Morphological identification and molecular level confirmation of *Heterometrus swammerdami* (Scorpiones; Scorpionidae) Simon, 1872 in Jaffna peninsula

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Abstract- This current study deals with the identification and the confirmation of the ***Heterometrus swammerdammi*** morphologically and molecularly from Jaffna peninsula. ***H. swammerdami*** is one of the scorpion species belongs to the family Scorpionidae. But, this is not an endemic species to Sri Lanka.

In this present study, random sampling was implemented during the weekends around Jaffna Peninsula from the first week of April 2014 to March 2015. Morphological identification of this species was done by using standard keys by Frantisek Kovarik. Further confirmation was done using molecular analysis using DNA extraction, PCR and sequencing. DNA extraction was followed manually by the traditional phenol – chloroform method. DNA templates were amplified by the PCR reactions using the primer 16S (sequence range from 314 to 322 bp in length) and the PCR products were sequenced.

The morphological aspects and the molecular level sequencing confirm that the scorpion is ***Heterometrus swammerdammi.*** As ***Heterometrus swammerdammi*** shares some characteristic features with ***Heterometrus flavimanus*** in its pre maturity stage, the molecular analysis plays a wagon wheel in the confirmation of this species from other scorpion species.

Heterometrus swammerdammi is considered0 as less toxic scorpion to human beings, but there is no report to date to ensure this belief. Therefore, it is more valuable to record the composition of its venom and to find out the nature of its toxicity in addition to the DNA sequence data.

Index Terms- Heterometrus swammerdami, 16S rRNA, Jaffna Scorpion, Heterometrus flavimanus

I. Introduction

As far as fauna is considered, most of them are movable. Therefore it is difficult to focus much on the diversity of movable faunas comparing with floras. As Kovarik mentioned in his publications, the reviews for some of the invertebrates such as butterflies and beetles are available high in number, but only few reviews are exist for scorpion. There are 16 species of scorpions available in Sri Lanka and 10 species are endemic out of that 16 species (Ranawana, 2013). To date, only a few studies were carried out on scorpions in Sri Lanka, but none of the studies dealt with the establishment of the molecular sequence for the scorpions. This indication awakes the curiosity to work more on scorpions. Therefore, this study was undertaken to establish a molecular data base for the common scorpion species, *Heterometrus swammerdammi*.

The scorpions are nocturnal animals with venomous stings. About 1750 species of scorpions are recorded so far; among them only a few numbers of scorpions are lethal to human. Normally scorpions prefer the dark environments such as bushes, burrows and heap of stones. But, it is recorded that some species of scorpions could survive in extreme conditions such as under the snow cover, at the desert region at about 80^{o}C and in the conditions where the temperature exceeding minus 20 ^{o} C. In wild, the scorpions give the preference to some of the invertebrates such as beetles, butterflies, cockroaches, crickets, stinkbugs and the various nymphal and larval stages of some insects as their food sources (Kovarik.F 2009). Scorpions can be available in large number in the places with the adlibitum food source, water and habitat.

Jaffna Peninsula (9 ^{o} 40’ 0” N 80 ^{o} 0’ 0” E) is situated in the northern most region of the Island Sri Lanka and it is situated in the southern part of Indian sub-continent with the area of 1025.6 km^2. The mean annual temperature and the rain fall are 27.190^{o}C and 1811.8mm respectively. The topography of Jaffna Peninsula is almost flat with the elevation of 10.5 m above the sea level in most of the areas except Tellipalai (Veronika K et al 2013). Elephant pass is being as a connector for Jaffna Peninsula with other part of the country. The flat topography of the Peninsula is characterised with the lime stones. This is a unique feature of Jaffna Peninsula comparing with other parts of the Island. Even though, Jaffna Peninsula has these particular required blessings for the survival of scorpion species, the scorpion fauna is poorly documented (Veronika K et al, 2013).

According to Pocock (1990), 16 species of scorpions from three families were reported from Sri Lanka. Out of those 16 scorpions, there are three species of scorpions, namely *Heterometrus swammerdammi*, *Hottentotta tamulus* and *Isometrus maculatus* reported from Jaffna Peninsula. All these reports were based on the morphological taxonomy (Veronika K et al, 2013).

II. Materials and Methods

Random sampling was implemented in selected places around Jaffna Peninsula. 7 live samples were collected from Chavakachcheri (9^{o}39’07.1” N 80^{o}15’33.7” E), Kopay

($9^o42'45.4"$ N $80^o03'25.6"$ E), Siruppitty ($9^o44'12.7"$ N $80^o05'38.8"$E) and Thamparsity (9^o 49'42.9" N $80^o13'28.6"$ E) area from the first week of April 2014 to last week of February 2015(Fig 1). The tongs, sample collection bottles, markers and forceps were used in the sampling. The photographs of the sampling areas as well as the samples were taken by using the digital camera (FUJIFILM, FINEFIX HS 50 EXR). The collected samples were given with the sample name by mentioning their collection order. At the same time, the Global Positioning System (GPS) was used to locate the place of collection. The soil samples and the stones were brought to the animal house from those areas where the scorpions were caught.

The collected specimens were brought to the Animal house of Department of Zoology and they were reared in artificially constructed environment. Adlibitum supply of water and the nymphal stages of cockroaches were given twice a week to the scorpions in the captivity. Three scorpions were alive still now and the dead samples were preserved in the -4^o C in polythene shielded bags. The dead samples were measured using the Venire caliper for their significance standard length for the identification. the present study was ethically approved by the Department of Zoology, University of Jaffna.

III. DNA EXTRACTION PROTOCOL

Preserved scorpions were used for the DNA extraction. 180 mg of scorpion muscular parts (Normally pedipalps and legs) or if none feeding stage the whole specimen was crushed with 4 ml lysis buffer and the crushed mixer was transferred into 50 ml falcon tube and incubated at 50 ^{o}C for five hours with occasional shaking. The digest was then extracted with an equal volume of phenol chiasm, followed by chiasm extraction and centrifuged at 12000 rpm for 10 min. The aqueous phase was separated and the DNA was precipated with $1/10^{th}$ volume of 3M solution of acetate (pH 5.2) and double the volume by ice cold absolute ethanol. The DNA pellet was collected by centrifugation at 12000 rpm for 15 min and washed twice with70 % ethanol. The washed pellet was collected by centrifugation at 12000 rpm for 5 min. The final DNA pellet was air dried and reconstituted in TE buffer (pH 8.0) and stored at -20 ^{o}C until further use.

Amplification of the extracted DNA templates were carried out in 25 micro litres of reactions containing 4.80 micro litres of template DNA, 5x buffer, 2mM $Mgcl_2$, 2 micro molar each of dATP, dCTP,dGTPand dTTP, 2 pM each primers. The primer 16S rRNA was used to amplify the templates (Borges A et al 2010).

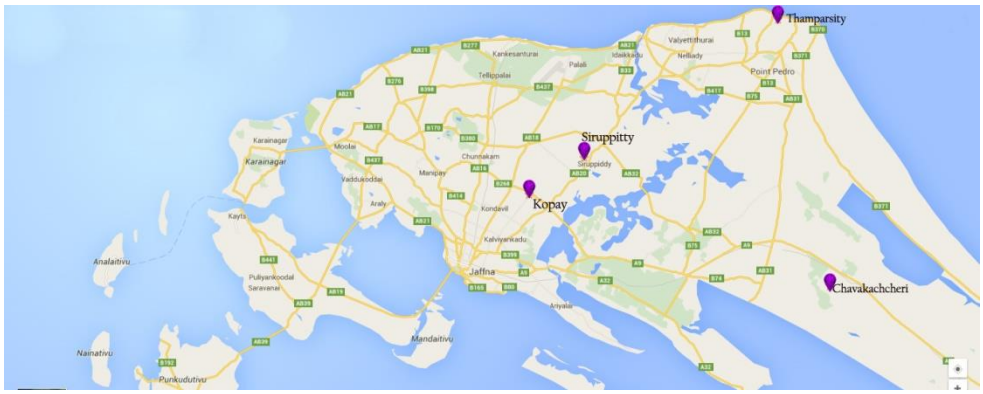

Fig 1. This Google map shows the locations of Sampling sites in the Jaffna peninsula, Northernpart of Sri Lanka(Source: Google Map)

IV. RESULTS AND DISCUSSION

Figure 02 A: Dorsal view of *Heterometrus swammerdammi* with scale.

Figure 02 B: Ventral view of *Heterometrus swammerdammi* with scale.

Table: 01. Details of Morphometric features used in the identification of *Heterometrus swammerdammi*

Characters	**Female**	**Male**
Prosoma length Prosoma anterior width Prosoma posterior width	18.60mm 10.90mm 11.46mm	15.30mm 08.44mm 09.80mm
Mesosoma length Mesosoma anterior width Mesosoma posterior width	32.88mm 11.46mm 07.36mm	36.00mm 09.80mm 06.00mm
Metasomal segment –I Length Width	 12.00mm 09.20mm	 08.50mm 07.40mm
Metasomal segment –II Length Width	 13.30mm 08.30mm	 10.30mm 06.68mm
Metasomal segment –III Length Width	 14.26mm 08.66mm	 11.00mm 06.50mm
Metasomal segment –IV Length Width	 15.40mm 06.94mm	 12.40mm 06.00mm
Metasomal segment –V Length Width	 18.10mm 06.50mm	 15.60mm 05.70mm
Number of basal teeth	01	01
Number of pectinal teeth	18	18

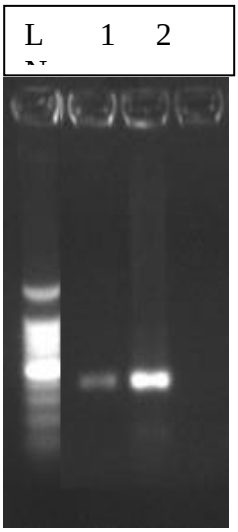

Figure 03: Agarose Gel picture (1%) illustrates the PCR products amplified with 16S rRNA primer under the UV gel documentation system.
Where, L,1,2 and the N were the different wells, 1-2 were the samples with the DNA templates, N is the negative control where the autoclaved double distilled water was used instead of template DNA and the L is the 100bp ladder.

Range 1: 107 to 375 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
337 bits(182)	1e-88	242/270(90%)	7/270(2%)	Plus/Plus

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Query  11   TGG-ATG-ACGGTTAGACCGAGTGATTTTGTCTTGGGGTT-TATTTTG-ATTTTATATTT  66
            ||| ||| ||||||||||||||||| ||||||||| ||||| ||||||| ||||||||||
Sbjct  107  TGGAATGAACGGTTAGACCGAGTGGTTTTGTCTTTGGGTTATATTTTGAATTTTATATTT  166

Query  67   TAGTAAAAAAAGCTAAAATTTATTTCAGGGACGAGAAGACCCTGTCAAACTTTACAAGCTA  126
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||   ||
Sbjct  167  TAGTAAAAAAAGCTAAAATTTATTTCAGGGACGAGAAGACCCTGTCAAACTTTACAAATTA  226

Query  127  ATCTTTCTCTCTTTGG-TAGGGTAA-TTTGTTTTTACTGGGGCAGTAAGCAATTAAATTT  184
            | |||||||||||| | |||||||| |||| ||||||||||||||||| ||| | ||||||
Sbjct  227  ACCTTTCTCTCTTTAGATAGGGTAAGTTTG-TTTTACTGGGGCAGTAGGCAGTAAAATTT  285

Query  185  TGCTTTATTATTATGAAGTTTCTTCATGTAAAAGCAGGAGTTTCCAACCAAAAAATAACT  244
            |||||||||||| || | ||||||| ||||||| | ||||||||| |  ||||||||| |
Sbjct  286  TGCTTTATTATTGTGGATTTTCTTCTTGTAAAATCGGGAGTTTCCTAAGAAAAAATAAGT  345

Query  245  GCCTGCAGGGATAACAGCGTGATTCTTTTT  274
              |||||||||||||||||||||||||||||
Sbjct  346  TACTGCAGGGATAACAGCGTGATTCTTTTT  375
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Fig 4. Evidence of 90% of alignment of the obtained sequence with 16S ribosomal RNA gene

The sampling was being as the initial step in this study. The positive outcome of sampling was observed during early morning times. The morphological identification of the scorpions was done initially to find the family and the genera of the scorpion. During the genera level identification, there was a struggle with the scorpion in between the genus *Heterometrus* and the genus *Pandinus*. The *Heterometrus* and *Pandinus* of sub family Scorpioninae are comprised of large species and are chosen as pilot genera because many naturalists keep and propagate them. Therefore the live specimen were kept at the animal house of Department of Zoology, University of Jaffna where a mimic natural environment to its original habitat with the same soil in the locality and stones were provided. Finally, the genus of the scorpion was confirmed as *Heterometrus.*

Genus: *Pandinus*

Total length 60-220 mm. Pedipalp femur with three tricobotria, only one of them on internal surface. Pedipalp patella with 13 external and numerous (usually about 30) ventral tricobothria. Retrolateral pedal spurs absent. Lateroapical margins of tarsi produced into round lobes. Metasomal segments I-IV with paired ventral submedian carinae. Stridulatory organ located on opposing surfaces of pedipalp coxa and first leg.

Genus : *Heterometrus*

Pedipalp femur with three tricobothria, of them only one on internal surface. Patella of pedipalp with 19 tricobothria, three on ventral and 13 on external surface. Chela of pedipalp with 26 tricobothria. Retrolateral pedal spurs absent. Lateroapical margins of tarsi produced into rounded lobes. Metasomal segments I to IV with paired ventral submedian carinae. Stridulatory organ located on opposing surfaces of pedipalp coxa and first leg. Total length is 60 to 180 mm.

Further, the key for the species level identification was carried out using the species level identification keys. Here too, confusions were faced with the overlapping of characters in between two species namely *Heterometrus swammerdami* and *Heterometrus flavimanus* where many morphological characters and the patterns of colouration were overlapped with each other and the above two species have shared a same clade in the dichotomous key.

Heterometrus swammerdami

Adults 130-176 mm long. Base colour uniformly reddish brown to reddish black. Juveniles may be red with yellow telson. Pecinal teeth number 16 -20. Sexual dimorphism in proportions of pedipalps not noticeable. Chela strongly lobiform, its length to width ratio 1.6- 1.8 in both sexes (Table 01). Entire manus covered by large, rounded granulaethat do not form true carinae. Patella of pedipalp without pronounced internal tubercle. Carapace with disc smooth, margins and posterior portions granulate, and anterior portion granulate and tuberculate; occasionally entire surface sparsely granulate. Fifth segment of metasoma longer than femur of pedipalp, fourth segment of metasoma approximately as long as femur of pedipalp. Telson bulbous , vesicle as long as or longer than aculeus (Fig 2 A & B).

Heterometrus flavimanus

Adults 110- 150 mm long. Base colour uniformly reddish brown, manus of pedipalp,legs and telson reddish to yellow. Pectinal teeth number 19-22. Sexual dimorphism in protions of pedipalp not noticeable. Chela strongly lobi form, its length to width ratio about 1.7 in both sexes. Entire manus covered by large, rounded granules that do not form true carinae. Patella of pedipalp without pronounced internal tubercles. A carapace usually with disc smooth and all margins granulate and turbecuate (in some specimens entire carapace sparsely granulate). Fifth segment of metasoma longer than femur of pedipalp, fourth segment of metasoma approximately as long as femur of pedipalp. Telson bulbous, vesicle longer than aculeus.

The DNA extraction is followed by the traditional Phenol chloroform method. The dead and ice preserved adult scorpion samples were used for the extraction purpose. But, it gives colour pigmented DNA. Therefore the samples preserved in 70% of alcohol were taken for the extraction. However scorpiolings were shown better quality of DNA i.e. non pigmented, clear and good quantity of the DNA. The quantity of the DNA obtained from juvenile scorpion was considerably good . The extracted DNA was then used in the PCR reactions. The 16S primer was used to amplify the PCR reactions (Fig 3).

The mitochondrial 16S rRNA (n=32) gene sequences were determined for our species. The length of the 16S sequences ranged from 314 to 322 bp with a nucleotide combination of 13.6% guanine, 32.3% adenine, 40.2% thymine, and 13.8% cytosine. It has shown 90% of identities (Fig 4) with the sequence of *Heterometrus swammerdami* which was already deposited in the GenBank (Sequence Id: gb/AY156560.1). Based on this alignment it has been confirmed that the name of the species is the *Heterometrus swammerdami* .

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