Koedoe 28: 35-45 (1985)

A RE-APPRAISAL OF THE ROCK SCORPIONS (Scorpionidae: *Hadogenes)*

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2000

Abstract — The morphological similarity between species of the rock scorpion genus *Hadogenes* has given rise to a great deal of controversy amongst taxonomists over the last eighty years. To resolve these difficulties, species of the genus were re-appraised in terms of their chromosome number and an electrophoretic analysis of venom proteins. The relationships arising from these data were integrated with morphological characteristics in order to get a more realistic appreciation of the genus. It emerges that the genus consists of 14 recognised species, some of which represent as yet unnamed species complexes. Taxonomic changes proposed are *H. lawrencei* sp. res., *H. zuluanus* stat. nov, *H. gracilis fluvianus* and *H. gracilis namaquensis* = *H. phyllodes* syn. n. A new key to the species is provided.

Introduction

Scorpions of the genus *Hadogenes* Kraepelin 1894, commonly known as 'rock scorpions', are restricted to the southern half of the Afrotropical region where they are widespread and common. Since Kraepelin erected the genus in 1894, five checklists and keys have been published *viz.* Kraepelin 1899; Hewitt 1918; Lawrence 1955; Newlands 1972 and Lamoral 1979.

Both Hewitt (1918) and Lawrence (1955) employed the degree of curvature of the carapace anterior margin to subdivide the genus into 3 species groups. Newlands (1970) demonstrated that the degree of curvature is extremely variable, even in a specified population. Accordingly, the keys of Hewitt and

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Lawrence can no longer be considered valid. The checklists and keys of Newlands (1972) and Lamoral (1979) were devised solely for the fauna of Namibia and are therefore of limited application.

Hadogenes includes some of the smallest scorpionids as well as the world's largest, which reach lengths of up to 210 mm. These species are highly specialized being found exclusively in rock cracks and crevices. It follows therefore, that the distribution of the various allopatric species is limited to distinct rocky outcrops and mountain ranges. Examples of this insular distribution are frequent. In the Pretoria area, *H. gunningi* inhabits the Magaliesberg while *H. gracilis* is found only on a series of rocky outcrops 2-3 km north of the Magaliesberg. The narrow valley between them acts as a natural barrier across which gene flow has not been demonstrated. Further numerous examples are to be found in the Namib desert with members of *H. tityrus* species group each occupying a discrete mountain range or rocky outcrop.

Using a traditional morphological approach to the taxonomy of the group, the fact that most species are morphologically similar and occur allopatrically, could be used to argue that *Hadogenes* consists of a few polymorphic species with a wide distribution. The present study however, presents evidence to support the contrary view that the genus consists of a large number of allopatric species, each with a restricted distribution. Three criteria were used to assess the taxonomic status of members of the genus, notably morphology, chromosome analysis and electrophoretic examination of the venom proteins.

Studies of trichobothrial patterns have been in vogue since Vachon (1973) stated that trichobothria (mechano-receptive setae on the pedipalps of scorpions) are extremely constant within scorpion genera and species. The present study investigated whether this is valid for *Hadogenes*. *Hadogenes* has never been studied as regards karyotype or the specificity of the venom proteins.

Materials and Methods

For the taxonomic studies, material was borrowed from local and overseas museums (Newlands 1980). Vachon's (1973) procedure for characterizing scorpion trichobothrial patterns could not be applied to *Hadogenes* as this genus has so many trichobothria that patterns were not discernible. Accordingly, counts for each palpal segment were recorded separately for left and right palps.

The method of Crozier (1968) was used for preparing chromosomal spreads from freshly collected material accumulated during field trips throughout South Africa, Namibia and Zimbabwe. Crozier's method was modified in that 0,5% sodium citrate was substituted for the 1% hypotonic solution used to swell the chromosomes. In the majority of cases, spreads could be obtained from male gonads only.

The venoms used in the electrophoretic study were obtained by electric stimulation using the method of Wittemore, Keegan, Fitzgerald, Bryant & Flanigan (1963). The samples were dissolved in 0,2 ml of a solution

containing 1% sodium dodecyl sulphate (SDS), 1% 2-mercapto-ethanol and 8M urea and heated in a boiling waterbath for 1 min. Electrophoresis was carried out in vertical 7,5% polyacrylamide slabs containing 1% SDS and stained with Coomassie Blue R250 (Newlands 1980).

Results

Trichobothrial analysis

Vachon (1973) has stated categorically that the number and position of the trichobothria are constant for a given species. Examination of all South African scorpion genera, except *Hadogenes,* has confirmed Vachon's statement (Newlands, unpublished data). In species of *Hadogenes,* the numbers and position of trichobothria are not constant except for those on the femur (Table 1). On the patella and chela, the number and position of the trichobothria invariably differ from specimen to specimen and frequently from left to right pedipalp.

Table 1

Trichobothrial counts on the right pedipalp of species of Hadogenes

SPECIES	MEAN COUNTS					
	Femur	Patella	Chela	Total for Pedipalp	No. of specimens	Range
H. tityrus species complex	3	62	72	137	52	109-182
H. lawrencei	3	62	68	133	7	125-142
H. minor	3	58	127	188	23	182-203
H. trichiurus	3	76	70	149	17	124-191
H. zuluanus	3	80	84	167	10	141-193
H. bicolor	3	85	94	182	47	171-192
H. granulatus	3	86	93	182	65	161-202
H. gunningi	3	81	74	158	55	143-172
*H. paucidens**	3	111	139	253	1	— —
H. taeniurus	3	99	100	202	20	188-212
H. troglodytes	3	88	83	174	288	161-190
H. gracilis	3	94	89	186	232	172-204
H. phyllodes	3	81	74	158	80	137-184
H. zumpti	3	90	91	184	5	175-197

*Species known from a single type specimen

Chromosome analysis

Eleven of the fourteen species were analysed in terms of chromosome number. Many tissues were investigated as a source of somatic chromosomes to avoid total reliance on testicular tissue where meioses may cloud the issue. Tissues used repeatedly were digestive gland, ganglia, muscle, coxal gland, gonads and developing embryos. Spreads were found with such low frequency in all but testicles as to make the other tissues impractical. The reasons for this are not clear but these scorpions take 8-10 years to reach

maturity and have a gestation period of up to 18 months. This extended development may well be responsible for the low somatic cellular division rate observed. In cases where somatic chromosomes were obtained, the chromosome number agreed with that determined from testicular tissue.

In examining the chromosomal spreads, karyotyping was not attempted. In Fig. 1 typical examples of high and low chromosome numbers are depicted and illustrate the futility of recognising and characterizing specific bivalents for karyotyping.

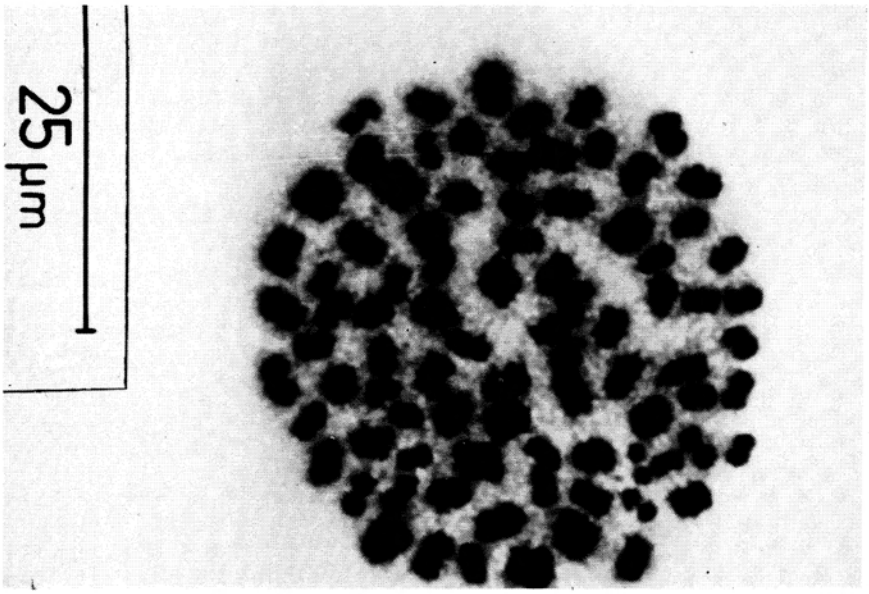
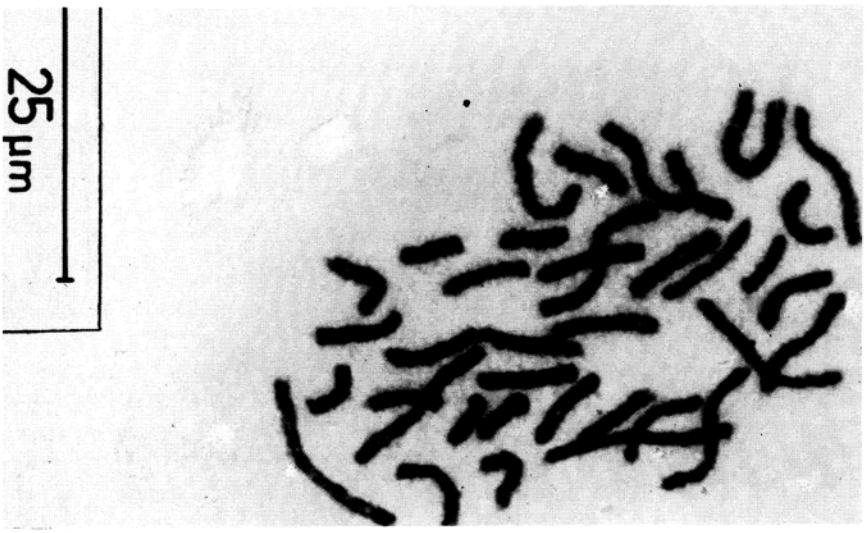


Fig. 1. Typical examples of chromosomal spreads showing (a) high *(H. tityrus* sibling) and (b) low *(H.phyllodes)* counts.



Chromosome numbers for species of *Hadogenes* ranged from 2n = 60 in *H. zumpti* to 2n = 174 in the case of an as yet unnamed sibling species in the *H. tityrus* complex. Except for *H. bicolor* and *H. granulatus* (2n = 96), all other species were found to have differing but constant chromosome numbers.

Venom proteins

In Figs 2 to 5 a number of polyacrylamide slab gel electrophoregrams are presented. Combinations of specimens have been run simultaneously to illustrate specific points of similarity or divergence between species. The interpretation of such gels has limitations and, as such, differences between specimens may be missed. However, for a given species, the venom protein patterns are remarkably constant and so when interspecies differences are obtained, these can confidently be interpreted as reflecting species' specific characteristics.

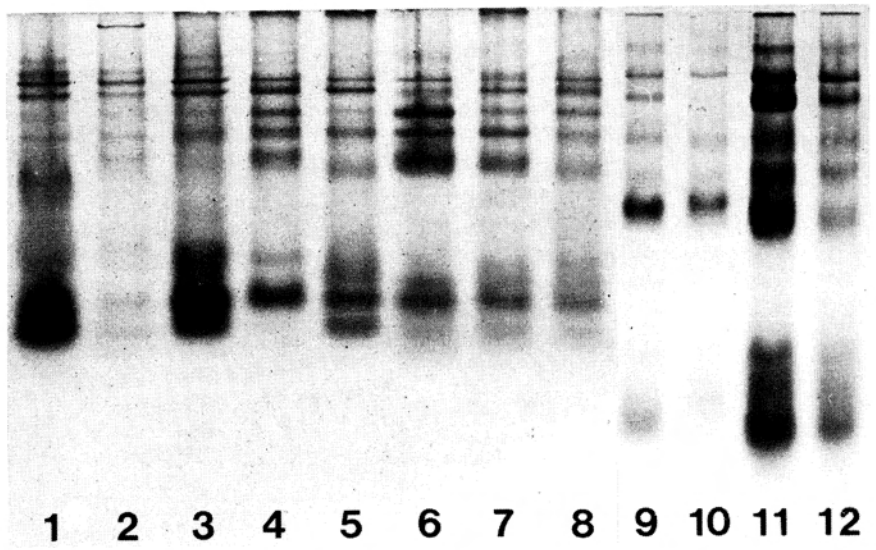


Fig. 2. Selected members of the *H. tityrus* complex, composite of two gels. 1-2. *H. tityrus* sibling from Windhoek: 3-4. *H. tityrus* sibling from Lekkersing: 5-8. *H. tityrus* sibling from Helskloof: 9-10 *H. lawrencei* from Hauchab: 11-12. *H. tityrus* sibling 25 km north of Lekkersing.

Discussion

The trichobothrial analysis showed that *Hadogenes* is unique in having the highest counts ever recorded for a scorpion genus. The trichobothria differ in number and position from individual to individual, and from left to right pedipalp in the same individual, in a random fashion. In all other species and genera examined by Vachon (1973) the number and position of trichobothria was constant for given taxa. Certain species are prone to considerable trichobothrial variation, in particular *H. tityrus, H. trichiurus, H. zuluanus* and *H. phyllodes*. In the case of all except *H. zuluanus,* it would seem that these species might well represent groups of sibling species. This assertion is

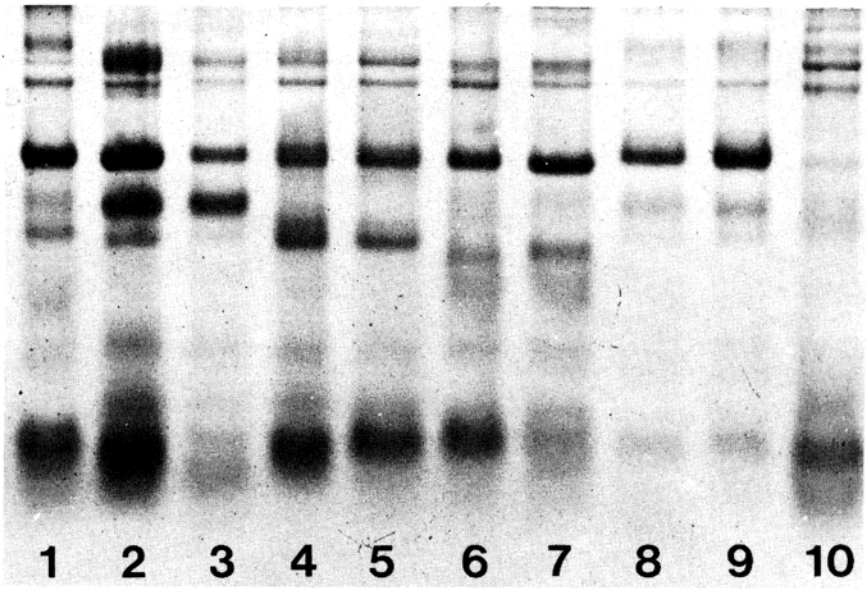


Fig. 3. Gel showing differences in the venom proteins of a group of species which are morphologically very similar. 1. *H. gunningi,* Pretoria: 2-3. *H. bicolor* Bronkhorstspruit: 4-5 *H. bicolor.* The Downs: 6-7. *H. zuluanus* Magudu: 8-9. *H. trichiurus* Rooinekpass. 10. Representative of the *H. tityrus* group from Windhoek included for comparison.

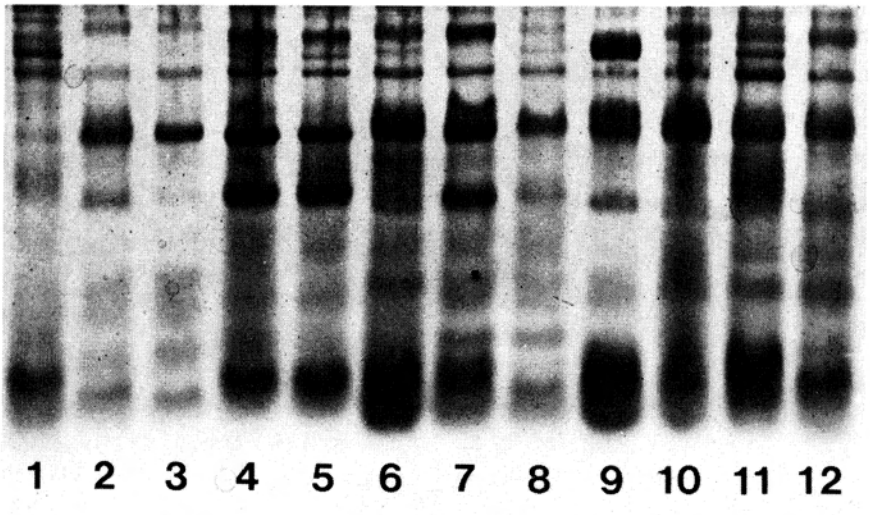


Fig. 4. Venom proteins of the *H. gracilis — H. troglodytes* group compared to the *H. tityrus* pattern. 1. *H. tityrus,* Windhoek: 2. *H. troglodytes,* Tuli Block: 3. *H. troglodytes* Gaborone: 4-5. *H. gracilis,* Brits: 6,10,11. *H. phyllodes* Keimoes: 7-8. *H. phyllodes,* Griekwastad: 9. *H. phyllodes* Kamieskroon: 12. *H. phyllodes,* Zeekoebaardsnek.

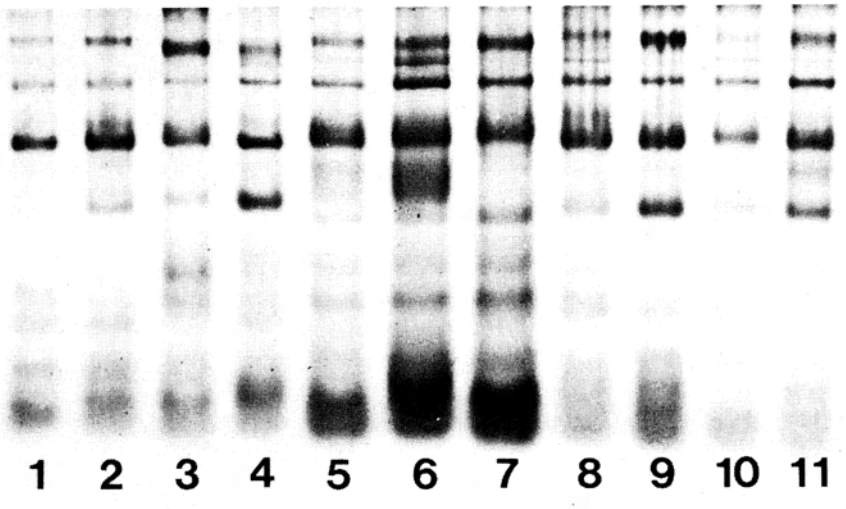


Fig. 5. Venom proteins of members of the *H. troglodytes* and *H. phyllodes* groups. 1-2. *H. troglodytes* from Botswana: 3-4. *H. gracilis* from Brits: 5-6. *H. phyllodes* from Keimoes. 7. *H. phyllodes* from Zeekoebaardsnek. 8. *H. phyllodes* from Leerkrans: 9-10. *H. phyllodes* Griekwastad: 11. *H. phyllodes* from Bergenaarspad.

borne out in part by the electrophoretic data obtained for *H. phyllodes*.

Members of the *H. tityrus* complex have the highest chromosome numbers reported for scorpions. Previously 2n = 120 was the highest number on record (Guénin 1957). In Table 2, three members of the *H. tityrus* complex have chromosome numbers higher than 2n = 120. It appears that the more specialized species of the genus (the larger species with considerable sexual dimorphism, higher pectinal tooth counts, etc.) have the lowest chromosome numbers. In all specimens examined in this study, those species most similar morphologically had very similar chromosome numbers. Accordingly in this genus it would appear that chromosome number accurately reflects phylogenetic relationships.

The results of the electrophoretic study of venom proteins also exhibit a similar relationship in that those species most closely related on morphological and chromosomal grounds have similar protein electrophoregrams.

The venom patterns illustrate a number of other interesting relationships. For example, Lamoral (1979) inferred from morphology alone that *H. tityrus* was a polymorphic species with a wide distributional range, and in so doing he included *H. lawrencei* as a synonym of *H. tityrus*. The fact that three members of the *H. tityrus* complex which were karyotyped, differed considerably in chromosome number (Table 2) indicates that three distinct species are involved. In Fig. 2, this is further substantiated by the venom protein patterns. *H. lawrencei* differs clearly from three other sibling species found at

Table 2

Diploid chromosome numbers for species of Hadogenes

H. species*	174
H. tityrus	168
H. lawrencei	132
H. minor	106
H. taeniurus	100
H. bicolor	96
H. granulatus	96
H. gunningi	88
H. troglodytes	84
H. gracilis	80
H. phyllodes	72
H. zumpti	60

*As yet unnamed species of the *H. tityrus* species complex collected at Lekkersing, Richtersveld.

distinct localities in the Richtersveld (Lekkersing and Helskloof), and at a third near Windhoek on the farm Aapies. In view of the above we have no hesitation in resurrecting the species *H. lawrencei.*

Morphologically *H. granulatus, H. bicolor, H. gunningi* and *H. trichiurus zuluanus* are all very similar. In this study only two could be examined and gave identical chromosome numbers. In view of this, the electrophoretic results were of considerable interest. In Fig. 3, it is evident that the four species examined are distinct. Of interest here is that specimens of *H. bicolor* from two localities (*viz.* The Downs and Bronkhorstspruit) were distinctly different. The fact that no morphological differences between these specimens could be detected, suggests that *H. bicolor* may comprize a species complex. The subspecies *H. trichiurus zuluanus* gave a venom pattern quite distinct from the typical *H. trichiurus* and morphologically the subspecies was found to be more similar to the *H. granulatus/H. bicolor* group. Accordingly we elevate *H. zuluanus* to full species status.

In the *H. troglodytes* group, inadequate live material was available to resolve all the problems. Although morphologically almost identical, *H. troglodytes* and *H. gracilis* proved quite distinct chromosomally (Table 1). However, the subspecies of *H. gracilis (viz. H.g. fluvialis* and *H.g. namaquensis)* represent a problem as, except for colour, they could not be distinguished from what has come to be accepted as *H. phyllodes* (see Lamoral 1979). Accordingly, for the purpose of this study, these subspecies have been regarded as conspecific with *H. phyllodes.* It is unlikely that these can be regarded as subspecies of *H. gracilis,* as *H. gracilis* has a very restricted distribution (approximately 3 x 50 km strip east of Rustenburg) and *H. gracilis* is almost certainly a sibling species of *H. troglodytes.* At least 600 km of rockless savannah separate *H. gracilis* from its described subspecies. Figure 4 portrays the similarities of the *H. troglodytes/H. phyllodes* group in contrast to a member of the *H. tityrus* group. That *H. phyllodes* possibly consists of a species complex becomes evident on examination of the gel depicted in Fig.

5. Venom samples representing various geographically distinct populations of *H. phyllodes* from the north-western Cape show regional variation (*e.g.* Keimoes, Springbok, O'Kiep, Louisvale and Limewall near Griekwastad). This variation is evidence of a probable species complex. Detailed studies need to be conducted on this group before final conclusions can be reached. A new species to be named *H. zumpti* from the Richtersveld (Newlands, *in prep.)* also forms part of the *H. troglodytes/H. phyllodes* group.

The data obtained from the present study have made it possible to compile the following key to the species of *Hadogenes*.

Key to the species of *Hadogenes*, Kraepelin, 1894.

1. Movable finger of chela longer than length of chela as measured along the posterior-ventral keel 2
   Movable finger of chela shorter than length of chela as measured along the postero-ventral keel 3
2. Chela with 1-3 accessory trichobothria between *it* and *ib* on the anterior surface (= interior surface of Vachon, 1973) of chela; anterior margin of carapace deeply excavated with well-rounded frontal lobes. Males with terminal spiniform granules on the dorso-lateral keels of metasomal segment IV ..... *H. zuluanus* Lawrence
   Chela without accessory trichobothria between *it* and *ib* on anterior surface of chela; anterior carapace margin almost straight or weakly concave, male without spiniform granules of dorsal surface of metasomal segment IV ..... *H. gracilis* Hewitt
3. Metasoma considerably shorter than mesosoma and prosoma combined. Total number of trichobothria less than 140 per pedipalp and diploid chromosome number in excess of 130 4
   Metasoma longer than mesosoma and prosoma combined, more than 150 trichobothria per pedipalp. Diploid chromosome number less than 129 5
4. length of pedipalpal patella shorter than carapace length, length of pedipalpal femur equal to the carapace length. Pectinal teeth: female, 9-13; male, 13-16. Diploid chromosome number 168
   ..... *H. tityrus* complex
   Length of pedipalpal patella longer than the carapace length, length of pedipalpal femur 1,3 times the carapace length. Pectinal teeth: female, 6; male, 11. Diploid chromosome number 132
   ..... *H. lawrencei* Newlands
5. Adult males and females without basal lobe on the chela tarsus, length of the chela more than 5 times its width. Dorsal surface of the pedipalpal patella with two trichobothria ... *H. zumpti* Newlands
   Adults males and females with basal lobe on the chela tarsus, length of chela less than four times the width. Dorsal surface of pedipalpal patella with at least four trichobothria 6
6. Triangular inset positioned well back on carapace such that the frontal lobes are exposed as a pair of well-rounded protruding structures 7

Triangular inset positioned anteriorly on the carapace such that the anterior margin is straight or slightly concave and that the frontal lobes do not protrude 8

7. Dorsal keel of patella distinct and granular. Lateral ocelli slightly smaller in size than the median ocelli. Metasoma of adult male not elongated ..... *H. bicolor* Purcell
Dorsal keel of patella absent, lateral ocelli distinctly larger than the median ocelli; metasoma of adult male considerably elongated ..... *H. trichiurus* (Gervais)

8. Metasomal segment I wider than high 9
Metasomal segment II higher than wide 12

9. Subspiniform granules present on the venter of metasomal segment II of equal size or larger than those present on the venter of metasomal segment V 10
If present, weak or subspiniform granules on the venter of metasomal segment II considerably smaller than those on the venter of metasomal segment V 11

10. Legs and sternite much lighter in colour than tergites. Carapace length of female distinctly shorter than the combined length of metasomal segments I and II ..... *H. taeniurus* (Thorell)
Legs and sternites the same colour as the tergites. Carapace length of female equal in length to the combined length of metasomal segments I and II. Male unknown ..... *H. paucidens* Pocock

11. Species small (80-130 mm), more than 230 trichobothria per pedipalp. Female with less than 12 pectinal teeth. Granules of dorso-lateral and ventro-lateral keels of metasomal segment V larger than those of the antero-dorsal keel of the pedipalpal femur ..... *H. minor* Purcell
Species large (140-180 mm), less than 200 trichobothria per pedipalp. Female with more than 14 pectinal teeth. Granules on dorso-lateral and ventro-lateral keels of metasomal segment V much smaller than those of the antero-dorsal keel of the pedipalpal femur ..... *H. granulatus* Purcell

12. Dorso-lateral keels of metasomal segments II and III without an enlarged terminal granule distally ..... *H. troglodytes* (Peters)
Dorso-lateral keels of metasomal segments II and III terminate distally with an enlarged granule or spiniform granule 13

13. Female with pair of deep oval depressions distally on sternite VII, metasomal segment V shorter than the carapace. In male, metasomal segment V less than 1,25 times the carapace length ..... *H. gunningi* Purcell
Female without distal, oval depressions on sternite VII, metasomal segment V equal in length to carapace length. In male, metasomal segment V more than 1,5 times the carapace length ..... *H. phyllodes* (Thorell)

Acknowledgements

We are grateful to Mrs. C.B. Martindale and Mrs. M.M. Cawood for expert technical assistance.

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