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Research Article

Direct chromosomal preparation protocol from old world tarantulas (Araneae, Theraphosidae)

Paveen Piyatrakulchai[1,2], Alongklod Tanomtong[1,2], Phichaya Buasriyot[2], Narin Chomphuphuang[3*]

[1] *Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand*

[2] *Animals Cytogenetics Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand*

[3] *Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand*

[*] *Name Corresponding Author: Narin Chomphuphuang (Narich@kku.ac.th)*

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Abstract- The Theraphosidae (Thorell, 1869) is the most prevalent family in the suborder Mygalomorpae, but little is known about their cytogenetics, nor have the chromosomes of old-world tarantulas been studied. This study aims to develop an efficient protocol for chromosomal tarantula preparation in which chromosomes are extracted directly from the internal organs of spiders following in vivo colchicine treatment. *Chilobrachys* (Karsch, 1892) and *Cyriopagopus* (Simon, 1888), which belong to different subfamilies, were chosen as validation models. The results indicate that the protocol for preparing chromosomes from both subfamilies is effective, suggesting that Southeast Asian tarantulas are probable to have bi-arms chromosomes and a high proportion of diploid number (*2n*). Interestingly, diploid chromosomes contain chromosomal gaps on many chromosomes, which will be investigated in the future to facilitate a greater understanding.

Keywords: Chromosome, *Chilobrachys*, *Cyriopagopus*, Selenocosminae, Ornithoctoninae

1. Introduction

Spiders (Clerck, 1757) represent one of the largest orders of arachnids. There are approximately 50,000 species in the order Araneae, which is divided into two suborders, Mesothelae and Opisthothelae. The latter is classified into two infraorders: Mygalomorphae and Araneomorphae (World Spider Catalog, 2022).

Karyotype data can be a valuable source of information for evolutionary studies, and it can aid in identifying taxonomic homologies (Dobigny *et al.,* 2004). Araneae display multiple heterogamy systems, with the majority of spiders exhibiting the X_1X_20 system (Araujo *et al.*, 2008, 2012; Dolejš *et al.*, 2011; Král *et al.*, 2013; Kumbiçak *et al.*, 2013, 2014; Neto *et al.*, 2020; Paula-Neto *et al.*, 2013; Souza *et al.*, 2017, 2021; Stávale *et al.*, 2011). Almost all chromosomal studies on spiders currently encompass only Araneomorphae. Despite the fact that the family Theraphosidae, which contains over a thousand species of tarantulas, is the most abundant in the infraorder Mygalomorphae (World Spider Catalog, 2022), the protocols for chromosome preparation are insufficient due to the paucity of tarantula chromosome research (Král *et al.*, 2013). Consequently, to enhance the cytogenetic data of tarantula, the effective chromosome preparation in tarantula needs to be developed.

Certainly, chromosome preparation is the foundation of cytogenetics research. The direct chromosome preparation technique is inexpensive and applicable to invertebrate chromosome preparation. It typically selects target organs from the gonad, which frequently exhibits cell division. The chromosomal spider protocol is largely derived from the insect protocol in which spiders die after chemicals are added; however, due to the larger size of tarantulas, they are more resistant to the toxicity of chemicals that inhibit cell division. Therefore, techniques that are both reliable and suitable for isolating tarantula chromosomes should be produced.

This study proposes an efficient protocol for harvesting metaphase cells by injecting colchicine solution directly into the tarantula's abdomen and collecting all internal organs for a cell suspension mixture, thereby reducing the difficulty of locating the gonad within the spider. The method uses two genera of old-world tarantulas as models, including *Chilobrachys* Karsch, 1892 and *Cyriopagopus* Simon, 1888, which belong to different subfamilies to validate protocol results. The research will be utilized to support and advance cytogenetic tarantula studies involving spider taxonomy, evolutionary systematics, and sex determination systems.

2. Materials and method

2.1 Sample collection

Individual specimens will be gathered from the wild and donated by tarantula keepers. Mature male tarantulas were fed until they reached maturity. Before chromosomal preparation, the tarantula must remain undisturbed. In protocol testing, two genera (three species) of tarantulas are employed: *Chilobrachys Chilobrachys huahini* Schmidt & Huber, 1996 and *Cyriopagopus*. (*Cyriopagopus albostriatus* (Simon, 1887) and *Cyriopagopus lividus* (Smith, 1996)

2.2 Chromosomal tarantula preparation protocol

1. Before a mature male tarantula produces a sperm web, inject a 0.1% aqueous colchicine solution into its lateral abdomen. To avoid fatal injuries to internal organs such as the heart located dorsally in the abdomen.

2. Keep samples in exotic pet box for more than 16 hours, and then sacrifice them after anaesthetization in freezing temperature.

3. Place the samples in a ventral position and open the abdomen from pedicel (isthmus between the cephalothorax and abdomen). Internal organs are washed in 0.075 M KCl to remove fat.

4. Put internal organs in a mixed solution (0.075 M KCl and 0.05% colchicine) for 5 minutes.

5. Cut the internal organs of a tarantula with medical scissors in a solution mixture until the tissue is completely shattered

6. Transfer the mixture to a centrifuge tube for a 30-minute incubation at room temperature. Due to its effective quality in the centrifuge, the volume of the mixture should be 7 ml/tube.

7. Centrifuge the cell suspension for 10 minutes at 1000-1200 rpm. The supernatant solution is discarded before cells are fixed in fresh Carnoy's fixative (Methanol: acetic acid; 3:1). Fixative addition should be meticulously added before being centrifuged again.

8. Centrifuge cells in fixative again for 10 minutes at 1000-1200 rpm. The supernatant solution is discarded. Repeat this step 3—4 times.

9. Store the cell suspension in a centrifuge tube which is kept at a frozen temperature. Before beginning the cytogenetic technique, the cell suspension will be centrifuged, and the supernatant will be removed.

10. Using a Pasteur pipette, apply 3 to 4 drops of cell suspension to various regions of a slide placed on a hot plate heated to 45-60 ºC

Sixth through tenth steps of the protocal, as modified from Juntaree & Supiwong (2000) and Srisamoot *et al*. (2021).

2.3 Chromosomal staining

Chromosomes were investigated using conventional staining. Slides with metaphase cells are stained using Giemsa's solution for 30 minutes. Stained chromosomes were investigated by an Olympus CH30 light microscope, and chromosomal pictures were taken by a Nikon D5300.

3. Results

Chilobrachys huahini Schmidt & Huber, 1996

Chromosomes were recognizable in the cells of *C. huahini* that had been stained with Giemsa (Figure 1). Due to the presence of gapped chromosomes in metaphase cells, this species may possess bi-arm chromosomes (Figure 1). This method treats *C. huahini* with colchicine for 23 hours.

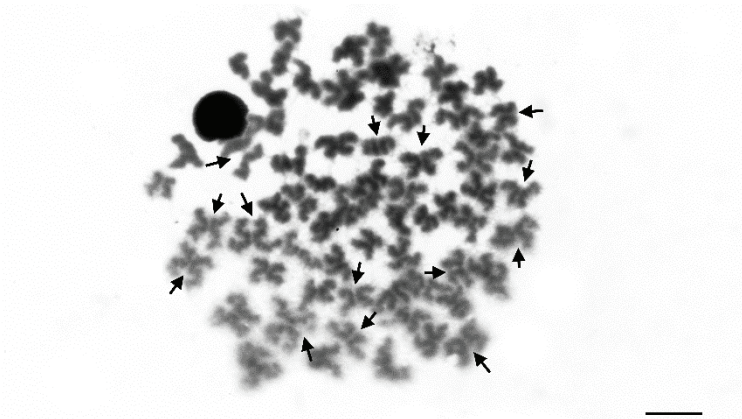

Figure 1. Male *C. huahini* chromosomes using conventional staining technique; chromosomes with chromosome gap (arrows), Scale bar: 5 μm

Cyriopagopus albostriatus (Simon, 1887)

Cy. albostriatus is thought to possess bi-arm chromosomes (Figure 2A). The occurrence of polyploidy in this species is revealed by the presence of numerous chromosomes in cells that more than double the number of diploid chromosomes (Figure 2B). This method treats *Cy. albostriatus* with colchicine for 25 hours.

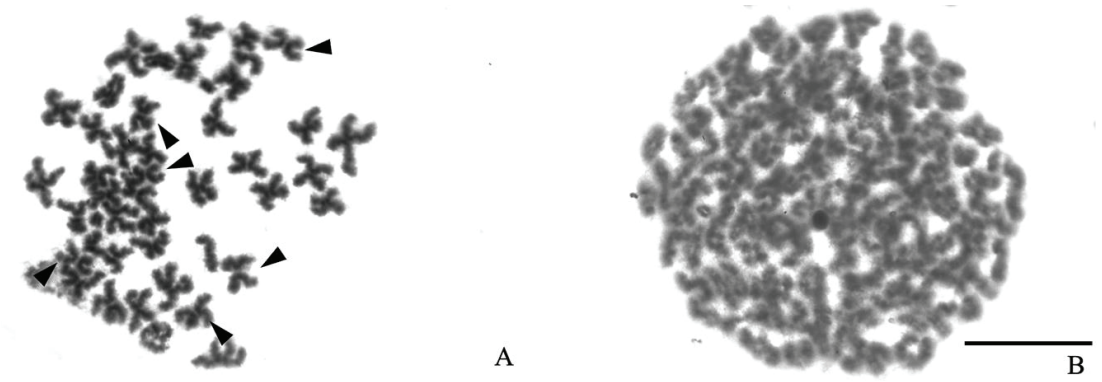

Figure 2. Male *Cy. albostriatus* chromosomes using conventional staining technique; (A) chromosomes with chromosome gap (arrowheads); (B) *Cy. albostriatus* polyploid, Scale bar: 5 μm

Cyriopagopus lividus (Smith, 1996)

The chromosomes of *Cy. lividus* cells stained with Giemsa are represented in Figure 3.

The chromosome characteristics of *Cy. lividus* are similar to those of *Cy. albostriatus*, a member of the same genus, but *Cy. lividus* does not exhibit polyploid chromosomes. The bivalent chromosomes of *Cy. lividus* are shown in Figure 3B. Although *C. huahini* (Subfamily Selenocosmiinae) and *Cyriopagopus* (Subfamily Ornithoctoninae) belong to different subfamilies, they share numerous chromosome gaps in their cells (Figure 1-3). This method treats *Cy. lividus* with colchicine for 23 hours.

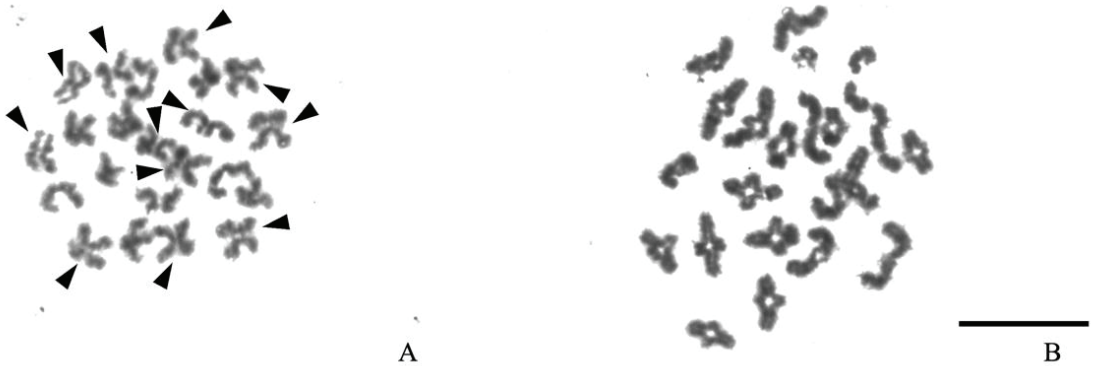

Figure 3 Male *Cy. lividus* chromosomes using conventional staining technique; (A) chromosomes with chromosome gap (arrowheads); (B) bivalent chromosomes, Scale bar: 5 μm

4. Discussions and conclusions

This protocol is effective for collecting metaphase cells from old-world tarantulas belonging to the subfamilies Selenocosminae (Simon, 1889) and Ornithoctoninae (Pocock, 1985) which are distributed throughout Asia. All species of tarantulas in this study are suspected to have bi-arms chromosomes, and their chromosomes are morphologically similar to those of other mygalomorphs (Král *et al.*, 2011, 2013). The results from the *Cy. lividus* chromosome (Figure 2B) resemble those of the scorpion chromosome, which may have been caused by heterozygous translocations or bivalent chromosomes (Ubinski *et al.*, 2018). The investigation reveals the presence of a chromosome gap, the significance of which requires further study. The protocol has the advantage of only requiring a small number of samples in order to obtain a large number of metaphase cells, which results in cost savings for the cytogenetic study. Almost tarantulas are a big spider. Thus, they are more tolerant than other spiders to the toxicity of chemicals that inhibit cell division. This study proposes a method for efficiently harvesting metaphase cells from tarantulas by injecting colchicine directly into the lateral abdomen of living tarantula. The results suggest that colchicine should not be treated for more than 24 hours, as this chemical's effect can result in the duplication of chromosomes, which could lead to polyploid *Cy. albostriatus* chromosomes. Chromosome preparation should select target organs with cell division, as arthropod gonads frequently exhibit cell division. Because the internal organ of the spider's abdomen is rather fluid, it is quite difficult to collect only the gonad. As a result, we apply using all internal organs, excluding liquid and fat, to extract isolated chromosomes from cells tissues. However, the protocol has the limitation of requiring the collection of chromosomes from adult male tarantulas prior to the production of sperm webs. Male tarantulas are typically relatively rare and can only be found during some periods of the year, particularly during mating season. Alternatively, male tarantulas can be obtained by collecting juveniles and raising them to maturity.

This study is the first attempt to develop a protocol for chromosome preparation of old-world tarantula that can improve chromosomal tarantula research

by predicting the sex determination system of spiders and determining the fundamental trends of karyotype evolution in order to classify and identify tarantula.

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