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## **Phylogenetic Relationships of Two Earth Tiger Tarantulas, *Haplopelma lividum* and *H. longipes* (Araneae, Theraphosidae), within the Infraorder Mygalomorph Using 28S Ribosomal DNA Sequences**

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### **ABSTRACT**

*Haplopelma lividum* and *H. longipes* (Araneae: Mygalomorphae: Theraphosidae) are tarantulas that are distributed throughout Southeast Asia and are important carnivorous predators in ecological systems. The present study aimed to examine the phylogenetic relationships between Mygalomorph spiders using 28S ribosomal DNA sequences. The molecular results supported the placement of both species within a common theraphosid taxon. However, when considering relationships between *Haplopelma* spp. and related genera, *H. schmidtii*, *H. lividum* and *H. longipes* were not monophyletic, suggesting that molecular data are incongruent with phylogenies based on morphological characteristics. These results provide molecular data to help elucidate the phylogenetic relationships between theraphosid tarantulas.

**Key words:** *H. lividum*, *H. longipes*, tarantula, 28S rRNA gene sequences

### **INTRODUCTION**

Theraphosid tarantulas (infraorder Mygalomorphae) are known to be important predators in ecological webs, are kept commercially as pets and are used as research tools in the fields of toxicology, pharmacology, systematic biology and molecular phylogeny (Escoubas and Rash, 2004; Tang *et al.*, 2010; Bertani, 2012). The Earth tiger tarantula is a mid-to large-size venomous spider in the subfamily Ornithoconinae, belonging to the family of Theraphosidae (Zhu and Zhang, 2008). The *Haplopelma* tarantula is a genus in Ornithoconinae and includes, for example, *H. albostriatum*, *H. hainanum*, *H. schmidtii*, *H. lividum* and *H. longipes* (Simon, 1892). *Haplopelma* are widely distributed within the tropical environments of South China and Southeast Asia, inhabiting underground burrows and hilly areas protected by flat sheet webs (Pan and Yu, 2010).

*H. lividum* (Smith, 1996) (which are commonly called cobalt blue tarantulas, are mainly found in Myanmar and Thailand and *H. longipes* (Von Wirth and Striffler, 2005) are distributed throughout Thailand and Cambodia. However, few studies have been reported on these species. Takaoka *et al.* (2001) described two case studies of finger bites from *H. lividum* which appeared

relatively harmless compared to bites from other tarantulas. Moore *et al.* (2009) characterized the crude venom of *H. lividum*, detecting glutamic acid, histamine, adenosine and polyamine spermine. Von Wirth and Striffler (2005) also described systematic identification of *H. longipes* using morphological characteristics of the whole body. Within the genus *Haplopelma*, 28S ribosomal RNA (rRNA) sequence data have only been reported for *H. schmidtii*, placing it within the phylum Chelicerata (Arabi *et al.*, 2012). Phylogenetic relationships and rRNA sequence data for *H. lividum* and *H. longipes* have not been evaluated.

Therefore, the present study aimed to examine the phylogenetic tree of two tarantulas (*H. lividum* and *H. longipes*, Fig. 1b, respectively) in relation to other species of the suborder Mygalomorphae, based on 28S rRNA gene sequences. This study contributes to the understanding of the evolutionary relationships among theraphosid spiders.

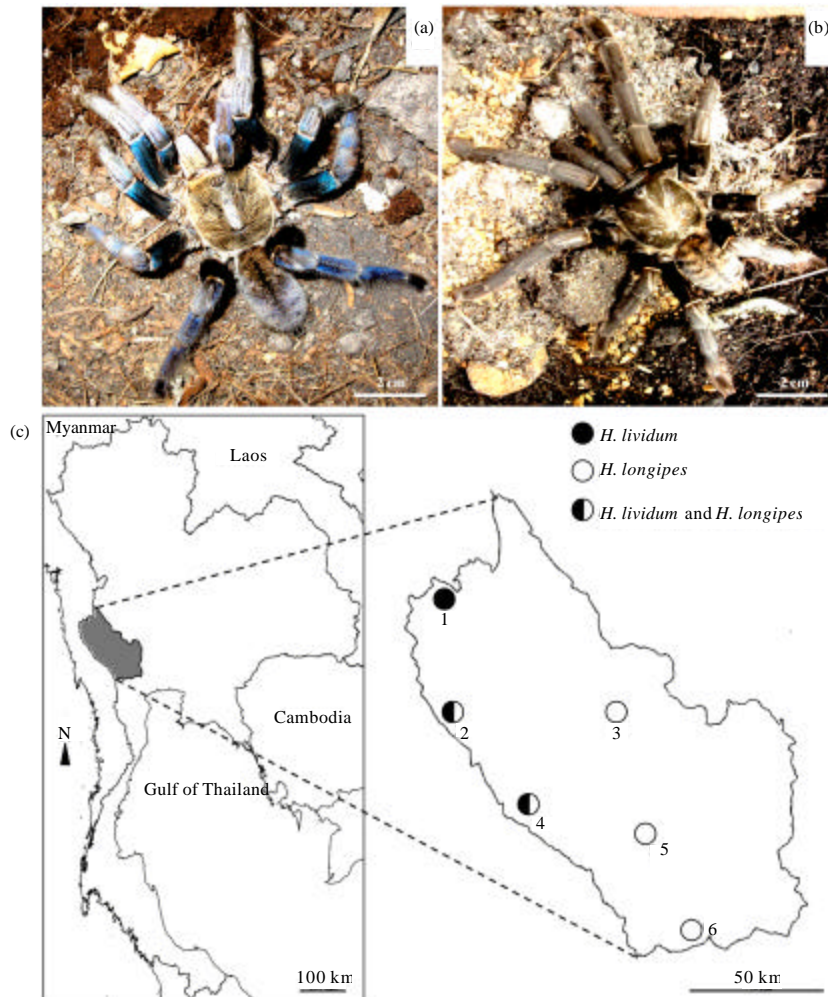


Fig. 1(a-c): (a) A ventral views of adult *H. lividum*, (b) *H. longipes* and (c) Inhabiting a ground of natural environment. A map showed the six localities of Kanchanaburi Province for tarantula collections. Black circle indicates the localities which observed for *H. lividum* and white circles for *H. longipes*. Black and white circles indicate the localities which found both two tarantulas

## MATERIALS AND METHODS

**Tarantula sampling:** Adult *H. lividum* and *H. longipes* (Fig. 1a-b) inhabiting ground burrows were caught from six localities in Kanchanaburi Province, Thailand (15°9'21"N 98°27'13"E, 14°44'45"N 98°37'30"E, 14°35'57"N 99°6'50"E, 14°6'56"N 99°8'40"E, 14°0'12"N 99°33'0"E and 13°51'13"N 99°24'38"E) (Fig. 1c). The species of those tarantulas was identified according to the criteria of Smith (1996) and Von Wirth and Striffler (2005).

**28S rDNA sequences amplification:** Total genomic DNA was extracted from each specimen using a Dneasy Tissue kit (Qiagen, Germany). The 28S primers for amplification were Hap1 (5'-GTACGTGAAACTGCTCAGAGGC-3') and Hap2 (5'-GTCCAGGTTCTGGGAATATTGACC-3') which produced approximately 1600 bp PCR products. Hap3 (5'-TAAGGACGGGCACCGGAAG-3') and Hap4 (5'-CGTGTTTCAAGACGGGTCCG-3') produced 300 bp fragments within the above products. PCR cycling included an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec and extension at 72°C for 1 min. Final extension was performed at 72°C for 10 min. DNA from the PCR products was purified using the QIAquick Gel Extraction Kit (Qiagen).

**DNA sequencing analysis:** DNA sequencing was performed by Macrogen DNA Sequencing Service, Korea. The 28S rDNA sequences have been deposited in the NCBI database (accession numbers KC881567 and KC881568). Phylogenetic analysis was performed using the bootstrap method with 1000 replications by the MEGA 5.10 software package (Tamura *et al.*, 2011). The local similarity of the DNA sequences was analyzed using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

## RESULTS AND DISCUSSION

The phylogenetic tree of *H. lividum* and *H. longipes* showed a common cladistic form for mygalomorph relationships as assessed using partial 28S sequences (Fig. 2a). Molecular results confirmed that *H. lividum* and *H. longipes* were classified in the family Theraphosidae as a monophyletic group and clearly separated from other families within the mygalomorphs. Moreover, this phylogeny corresponded to the general taxon of tarantulas constructed by using morphological classification (Bertani, 2012; Zhu and Zhang, 2008). In contrast, the genus of *Haplopelma* was not monophyletic group within the subfamily of Ornithoconinae. The *H. lividum* and *H. longipes* were more closely related to *Cyriopagopus schioedtei* than either was to *H. schmidti*. Hedin and Bond (2006) reported that a preliminary Mygalomorph phylogeny inferred from 18S and 28S rRNA genes conflicted with morphological hypotheses. Therefore, these molecular data did not support a morphological taxonomy between *Haplopelma* spp. and *C. schioedtei*.

Local alignments revealed that the partial 28S rDNA sequences of *H. longipes* shared 99% homology with those of *H. lividum*, *H. schmidti* and *C. schioedtei* (Fig. 2b). The *H. lividum* and *H. longipes* were clearly separated from *Lasiadora parahybana* and *Chilobrachys huahini*, sharing only 91 and 92% homology, respectively. The nucleotide sequences of *H. lividum* were 99, 98, 99, 91 and 91% of homologous to those of *H. longipes*, *H. schmidti*, *C. schioedtei*, *L. parahybana* and *C. huahini*, respectively. The sequences of *H. longipes* were 99, 99, 92 and 92% homologous to those of *H. schmidti*, *C. schioedtei*, *L. parahybana* and *C. huahini*, respectively. These results suggest that the 28S partial rDNA sequences of *H. lividum* and *C. schioedtei* are more closely conserved with each other than either is with those of *H. schmidti*. The *C. schioedtei* (known as the

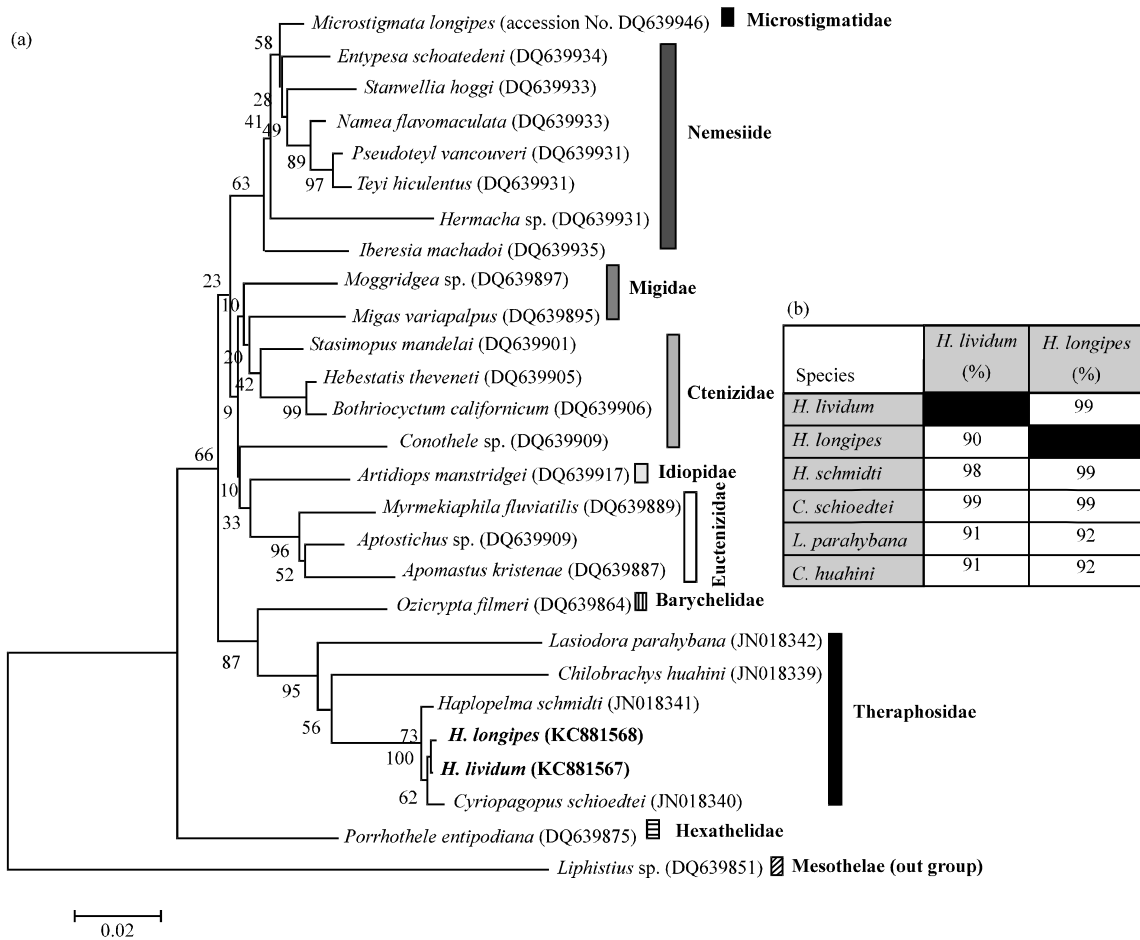


Fig. 2(a-b): (a) Phylogenetic tree showing the relationships of *H. lividum* and *H. longipes* within 25 species of Mygalomorph based on the 28S rDNA gene and (b) Local similarity of the DNA sequences in Theraphosidae

Malaysian earth tiger tarantula) is a native species in Peninsular Malaysia (Song *et al.*, 2002). Of the three *Haplopelma* considered here, *H. schmidtii* is primarily found in Vietnam (Arabi *et al.*, 2002); the distributions of *H. lividum* and *H. longipes* were explained in the introduction. In this study, however, the molecular evaluation was incongruent with the relationships suggested by taxonomy and geographical distributions since *H. lividum* exactly related to *C. schioedtei* by using 28S rDNA sequences.

## CONCLUSION

In summary, the present study has determined partial 28S rDNA sequences for *H. lividum* and *H. longipes* and used these sequences to study the spiders' phylogenetic relationships within the infraorder Mygalomorphae. The resulting molecular phylogeny was partially congruent with the phylogeny based on morphological descriptions of tarantulas but there were some inconsistencies between the two methods. The complete rDNA sequences remain to be evaluated and may help to resolve this incongruence.

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