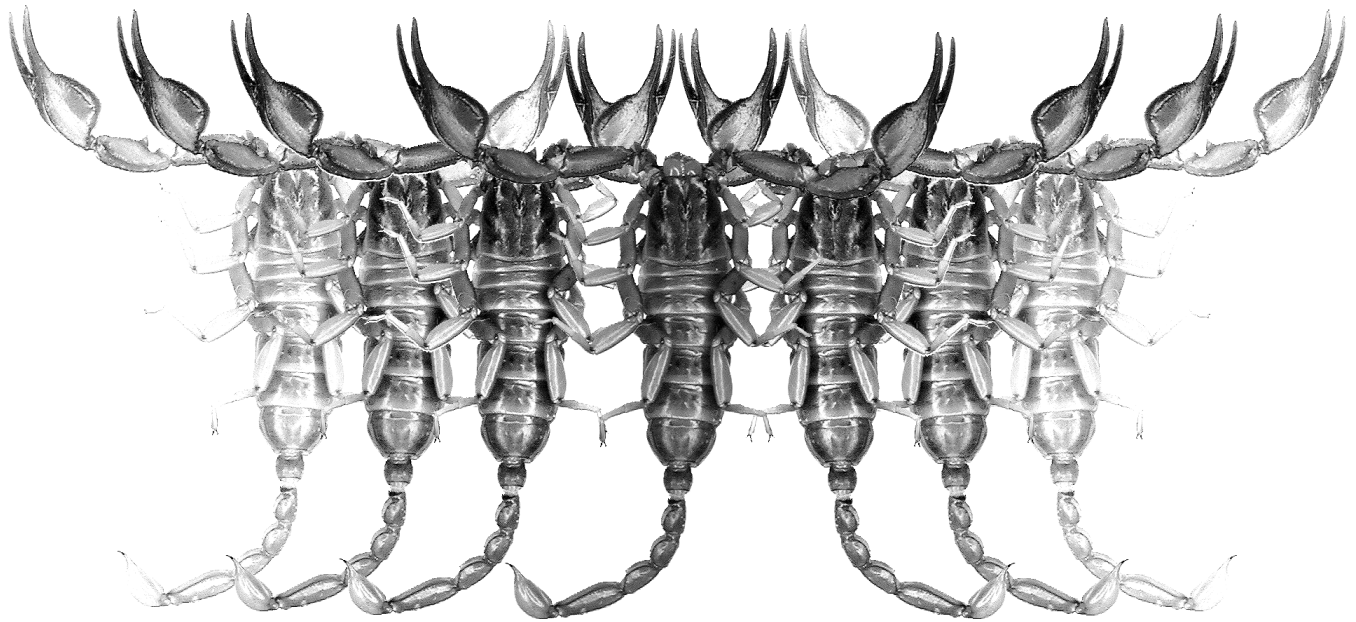


# *Euscorpilus*

Occasional Publications in Scorpiology



## **The First Molecular Phylogeny of Buthidae (Scorpiones)**

**Victor Fet, Benjamin Gantenbein, Alexander V. Gromov,  
Graeme Lowe and Wilson R. Lourenço**

February 2003 — No. 4

# *Euscorpius*

## Occasional Publications in Scorpiology

*EDITOR*: Victor Fet, Marshall University, 'fet@marshall.edu'

*ASSOCIATE EDITOR*: Michael E. Soleglad, 'soleglad@la.znet.com'

*Euscorpius* is the first research publication completely devoted to scorpions (Arachnida: Scorpiones). *Euscorpius* takes advantage of the rapidly evolving medium of quick online publication, at the same time maintaining high research standards for the burgeoning field of scorpion science (scorpiology). *Euscorpius* is an expedient and viable medium for the publication of serious papers in scorpiology, including (but not limited to): systematics, evolution, ecology, biogeography, and general biology of scorpions. Review papers, descriptions of new taxa, faunistic surveys, lists of museum collections, and book reviews are welcome.

### Derivatio Nominis

The name *Euscorpius* Thorell, 1876 refers to the most common genus of scorpions in the Mediterranean region and southern Europe (family Euscorpiidae).

*Euscorpius* is located on Website '<http://www.science.marshall.edu/fet/euscorpius/>' at Marshall University, Huntington, WV 25755-2510, USA.

---

The International Code of Zoological Nomenclature (ICZN, 4th Edition, 1999) does not accept online texts as published work (Article 9.8); however, it accepts CD-ROM publications (Article 8). *Euscorpius* is produced in two *identical* versions: online (ISSN 1536-9307) and CD-ROM (ISSN 1536-9293). Only copies distributed on a CD-ROM from *Euscorpius* are considered published work in compliance with the ICZN, i.e. for the purposes of new names and new nomenclatural acts. All *Euscorpius* publications are distributed on a CD-ROM medium to the following museums/libraries:

- **USNM**, United States National Museum of Natural History (Smithsonian Institution), Washington, DC, USA
- **AMNH**, American Museum of Natural History, New York, USA
- **CAS**, California Academy of Sciences, San Francisco, USA
- **FMNH**, Field Museum of Natural History, Chicago, USA
- **MCZ**, Museum of Comparative Zoology, Cambridge, Massachusetts, USA
- **MNHN**, Museum National d'Histoire Naturelle, Paris, France
- **NMW**, Naturhistorisches Museum Wien, Vienna, Austria
- **BMNH**, British Museum of Natural History, London, England, UK
- **MZUC**, Museo Zoologico "La Specola" dell'Universita de Firenze, Florence, Italy
- **ZISP**, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia
- **WAM**, Western Australian Museum, Perth, Australia
- **NTNU**, Norwegian University of Science and Technology, Trondheim, Norway

---

Publication date: 26 February 2003

## The first molecular phylogeny of Buthidae (Scorpiones)

Victor Fet<sup>1</sup>, Benjamin Gantenbein<sup>2</sup>, Alexander V. Gromov<sup>3</sup>, Graeme Lowe<sup>4</sup>  
and Wilson R. Lourenço<sup>5</sup>

<sup>1</sup>Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2510, USA

<sup>2</sup>Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, United Kingdom

<sup>3</sup>Institute of Zoology, Al-Farabi 93, Akademgorodok, Almaty 480060, Kazakhstan

<sup>4</sup>Monell Chemical Senses Center, 3500 Market St., Philadelphia, Pennsylvania 19104-3308, USA

<sup>5</sup>Département de Systématique et Evolution, Section Arthropodes (Arachnologie), Muséum National d'Histoire Naturelle, 61, rue de Buffon, 75005 Paris, France

---

### Summary

The first partial phylogeny of family Buthidae (17 genera) is presented, based on molecular data (16S rRNA mitochondrial DNA). The strong support for a monophyletic Old World group of 13 genera (mainly Palearctic desert forms) is demonstrated, while representative genera from Madagascar (*Grosphus*) and Southeast Asia (*Lychas*) group outside, as well as New World genera *Centruroides* and *Rhopalurus*. A very strong support is observed for the first time for three groups of Old World genera: (a) *Compsobuthus*, *Mesobuthus*, *Liobuthus*, *Kraepelinia*; (b) *Hottentotta*, *Buthacus*; (c) *Orthochirus*, *Anomalobuthus*. Phylogenetic hypotheses are discussed.

---

### Introduction

All scorpion species with highly potent, mammal-specific neurotoxins belong to the family Buthidae, which therefore has medical importance. Buthidae is the largest family of extant scorpions: Fet & Lowe (2000) listed 73 genera and 529 species in the "Catalog of Scorpions of the World", and it is likely that many more species exist in nature. Buthids are ecologically diverse and successful, widely spread across the globe (Nenilin & Fet, 1992; Lourenço, 1996, 2000), and known from the fossil record since Paleocene-Eocene (Baltic amber). This family also represents one of the fundamental evolutionary lineages of extant scorpions, so-called orthobothriotaxic Type A (Vachon, 1974; Sissom, 1990; Sologlad & Fet, 2001).

The existing keys for the family Buthidae (Stahnke, 1972; Sissom, 1990) make use of a number of variable morphological characters, including carapacial and metasomal carination, leg spination, dentition of chelicerae

and pedipalp chela, and patterns of trichobothria. Although morphology of the family is relatively well studied (see e.g. Levy & Amitai, 1980), there has been no attempt to produce a modern cladistic phylogeny of Buthidae. Historical suggestions to divide Buthidae into two or more groups have not been consistent, and no subfamilies or tribes are recognized at the moment (for a detailed discussion, see Fet & Lowe, 2000: 54).

It is well known (Fet & Lowe, 2000) that the genus-level diversity in Buthidae is much higher in the Old World than in the New World, although species-level diversity is higher in the New World. To some extent it could be an artifact of "splitting" tendencies of buthid taxonomists who worked in the Old World. On the other hand, arid (desert) Old World scorpion taxa often present clearly derived morphological characters, which have been traditionally used for delineation of genera in scorpions. Many of those characters are undoubtedly a result of parallelism, e.g., sand-living adaptations (Fet et al., 1998).

The arid Buthidae of the Palearctic biogeographic region have been extensively studied - first as members of a "catch-all" genus *Buthus* (Pocock, 1893; Kraepelin, 1899; Birula, 1917a, 1917b); then, due mostly to the detailed studies of Vachon (1952), as members of many genera (of which some are monotypic). Since the 1950s, there has been a considerable increase in description of new forms and regional faunas of Buthidae, which proved to be especially rich in the Palearctic, Afrotropic and Neotropical regions.

We present here the first data supporting a phylogeny of 17 genera of Buthidae, focusing on the Palearctic fauna (13 genera), and based on the sequence analysis of mitochondrial DNA (16S rRNA gene). We included representatives of all most common, widely distributed buthid genera from the Palearctic region (such as *Androctonus*, *Buthus*, *Hottentotta*, *Mesobuthus*). These genera have wide geographic ranges (e.g. *Mesobuthus*, found from Greece to China; *Androctonus*, from Morocco to India), and their radiation has been associated with aridification of the Palearctic deserts (Fet, 1994).

## Methods and Material

**Material.** Data on the specimens and DNA sequence accession numbers are presented in Table 1. Detailed data on locality and specimens are available from the authors. We previously published sequence of *Centruroides exilicauda* (Gantenbein et al., 2001). *Lychas mucronatus* sequence was taken from GenBank; it was published in Giribet et al. (2001) without locality data.

**DNA Analysis.** For DNA analyses, the total DNA was extracted from fresh or preserved (94-98% ethanol) muscle tissue (usually pedipalp or metasoma) using standard extraction methods. We amplified a 400-450 base pair (bp) fragment of the 16S rRNA mitochondrial (mt) DNA by polymerase chain reaction (PCR) using the primers and conditions as described in Gantenbein et al. (1999). PCR primers were removed by polyethylene glycol (PEG 8000) precipitation or by Qiagen purification kit, and templates were directly sequenced on one strand using one of the PCR primers and the BigDye sequencing kit (Version 2, Roche). Sequencing reactions were ethanol/sodium-acetate precipitated and were run on an ABI377XL sequencer. All sequences were checked manually for sequencing errors. All sequences were deposited in the GenBank nucleotide sequence database (<http://www.ncbi.nlm.nih.gov>).

**Phylogenetic Analysis.** The DNA sequences were aligned using ClustalX (Thompson et al. 1997) and by eye. We applied Maximum Likelihood (ML) to the DNA sequence data. All ambiguities and gaps were removed (Felsenstein, 1981; Swofford et al., 1996), leaving 296 bp. Initially, a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) using JC69 distance (Jukes & Cantor, 1969) was

calculated for all three genes separately. This tree was then used for estimating the maximum likelihood (ML) score and parameters from 56 different substitution models. Maximum likelihood ratio tests were then performed in a nested design using the program MODELTEST 3.06 (Posada & Crandall, 1998) implemented in PAUP\* 4.0b10 (Swofford, 1998). Details about likelihood ratio tests (LRT) are given in Huelsenbeck & Crandall (1997) and Huelsenbeck & Rannala (1997). The tree space was explored by 100 heuristic tree searches and by randomizing the order of the sequence input in PAUP\*. The rate heterogeneity among sites was assumed to follow a gamma distribution (shape parameter  $\alpha$  was ML-estimated) with four categories, each represented by its mean (Yang, 1996). In a further step, the molecular clock hypothesis (i.e., equal rates across all sequences) was tested using the  $\chi^2$  approximated likelihood ratio test statistics. The stability of nodes was evaluated with NJ non-parametric bootstrapping of ML-distances using the parameters estimated from the heuristic tree search.

As an outgroup we selected *Pseudochactas ovchinnikovi* Gromov, 1998, the relict taxon of the monotypic family Pseudochactidae, which is considered to be the basal group to Buthidae (Soleglad & Fet, 2001).

## Results

The phylogeny resulting from the ML analysis is presented in Fig. 1. The hierarchical likelihood ratio testing revealed the TVM + I +  $\Gamma$  substitution model as most likely. The estimated parameters are given in the legend of Fig. 1. The molecular clock hypothesis was not rejected ( $2\delta = 2(\ln L_0 - \ln L_1) = 2(2721.21 - 2714.12) = 14.16$ ,  $P_{\chi^2} = 0.59$ ,  $df = 16$ ). Thus, the tree search was carried out enforcing a global molecular clock.

For distance matrix representing sequence divergence, showing both uncorrected  $p$  and TVM + I +  $\Gamma$ , see the **Appendix**. Strong support is demonstrated for a monophyletic group of 13 genera (bootstrap 83 %). Representative genera from Madagascar (*Grosphus*) and Southeast Asia (*Lychas*) group outside this clade, as well as New World genera *Centruroides* and *Rhopalurus*. The latter two genera formed a strongly supported clade (91 %) as well (Fig. 1). Within the "13 genera clade", two moderately supported (60-65 %) clades emerged. Within the first clade (6 genera), a very strong support was observed for two groups of genera: (a) *Compsobuthus*, *Mesobuthus*, *Liobuthus*, *Kraepelinia* (93 %), and (b) *Hottentotta* and *Buthacus* (85 %). Within the second clade (7 genera), most branching orders were weakly supported (54-56 %), except a very strongly supported (91 %) clade of *Orthochirus* and *Anomalobuthus*. Relationships were less defined within a cluster of five genera (*Buthus*, *Androctonus*, *Leiurus*, *Vachoniolus*, *Apistobuthus*).

Taxon	Country of origin	Collector(s)	GenBank sequence accession number
<b>Fam. Buthidae</b>			
<i>Androctonus amoreuxi</i> (Audouin, 1826)	Morocco	A. Scholl	AY226175
<i>Anomalobuthus rickmersi</i> Kraepelin, 1900	Kazakhstan	V. Fet & A. Gromov	AY226170
<i>Apistobuthus pterygocercus</i> Finnegan, 1932	Oman	G. Lowe	AY226178
<i>Buthacus yotvatensis</i> Levi, Amitai & Shulov, 1973	Oman	G. Lowe	AY226173
<i>Buthus occitanus</i> (Amoreux, 1789)	Morocco	A. Scholl	AY226172
<i>Centruroides exilicauda</i> (Wood, 1863)	Arizona, USA	J. Bigelow	AJ288640 (Gantenbein et al., 2001)
<i>Compsobuthus arabicus</i> Levy, Amitai & Shulov, 1973	United Arab Emirates	B. & I. Gantenbein	AY226177
<i>Grosphus madagascariensis</i> (Gervais, 1843)	Madagascar	W.R. Lourenço	AY226168
<i>Hottentotta jayakari</i> (Pocock, 1895)	United Arab Emirates	B. & I. Gantenbein	AY226176
<i>Kraepelinia palpator</i> (Birula, 1903)	Turkmenistan	A. Gromov	AY226181
<i>Leiurus quinquestriatus</i> (Ehrenberg, 1828)	Oman	G. Lowe	AY226174
<i>Liobuthus kessleri</i> Birula, 1898	Turkmenistan	V. Fet & A. Gromov	AY226180
<i>Lychas mucronatus</i> (Fabricius, 1798)	n/a (Southeast Asia?)	n/a	AF370855 (Giribet et al., 2001)
<i>Mesobuthus eupeus</i> (C. L. Koch, 1839)	Kazakhstan	A. Gromov	AY228141
<i>Orthochirus innesi</i> Simon, 1910	Morocco	A. Scholl	AY226171
<i>Rhopalurus abudi</i> Armas & Marcano Fondeur, 1987	Dominican Republic	D. Huber	AY226169
<i>Vachoniolus globimanus</i> Levy, Amitai & Shulov, 1973	Oman	G. Lowe	AY226179
<b>Fam. Pseudochactidae (outgroup)</b>			
<i>Pseudochactas ovchinnikovi</i> Gromov, 1998	Uzbekistan	V. Fet & A. Gromov	AY226167

**Table 1:** Data on specimens.

## Discussion

A variety of buthid genera inhabit North Africa, Middle East and Central Asia (Vachon, 1952; Levy & Amitai, 1980; Sissom, 1990; Fet & Lowe, 2000). These desert areas of the Palearctic have been a rich source of speciation for arid animal taxa (Kryzhanovskiy, 1965). It is assumed that extensive Tertiary aridification of the southern Palearctic region facilitated radiation in scorpions (Nenilin & Fet, 1992; Fet, 1994; Fet et al., 1998), as well as in other groups of organisms.

Since no morphology-based phylogenetic analysis exists for these taxa, all relationships supported within a monophyletic group of “13 genera” are being observed here for the first time. Of these 13 genera, most belong to the desert Palearctic fauna, and only *Hottentotta* and *Mesobuthus* have species found also outside of the arid

zone of the Palearctic region. Morphological studies of the analysed genera (e.g. Vachon, 1952; Levy & Amitai, 1980) did not contain any phylogenetic hypotheses on their relationships. Below, we briefly address the biogeographic details and evolutionary issues that the obtained DNA phylogeny appears to document and clarify.

### *Compsobuthus*, *Mesobuthus*, *Liobuthus*, and *Kraepelinia*

This strongly supported (93 % bootstrap) monophyletic group includes genera either monotypic and endemic for Central Asia and Iran (*Liobuthus*, *Kraepelinia*), or diverse and widely distributed in many Palearctic arid landscapes (*Compsobuthus* is found from North Africa to Iran; *Mesobuthus*, from the Balkans to China). The branching order of this group of genera

strongly supports *Compsobuthus* as a sister group to the other three genera. Note that of this group only species of *Compsobuthus* are found in Africa; others are almost exclusively Asian. Relationship of the ingroup *Mesobuthus*, *Liobuthus* and *Kraepelinia* was not resolved. Biogeographic observations in Turkmenistan (Fet, 1994) indicate that *Liobuthus* is a widespread endemic genus of Central Asian sand deserts, highly adapted to a psammophile way of life (Fet et al., 1998). Conversely, *Kraepelinia* is a reclusive small genus burrowing in clay soils of southern Turkmenistan and Iran, and known from only a couple of localities (Fet, 1984, 1989, 1994). It also has highly derived features including a remarkable “stocky” pedipalp chela (Vachon, 1974, Fig. 235-239). Both of these are highly specialized genera endemic to desert Central Asian deserts. On the other hand, *Mesobuthus* is widely distributed, with a center of its diversity in Iran and Central Asia. Phylogenetic studies are now under way of the genus *Mesobuthus* (Gantenbein et al., in progress), revealing an order of branching that implies western Palearctic forms of this genus are ancestral, and forms distributed further east and north in Central Asia, Mongolia and China are derived. Modern range of the genus *Mesobuthus* represents the northern limit of scorpion distribution in Asia (Gromov, 2001). This corresponds to the general trend of the evolution for Central Asian desert biota (Kryzhanovsky, 1965; Fet, 1994).

### ***Hottentotta* and *Buthacus***

Both genera are widespread in western Palearctic arid zone, in addition, a number of species of the diverse genus *Hottentotta* are also commonly found further south in the Afrotropical region in various ecological conditions. It remains to be seen whether the genus *Hottentotta* as accepted now is monophyletic. *Buthacus* includes eight psammophile species from North Africa and Middle East.

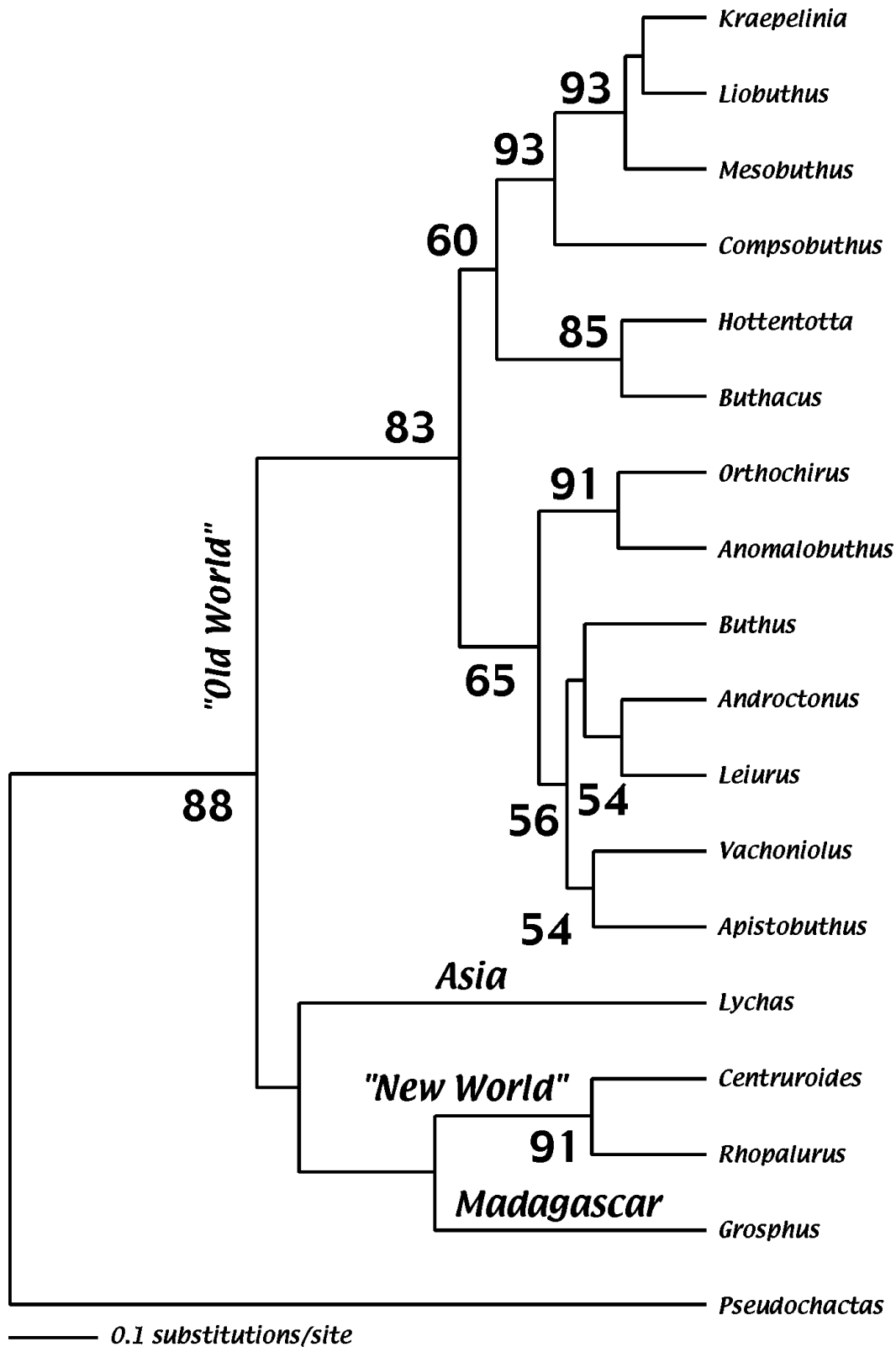
### ***Orthochirus* and *Anomalobuthus***

While the monotypic genus *Anomalobuthus* is a psammophile endemic of Central Asian deserts (Turkmenistan, Uzbekistan, Kazakhstan; Fet, 1994), species of the genus *Orthochirus* inhabit deserts from North Africa to Central Asia and India. The fact that these two genera are phylogenetically close is a new and important discovery. Earlier observations in nature (A. V. Gromov, pers. obs.) demonstrated some common behavioral traits of these species (the resting position of metasoma and its characteristic “jerky” movements). The genus *Orthochirus* has been under significant scrutiny by taxonomists for a long time, as it (as several other similar genera) possesses a number of unique features (morphosculpture of metasoma with “pitted” appearance due to small

punctations; reduced telson; shape of carapace; Vachon, 1952; Levy & Amitai, 1980). Birula (1917a, 1917b) even created a separate subfamily Orthochirinae based on this genus. A highly derived position of *Orthochirus* in our phylogeny casts a serious doubt on the possible validity of Orthochirinae in terms of phylogenetic classification, as its acceptance will require creation of at least several subfamilies to accommodate monophyly for other clades of Buthidae. It remains to be seen if a number of other buthid genera recently split off, or associated with, *Orthochirus*, are in fact closely related to this genus.

### ***Buthus*, *Androctonus*, *Leiurus*, *Vachoniolus* and *Apistobuthus***

This group, only weakly supported in our analysis, includes widespread *Buthus*, *Androctonus*, and *Leiurus*, all very typical for the western Palearctic deserts (North Africa, Middle East) (Vachon, 1952; Levy & Amitai, 1980; Sissom, 1994), and two psammophile genera from the Arabian Peninsula, *Vachoniolus* and *Apistobuthus* (two species each) It is worth noting that this loose group includes the most toxic of Old World scorpion genera, *Androctonus* and *Leiurus*. This result suggests that *Androctonus* and *Leiurus* could be more closely related to each other than to any other genus represented in our study, though their coupling is weakly supported in this analysis. This is consistent with the data on their venom (Loret & Hammock, 2001; see also below). The weak support for *Vachoniolus* and *Apistobuthus* could indicate their common origin as psammophile species in the Arabian deserts; *Apistobuthus* possesses some highly derived features (“flared” metasomal segments; Vachon, 1960). Placement of *Vachoniolus* within this clade, apart from *Buthacus*, was surprising because these two genera are closely similar in their external morphology, and indeed female *Vachoniolus* was originally confused with *Buthacus* (Levy et al., 1973). Both genera express relatively abbreviated pedipalps and the loss of carapacial and mesosomal carination. The data here implies these similarities may be a result of convergence, possibly associated with psammophilic lifestyles. However, the grouping of *Buthacus yotvatensis* (the example sequenced here) with *Hottentotta jayakari* is also correlated with distinctive characters separating this species from other members of *Buthacus*, i.e., large size and development of dense pilosity on the metasomal carinae, a feature typical of the “hirsute” group of *Hottentotta* sp., as exemplified by *H. jayakari*. The divergence of other diagnostic characters between *Hottentotta* and *Buthacus*, related to tarsal setation, are likely due to ecological radiation into different substrata (i.e., rock and gravel vs. sand). Molecular data from the smaller species of *Buthacus* may yet reveal a relationship to *Vachoniolus*.



**Figure 1:** Maximum Likelihood (ML) tree of Old World and New World Buthidae inferred from a 296 bp fragment of the mitochondrial ribosome 16S region. The DNA substitution model was TVM + I +  $\Gamma$ ; base frequencies:  $\pi_A = 0.34$ ,  $\pi_T = 0.42$ ,  $\pi_C = 0.14$ ,  $R_{\text{matrix}} = (A-G = 11.50, A-C = 2.95, C-G \sim 0, A-T = 2.44, G-T = 1, C-T = 11.50)$ , gamma shape parameter  $\alpha = 0.67$ , and proportion of invariable sites = 0.28, respectively. The tree was rooted using the outgroup species *Pseudochactas ovchinnikovi*.

## Other genera

Outgroups used in our analysis originate from the New World (genera *Centruroides* and *Rhopalurus*), Madagascar (endemic genus *Grosphus*) and Southeast Asia (*Lychas*). Not surprisingly, we see the expected, strongly supported close relationship of the genus *Centruroides* (North and Central America, Caribbean, northern South America) and *Rhopalurus* (South America and Caribbean). Absence from our analysis of additional genera from tropical Africa and Asia did not allow us to achieve phylogenetic resolution outside of the “13 genera clade”; it is most important, however, that both tropical Old World genera (*Grosphus* and *Lychas*) group outside of the “13 genera clade”, although their common clade with New World species is not supported. A possible evolutionary scenario for Buthidae could include separate evolution in Laurasia and Gondwana, after the split of Pangaea, as it was for many other groups of organisms. In this case the “13 genera clade” could represent lineages of northern (Laurasian) evolution. If support is obtained in the future for the clade of modern Neotropical and Afrotropical scorpions (represented by *Centruroides*, *Rhopalurus*, and *Grosphus* in our analysis), it would strongly suggest a typical Gondwanan “signature”, with New World buthids originating from South America as tropical forms. The latter hypothesis would correlate with patterns of modern diversity of arid scorpions in the New World, where scorpion radiation in the deserts of both Americas is largely observed in Bothriuridae, Vaejovidae and Iuridae. The few Buthidae which inhabit deserts of North America (species of *Centruroides*) could have dispersed to the arid landscapes from the New World tropics. It is worth noting that one of the major characters subdividing Buthidae at this moment, so-called alpha versus beta orientation of dorsal trichobothrial on pedipalp femur (Vachon, 1975; Sissom, 1990) also is consistent with our phylogeny, the “13 genera clade” all being beta pattern.

## Independent origin of psammophily

An important issue in scorpion evolution, discussed in detail by Fet et al. (1998) is parallelism in the highly adaptive features allowing psammophily (life in sand). For the first time, within the “13 genera clade”, we document an independent origin of buthid psammophily in *at least four clades*: (a) *Liobuthus* (Central Asia); (b) *Buthacus* (North Africa, Middle East); (c) *Anomalobuthus* (Central Asia); and (d) *Vachoniolus* and *Apistobuthus* (Middle East) (the latter two could have shared a psammophilic ancestor). Especially impressive is strongly supported independent evolution of psammophily in *Liobuthus* and *Anomalobuthus*, two virtually sympatric monotypic genera inhabiting the vast deserts of Central Asia from the Caspian Sea in Turkmenistan to

Syr-Darya River basin (Fet, 1989, 1994). A further study of molecular phylogeny of these genera is under work (Fet et al., in progress).

## Origin of mammal-specific toxins

Another important issue in scorpion evolution is, of course, evolution of toxins. Our phylogeny supports a common origin of Old World lineages of Buthidae with the most potent neurotoxic venom: the genera *Androctonus* and *Leiurus*. These and other Palearctic species in which toxins have been studied (*Buthus*, *Mesobuthus*, *Hottentotta*, *Orthochirus*) are all present in our “13 genera clade”. All these forms share separate mammal- and insect-specific neurotoxins specific for Na<sup>+</sup> channels (Loret & Hammock, 2001). At the same time, New World genera have potent toxins (*Centruroides*, *Tityus*) acting on both mammals and insects. We suggest that the origin of this feature could lie in the Laurasia versus Gondwana split as discussed above. Thus, the separate mammal-specific Na<sup>+</sup> toxins could have evolved during aridification of the Palearctic in the Tertiary period, when one of the most important selective factors was rapid radiation of small burrowing mammals (mostly rodents) in arid landscapes. Naturally, such newcomers to the scorpion environment as rodents would be a direct competitor for space (burrows) and in addition important nocturnal predators, as many of them are today (McCormick & Polis, 1990). Such pressure explains emergence of specific mammal-targeting toxins (used for defense, not for foraging) in predominantly burrow-living Palearctic buthids – as opposed to largely vegetation-inhabiting New World buthids.

Loret et al. (1991) discovered a toxin (AahIT4) with “ancestral” properties in a North African population of *Androctonus australis*. It is able to bind to both alpha and beta sites in both vertebrates and insects, as opposed to other, more specialized toxins. This led to a biogeographic hypothesis that buthid toxins acting on mammals “originated from North Africa” (Loret et al., 1991; Loret & Hammock, 2001). However, the currently accepted *A. australis* ranges from Mauritania to India (Fet & Lowe, 2000: 69), and the center of origin of this species is unknown; further, none of the congeneric *Androctonus* species or non-African Old World buthids were tested for the existence of the AahIT4 or related toxins. The constant reference to *A. australis* as the “Sahara scorpion” in toxicological literature leads to unjustified and superficial conclusions about evolution of both species and toxins, further confusing this complicated issue. According to our analysis, *Androctonus* does not have a basal position in buthid phylogeny, thus “ancestral” properties of *Androctonus* toxin should be a plesiomorphic character. Only an independently assessed evolutionary history of Buthidae will allow us to “plot” toxin features on the supported phylogeny. In



fact, some studies demonstrated that toxicity to both mammals and insects, as opposed to either mammal- or insect-specificity, could depend on minor amino acid changes (Kopeyan et al., 1993), thus a long evolutionary history is not required for explanation of toxin evolution.

### Further Directions of Research

Although our preliminary study covered only a selection of all existing buthid genera, we detected several important evolutionary trends in the radiation of Palearctic buthids, which include some of the most toxic scorpion species (genera *Androctonus* and *Leiurus*). In future, a combined morphological and molecular (DNA) phylogenetic analysis including more taxa will further clarify relationships within Buthidae. The major issues remaining to be addressed are the relationship between Palearctic, Afrotropical, and Oriental buthids in the Old World, and the origin of New World buthids (according to our hypothesis, from Gondwana). Many speciose buthid genera, first of all *Tityus* and *Centruroides* in the New World, have to be subjected to phylogenetic analysis, and could be eventually split into several genera. A large number of branches should be added to our preliminary tree in the future, many from exotic taxa in remote and difficult localities and hardly accessible. We are convinced that only the collaborative efforts of many scorpion taxonomists from many parts of the world will bring sufficient knowledge on evolution of Buthidae.

### Acknowledgements

We thank Michael E. Soleglad for his valuable advice in taxonomic issues. Iris Gantenbein, Mark Barker, Ian Towler, Elizabeth Fet, Joshua Greenwood and Erica Price all provided a skilled assistance in the laboratory. Adolph Scholl, Dietmar Huber, and Joe Bigelow kindly supplied us with specimens. Research of B.G. was supported by a grant No. 81BE-64218 from the Swiss National Science Foundation (SNF) and the Royal Society. Travel of V.F. and A.V.G. to Central Asia was supported by the National Geographic Society Research and Exploration grant No. 7001-01. V.F. and A.V.G. are grateful for the hospitality and help to Alex and Elena Kreuzberg, Victor Lukarevsky, Alexander Tarabrin, Ogulsoltan Ashirova, Gochmurat Kutlyev, and Sergei Morozov, and everybody else who assisted in field logistics in Turkmenistan, Uzbekistan, and Kazakhstan. G.L. thanks HH The Minister of National Heritage and Culture, Sultanate of Oman for sponsoring visits to study scorpions, Michael D. Gallagher, Said Ali Said Al-Farsi, Saddiqa Rhamdan, Khair Bin Antar Salim, and all past and present museum staff and collectors who helped with scorpion collections and research in Oman.

### References

- BIRULA, A. A. (Byalynitsky-Birula, A.A.) 1917a. *Arachnoidea Arthrogastra Caucasica. Pars I. Scorpiones*. Mémoires du Musée du Caucase, Tiflis: Imprimerie de la Chancellerie du Comité pour la Transcaucasie, A(5), 253 pp. (in Russian). English translation: 1964. *Arthrogastric Arachnids of Caucasica. I. Scorpions*. Jerusalem: Israel Program for Scientific Translations, 170 pp.
- BIRULA, A. A. (Byalynitskii-Birulya, A. A.) 1917b. Faune de la Russie et des pays limitrophes fondée principalement sur les collections du Musée Zoologique de l'Académie des Sciences de Russie. Arachnides (Arachnoidea). 1(1): xx, 227 pp. (in Russian). Petrograd. English translation: 1965. Fauna of Russia and Adjacent Countries. Arachnoidea. Vol. I. Scorpions. Jerusalem: Israel Program for Scientific Translations, xix, 154 pp.
- FELSENSTEIN, J. 1981. Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. *Evolution*, 35: 1229-1242.
- FET, V. 1984. New for the USSR genus and species of scorpions from Badkhyz: *Kraepelinia palpator* (Birula, 1903) (Scorpiones, Buthidae). *Proceedings of the Academy of Sciences Turkmen SSR (ser. biol. sci.)*, 4: 37-43 (in Russian).
- FET, V. 1989. A catalogue of scorpions (Chelicerata: Scorpiones) of the USSR. *Rivista Museo Civico di Scienze Naturali "E. Caffi" (Bergamo)*, 13(1988): 73-171.
- FET, V. 1994. Fauna and zoogeography of scorpions (Arachnida: Scorpiones) in Turkmenistan. Pp. 525-534 in V. Fet & K. I. Atamuradov (eds.). *Biogeography and ecology of Turkmenistan* (Monographiae Biologicae 72). Dordrecht: Kluwer Academic Publishers.
- FET, V. & G. LOWE. 2000. Family Buthidae. Pp. 54-286 in: Fet, V., W. D. Sissom, G. Lowe & M. E. Braunwalder. *Catalog of the Scorpions of the World (1758-1998)*. 690 pp. New York: New York Entomological Society.
- FET, V., G. A. POLIS & W. D. SISSOM. 1998. Life in sand: the scorpion model. *Journal of Arid Environments*, 39: 609-622.

- GANTENBEIN, B., V. FET, C.R. LARGIADÈR & A. SCHOLL. 1999. First DNA phylogeny of the genus *Euscorpium* Thorell, 1876 (Scorpiones, Euscorpidae) and its bearing on the taxonomy and biogeography of this genus. *Biogeographica* (Paris), 75: 59-72.
- GANTENBEIN, B., V. FET & M. D. BARKER. 2001. Mitochondrial DNA markers reveal a deep, divergent phylogeny in *Centruroides exilicauda* (Wood) (Scorpiones: Buthidae). Pp. 235-244 in: Fet, V. & P. A. Selden (ed.). *Scorpions 2001. In Memoriam Gary A. Polis*. Burnham Beeches, Bucks.: British Arachnological Society.
- GIRIBET, G., G. D. EDGECOMBE & W. C. WHEELER. 2001. Arthropod phylogeny based on eight molecular loci and morphology. *Nature*, 413 (6852): 157-161.
- GROMOV, A. V. 1998. A new family, genus and species of scorpions (Arachnida, Scorpiones) from southern Central Asia. *Zoologicheskyy Zhurnal*, 77(9): 1003-1008 (in Russian, English summary).
- GROMOV, A. V. 2001. On the northern boundary of scorpions (Arachnida: Scorpiones) in Central Asia. Pp. 301-307 in: V. Fet & P.A. Selden (Eds.). *Scorpions 2001. In Memoriam Gary A. Polis*. British Arachnological Society, Burnham Beeches, Bucks, UK.
- HUELSENBECK, J. P. & K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics*, 28: 437-66.
- HUELSENBECK, J. P. & B. RANNALA. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, 276: 227-232.
- JUKES, T. H. & C. R. CANTOR. 1969. Evolution of protein molecules. Pp. 21-132 in Munro, H. N. (ed.), *Mammalian protein metabolism*. Academic Press, New York.
- KOPEYAN, C., P. MANSUELLE, M. F. MARTIN-EAUCLAIRE, H. ROCHAT & F. MIRANDA. 1993. Characterization of toxin III of the scorpion *Leiurus quinquestriatus quinquestriatus*: a new type of alpha-toxin highly toxic both to mammals and insects. *Natural Toxins*, 1: 308-312.
- KRAEPELIN, K. 1899. Scorpiones und Pedipalpi. In F. Dahl (ed.), *Das Tierreich*. Herausgegeben von der Deutschen Zoologischen Gesellschaft. Berlin: R. Friedländer und Sohn Verlag, 8 (Arachnoidea): 1-265.
- KRYZHANOVSKY, O. L. 1965. *The composition and origin of the terrestrial fauna of Middle Asia (based primarily on Coleopteran material)*. Moscow & Leningrad: Nauka, 420 pp. (in Russian). English translation, 1980. New Delhi, India (Smithsonian Institution Translation Program).
- LEVY, G. & P. AMITAI. 1980. *Fauna Palaestina. Arachnida I. Scorpiones*. Jerusalem: The Israel Academy of Sciences and Humanities, 130 pp.
- LEVY, G., P. AMITAI & A. SHULOV. 1973. New scorpions from Israel, Jordan and Arabia. *Journal of the Linnaean Society of London. Zoology*, 52 (2): 113-140, figs. 1-48.
- LORET, E. & B. HAMMOCK. 2001. Structure and neurotoxicity of scorpion toxins. Pp. 204-233 in Brownell, P. H. & G. A. Polis (eds). *Scorpion Biology and Research*. Oxford: Oxford University Press.
- LORET, E. P., M. F. MARTIN-EAUCLAIRE, P. MANSUELLE, F. SAMPIERI, C. GRANIER & H. ROCHAT. 1991. An anti-insect toxin purified from the scorpion *Androctonus australis* Hector also acts on the alpha- and beta-sites of the mammalian sodium channel: sequence and circular dichroism study. *Biochemistry*, 30(3): 633-640.
- LOURENÇO, W. R. 1996. The biogeography of scorpions. *Revue Suisse de Zoologie*, Vol. hors série II : 437-448 (Proceedings of the XIIIth International Congress of Arachnology, Geneva, 3-8 September 1995; ed. V. Mahnert).
- LOURENÇO, W. R. 2000. Panbiogéographie, les familles des scorpions et leur répartition géographique. *Biogeographica* (Paris), 76: 21-39.
- MCCORMICK, J. & G. A. POLIS. 1990. Prey, predators, and parasites. Pp. 294-320 in Polis, G. A. (ed.), *Biology of Scorpions*. Stanford, California: Stanford University Press.
- NENILIN, A. B. & V. FET. 1992. Zoogeographical analysis of the world scorpion fauna (Arachnida: Scorpiones). *Arthropoda Selecta*, 1(2): 3-31 (in Russian).
- POCOCK, R. I. 1893. Notes on the classification of scorpions, followed by some observations on synonymy, with descriptions of new genera and species. *Annals and Magazine of Natural History*, (6), 12: 303-330.

- POSADA, D. & K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14: 817-818.
- SAITOU, N. & M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.
- SISSOM, W. D. 1990. Systematics, biogeography and paleontology. Pp. 64-160 in Polis, G. A. (ed.), *Biology of Scorpions*. Stanford, California: Stanford University Press.
- SISSOM, W. D. 1994. Descriptions of new and poorly known scorpions of Yemen (Scorpiones: Buthidae, Diplocentridae, Scorpionidae). *Fauna of Saudi Arabia*, 14: 3-39.
- SOLEGLAD, M. E. & V. FET. 2001. Evolution of scorpion orthobothriotaxy: a cladistic approach. *Euscorpilus*, 1: 1-38.
- STAHNKE, H. L. 1972. A key to the genera of Buthidae (Scorpionida). *Entomological News*, 83(5): 121-133.
- SWOFFORD, D. L. 1998. *PAUP\* Phylogenetic analysis using parsimony (\*and other methods)*. Version 4. Sunderland, Massachusetts: Sinauer Associates.
- SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL & D. M. HILLIS. 1996. Phylogenetic inference. Pp. 407-510 in Hillis, D. M., C. Moritz & B. K. Mable (ed.). *Molecular Systematics*. Sunderland, Massachusetts: Sinauer Associates.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN & D. G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24: 4876-4882.
- VACHON, M. 1952. *Etudes sur les Scorpions*. Algiers: Institut Pasteur d'Algérie, 482 pp.
- VACHON, M. 1960. Quelques remarques sur *Apistobuthus pterygocercus* Finnegan, Scorpion (Buthidae) habitant l'Arabie. *Archives de l'Institut Pasteur d'Algérie*, 38: 399-405.
- VACHON, M. 1974. Etude des caractères utilisés pour classer les familles et les genres de Scorpions (Arachnides). 1. La trichobothriotaxie en Arachnologie, Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. *Bulletin du Muséum National d'Histoire Naturelle, Paris*, 3(140): 857-958.
- VACHON, M. 1975. Sur l'utilisation de la trichobothriotaxie du bras des pédipalpes des Scorpions (Arachnides) dans le classement des genres de la famille des Buthidae Simon. *Comptes Rendus des Séances de l'Académie des Science, Paris, (D)*, 281(21): 1597-1599.
- YANG, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution*, 11: 367-372.

## Appendix Distance Matrix

Distance matrix of the sequence divergence (uncorrected  $p$ ) (lower left) and of TVM + I +  $\Gamma$  (upper right) calculated from pairwise comparisons of 16S mtDNA sequences among Old and New World buthids. Gaps were not considered, ML parameters were the same as designated in Fig 1.

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>
<b>1 Kraepelinia</b>	-	0.133	0.177	0.270	0.371	0.312	0.436	0.454	0.446	0.520	0.465	0.526	0.437	0.699	1.229	0.729	0.630	0.890
<b>2 Liobuthus</b>	0.101	-	0.185	0.331	0.422	0.315	0.428	0.413	0.488	0.474	0.445	0.496	0.495	0.749	1.209	0.895	0.688	0.795
<b>3 Mesobuthus</b>	0.122	0.125	-	0.326	0.466	0.417	0.513	0.561	0.482	0.593	0.658	0.669	0.582	0.841	1.335	0.873	0.831	1.010
<b>4 Compsobuthus</b>	0.159	0.176	0.179	-	0.395	0.352	0.448	0.482	0.428	0.521	0.573	0.418	0.491	1.122	1.283	0.788	0.736	0.906
<b>5 Hottentotta</b>	0.193	0.213	0.223	0.206	-	0.156	0.430	0.402	0.421	0.423	0.446	0.516	0.360	0.709	1.067	0.732	0.733	0.691
<b>6 Buthacus</b>	0.179	0.182	0.220	0.189	0.115	-	0.440	0.409	0.412	0.401	0.418	0.464	0.390	0.693	1.024	0.705	0.679	0.703
<b>7 Orthochirus</b>	0.206	0.209	0.230	0.206	0.209	0.206	-	0.172	0.249	0.323	0.355	0.301	0.427	0.926	1.540	0.597	0.519	0.693
<b>8 Anomalobuthus</b>	0.206	0.206	0.233	0.213	0.206	0.206	0.115	-	0.328	0.276	0.348	0.400	0.367	0.874	1.211	0.739	0.623	0.660
<b>9 Buthus</b>	0.209	0.226	0.223	0.206	0.216	0.206	0.149	0.179	-	0.243	0.315	0.218	0.277	0.894	1.234	0.658	0.599	0.613
<b>10 Vachoniolus</b>	0.223	0.216	0.233	0.226	0.206	0.199	0.176	0.162	0.152	-	0.221	0.252	0.213	0.980	1.093	0.763	0.717	0.593
<b>11 Apistobuthus</b>	0.223	0.226	0.260	0.243	0.216	0.199	0.193	0.193	0.176	0.142	-	0.310	0.272	0.952	1.610	0.709	0.688	0.571
<b>12 Androctonus</b>	0.230	0.226	0.253	0.206	0.230	0.209	0.166	0.189	0.139	0.149	0.169	-	0.201	1.181	1.131	0.739	0.690	0.678
<b>13 Leiurus</b>	0.199	0.226	0.236	0.230	0.182	0.189	0.203	0.189	0.162	0.132	0.155	0.128	-	0.978	1.124	0.796	0.790	0.640
<b>14 Lychus</b>	0.240	0.260	0.277	0.301	0.253	0.250	0.270	0.274	0.277	0.280	0.294	0.297	0.280	-	0.858	0.598	0.656	0.717
<b>15 Pseudochactas</b>	0.307	0.311	0.324	0.324	0.304	0.291	0.321	0.307	0.318	0.294	0.331	0.284	0.291	0.257	-	1.439	1.223	1.205
<b>16 Centruroides</b>	0.257	0.284	0.287	0.270	0.274	0.253	0.250	0.277	0.264	0.274	0.270	0.270	0.277	0.233	0.311	-	0.218	0.471
<b>17 Rhopalurus</b>	0.247	0.257	0.280	0.264	0.274	0.250	0.226	0.240	0.250	0.260	0.270	0.257	0.267	0.247	0.297	0.139	-	0.622
<b>18 Grosphus</b>	0.297	0.291	0.301	0.280	0.267	0.260	0.253	0.264	0.253	0.253	0.253	0.260	0.264	0.264	0.314	0.213	0.250	-