Morphology and sex-specific behavior of a gynandromorphic *Myrmarachne formicaria* (Araneae: Salticidae) spider

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Abstract

Behavioral studies of gynandromorphism, also called as sex mosaic, contribute to the understanding of the relationship between morphological gender and sexual identity of an animal. Few studies have focused on the behaviors of gynandromorphic spiders because of a scarcity of gynandromorphic individuals in the field. In this study, we collected a gynandromorphic spider, *Myrmarachne formicaria* (De Geer 1778) (Araneae: Salticidae), from the field and examined its morphology and sex-specific behavior in the laboratory. The right half of the gynandromorphic spider presented male characteristics, and the left half female characteristics. It showed courtship behavior to *M. formicaria* females and agonistic behavior to the males. These results indicate that the gynandromorphic spider's sexual identity is male. Our findings suggest that a spider can exhibit behaviors of male sexuality, although the external morphology has the characteristics of both sexes. To the best of our knowledge, this is the first report of a gynandromorphic individual and its behavior in the genus *Myrmarachne*.

Keywords

Agonistic behavior, ant-mimicking spider, bilateral gynandromorph, courtship behavior, sex mosaic, sexual identity

Introduction

Sometimes, an animal exhibits both male and female morphological characteristics and a chimeric phenotype. This phenomenon is known as gynandromorphism or sex mosaic, and it is attributable to several factors such as damage to the sex chromosome during cleavage, binucleation, and effects of symbionts (Narita et al. 2010). Gynandromorphic organisms have been reported in a wide range of animals, including vertebrates (e.g., birds: Peer and Motz 2014); however, most of the organisms are invertebrate species such as insects, crustaceans, and arachnids (Morgan 1905; Exline 1938; Narita et al. 2010). Studies on the behavior of a gynandromorph would help us to understand which organs are responsible for sex-specific behavior (Nissani 1977). The sexual behavior of gynandromorphic organisms has been mostly observed in insects that belong to Diptera (e.g., Nissani 1977), Hymenoptera (Matsuo et al. 2018; Sakagami and Takahashi 1955; Ugajin et al. 2016), and Orthoptera (e.g., Maeno and Tanaka 2007), whereas few studies on gynandromorphism in spiders have been focused on their behavior (e.g., Maekawa and Ikeda 1992).

Many species of spiders exhibit sexual dimorphism in body size, shape, color, and patterns (Foellmer and Moya-Laraño 2007; Lim and Li 2006). Jumping spiders (Salticidae) are remarkable examples, and they exhibit male-specific morphology and coloration. For instance, *Maratus* males have movable flaps on a brightly colored abdomen (Otto and Hill 2011), and *Myrmarachne* males have considerably elongated chelicera, which is as long as the carapace (Ono et al. 2009). In addition, salticid spiders exhibit sex-specific courtship behavior by using visual, chemical, and vibratory information

(Schneider and Andrade 2011).

To investigate a gynandromorph's behavior, it is necessary to capture it alive. However, most gynandromorphic spiders have been found after preservation (e.g., Kaston 1961; Baba et al. 2016). Salticid gynandromorphic spiders are more likely to be found alive because of their visually distinguishable sexual dimorphism (e.g., Maekawa and Ikeda 1992). This is a great advantage in investigating the relationship between the morphological gender and sexual identity of an animal. In this paper, we describe, for the first time, a gynandromorphic ant-mimic spider, *Myrmarachne formicaria* (De Geer 1778) (Araneae: Salticidae), from Japan. First, we compared the morphology of the gynandromorphic spider with that of normal (i.e., non-gynandromorphic) males and females to confirm its gynandromorphic pattern. Our aim was to determine the sexual identity of the gynandromorph. Therefore, we observed its behavior when it faced other male or female spiders of the same species and associated it with its gynandromorphic pattern.

Materials and Methods

Study species and specimen collection

The study species, *M. formicaria* (De Geer 1778), is a small ant-mimicking spider with a body length of 5-6 mm (female) or 4-5 mm (male) (Ono et al. 2009). *Myrmarachne formicaria* is distributed in the Palearctic region (World Spider Catalog 2018), and it was recently introduced in the United States (Bradley et al. 2006). This species is often found in grasslands and riverbeds, walking on the grass or ground surface (Suguro 2017).

One gynandromorphic *M. formicaria* was found in a grass field at Tennodai (36.1186786 N, 140.0990647 E), Tsukuba, Ibaraki, Japan, on October 22, 2016. This individual was one of many *M. formicaria* walking on the ground, and the other individuals were normal (non-gynandromorphic) spiders. Ten normal females and 27 normal males were obtained from four locations in Japan (i.e., Ibaraki, Tokyo, Yamagata, and Hokkaido). Three of the 10 females and 27 males were used for behavioral experiments, and all spiders were preserved in 80% ethanol. Ten females and 10 males were used for morphological measurement after the behavioral experiments. The 10 males were randomly chosen from the 27 male specimens.

Morphological comparison

To elucidate the morphological characteristics of the gynandromorph, 10 body parts of the specimen (body length, carapace length, carapace width, chelicera length, fang length, palp length, and length of leg I, leg II, leg III, and leg Ⅳ) were measured. Of these body parts, the chelicerae are sexual dimorphic (i.e., longer in the male than in the female) (Ono et al. 2009). Both left and right sides of the gynandromorph’s appendages were measured, but only the right (male) or left (female) appendages of the normal specimens were measured. The measurements were performed using a stereoscopic microscope (Nikon AZ100M; Japan) and microscope imaging software (Nikon NIS-Elements D 4.20.00 64-bit; Japan).

Behavioral experiments

The behaviors of a couple of spiders in male-male combination and male-female combination were observed to determine the sex-specific behavior of normal individuals (experiment 1). To determine the sexual identity of the gynandromorphic spider, its behavior when it encountered a male or female was observed (experiment 2). In each experiment, two normal individuals (experiment 1) or the gynandromorphic spider and a normal individual (experiment 2) were placed in a plastic cage (length: 90 mm, width: 90 mm, height: 80 mm). The behaviors of the spiders were recorded with a video camera (OLYMPUS TG-4; Japan) at 25 ℃. In experiment 1, male-male and female-male experiments were replicated 11 and 2 times, respectively. All spiders for the male-male experiments were used only once. The male-female experiments were conducted with the same couple because of the limited number of collected females. In experiment 2, observations were replicated four times for the gynandromorph-male combination and twice for the gynandromorph-female combination. The normal individuals used for this experiment were obtained from the same place where the gynandromorph was collected, and each spider was used only once.

Statistical analysis

Morphological comparisons between the normal females and males were tested for statistical significance by using the Mann–Whitney *U* test. All statistical analyses were performed in R (version 3.5.1; R Development Core Team 2018).

Results

Morphological comparison

The right half of the body of the individual exhibited male characteristics, and the left half, female characteristics (Figs. 1, 2); therefore, it was classified as a bilateral gynandromorph. The most distinguishable morphological characteristics of the gynandromorph were its chelicerae and fangs, i.e., the right chelicera and fang were elongated and the left ones were shorter than the right ones (right chelicera length: 1.74 mm, left chelicera length: 0.51 mm; right fang length: 1.90 mm, left fang length: 0.40 mm, Fig. 1a–d, Fig. 2). In the normal individuals, the chelicerae (1.39 ± 0.36 mm, mean ± SD) and fangs (1.26 ± 0.28 mm, mean ± SD) of the males were significantly longer than the chelicerae (0.54 ± 0.08 mm, mean ± SD) and fangs (0.35 ± 0.07, mean ± SD) of the females (no overlapping; Table 1). The chelicerae and fangs lengths of the gynandromorph fell within the range of both normal females (left) and males (right) (Fig. 2, Table 1), except the right fang, which was longer than the maximum value of the normal males (Fig. 2, Table 1). All other parts, body length, carapace length, carapace width, and palp and leg length, were not significantly different between the normal females and males ($P > 0.05$, Mann–Whitney *U* test; Table 1), and the lengths of those parts fall within the range of normal individuals.

The sexual organs of the individual were also bilateral gynandromorphic (Fig. 3). The right palp was developed as a mature palp organ, whereas the left one was that of a normal female (Fig. 3 a–d). The epigynum was formed only on the left side of the abdomen, and the spermatheca was located at an abnormal position when compared with the normal females (Fig. 3 e–f).

Behavioral experiment

The *M. formicaria* males showed agonistic behavior in the male-male experiments. When two normal males faced each other, they showed a recognizable behavior. The males bent both legs and moved repeatedly side-to-side (Fig. 4a; video S1). Their abdomens also bent to the right and left. During this phase, they maintained some distance and never touched each other. Six of 11 trials were finished at this phase, and five couples moved to the next phase. Both males raised their legs and opened the chelicerae in widthwise direction (Fig. 4a; video S1). Four of the five couples completed agonistic behavior and escaped at this phase. Only one couple showed heated battle. They began touching each other with the chelicerae and fangs, and one of the males tried to throw its opponent by using its chelicerae (Fig. 4a; video S1).

The courtship behavior of the spider was characterized in the male-female experiments, and it was distinguishable from the agonistic behavior. The males often showed a frontal approach to a female in both trials. The males stretched the legs forward and tried to touch a female's legs or body (Fig. 4b; video S2). When a male approached a female from behind or walked around it, the female turned around and faced the male. A female sometimes responded to a male's courtship behavior by stretching both of its forelegs and approaching the male, although that was rare (three of 19 male approaches in two trials). The females never accepted the males in the experiments.

In the gynandromorph-male experiments (n = 4), both the gynandromorph and opponent male bent their legs and moved side-to-side in all four trials (Fig. 4c; video S3). This behavior was similar to that of the males in the male-male experiments. The normal male ran away before the gynandromorph opened its chelicerae in all four trials. In the gynandromorph-female experiment (n = 2), the gynandromorph approached a female and stretched both of its forelegs forward, which was how the males behaved in the male-female experiments, and never bent its legs or side-stepped (Fig. 4d; video S4). The normal female escaped from the gynandromorph and did not show mating behavior.

Discussion

Not only non-gynandromorphic *M. formicaria* specimens examined in the taxonomic paper by Ono et al. (2009), but also our specimens showed sexual dimorphism in regard to length of chelicerae and fangs. Our gynandromorphic individual had a bilaterally asymmetric body, with the right and left halves of chelicerae, palps, and genitalia displaying male and female characteristics, respectively. To the best of our knowledge, this is the first report of a gynandromorphic individual and its behavior in the genus *Myrmarachne*.

The behavioral experiments revealed details of the agonistic and courtship behaviors of normal *M. formicaria* individuals for the first time, and the sex specificity of these behaviors was confirmed. The gynandromorphic spider showed courtship behavior to female spiders and agonistic behavior to males. These behaviors clearly indicated that the gynandromorph's sexual identity was male. Furthermore, the fact that normal males showed pre-fighting behavior to the gynandromorphic spider suggested that normal males may have also recognized the gynandromorph as a male. When the gynandromorph faced a female, it exhibited courtship behavior like a normal male, but the female tried to escape. However, we cannot conclude whether the normal female recognized the gynandromorph as a male with such a small number of observations (n = 2).

Previous studies have shown the relationship between the gynandromorphic pattern and sexual behavior of spiders (Gack and Helversen 1976 cited in Yoshikura 1987; Maekawa and Ikeda 1992; Table 2). Maekawa and Ikeda (1992) demonstrated that a completely bilateral gynandromorphic *Carrhotus xanthogramma* (Latreille 1819) (Araneae: Salticidae) showed male-specific behavior when it faced both a female and male, which is consistent with our results. On the basis of our results and those of previous studies, a spider may exhibit behaviors of male sexuality, although the external morphology has the characteristics of both sexes. Several studies on insects have also shown that gynandromorphs behaved like a male (Maeno and Tanaka 2007; Matsuo et al. 2018; Taniyama et al. 2018; Table 2), although further studies need to be performed.

For quantitative behavioral and physiological analyses of gynandromorphs, it would be necessary to obtain a large number of gynandromorphs from an established rearing colony (e.g., *Drosophila* *melanogaster*: Nissani 1977). However, in spiders, only one example of a gynandromorph obtained from a breeding colony has been reported (Laborda and Pérez-Miles 2017), and methods to produce gynandromorphic spiders under laboratory conditions have not yet been established. To understand the relationship between morphological gender and sexual identity of gynandromorphic spiders or non-insect arthropods, behavioral and physiological studies with wild gynandromorphic individuals in a natural population need to be performed.

In conclusion, we found a bilateral gynandromorphic spider behaving like a male to normal males and females, suggesting that its sexual identity was male. Our findings should encourage studies of gynandromorphism and sexual identity in non-model invertebrates.

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Fig. 1 External morphology of a *Myrmarachne formicaria* gynandromorph (a–d) and normal individuals (e-f). a, dorsal view; b, ventral view; c, enlarged dorsal view of gynandromorphic chelicera; d enlarged ventral view of gynandromorphic chelicera; e, enlarged ventral view of normal male chelicera; f, enlarged ventral view of normal female chelicera. Scale = 2 mm (a-b); 0.5 mm (c–f)

Fig. 2 Boxplots of the chelicera and fang lengths. a, chelicera length of each sex and the gynandromorph; b, fang length of each sex and the gynandromorph. Abbreviations: f, female; gf: left side of the gynandromorph; gm: right side of the gynandromorph; m, normal male. n = 10 (f, m), 1 (gf, gm)

Fig. 3 Sexual organs of the gynandromorph (a, c, e) and normal individuals (b, d, f). a-b, ventral view of the right palp; c-d, ventral view of the left palp; e-f, epigyne. The white arrow indicates the spermatheca at an abnormal position. Scale = 0.1 mm

Fig. 4 Flow diagrams of the behavioral sequences of agonistic behavior (a, c) and courtship behavior (b, d) performed by non-gynandromorphic individuals (a, b) and among gynandromorphic and normal individuals (c, d). Abbreviations: g, gynandromorph. Numbers within parentheses indicate the observed number of individuals that showed the behavioral elements per total number of observations.

Table 1 Morphological measurements of the gynandromorph and normal individuals of *M. formicaria*. Values of normal individuals indicate average and range within parentheses (in mm)

	Sexual type of the specimens				Mann–Whitney *U* test (normal female vs. normal male)	
Body part[1]	Gynandro-morph (left side)	Gynandro-morph (right side)	Normal female (N = 10)	Normal Male (N = 10)	Test statistic	p-value
Body length	4.49	4.49	4.56 (3.55–5.43)	4.12 (3.52–5.29)	72	0.10
Carapace length	2.12	2.12	2.00 (1.82–2.16)	1.92 (1.60–2.43)	65	0.28
Carapace width	1.12	1.12	1.03 (0.91–1.18)	1.02 (0.87–1.31)	59	0.53
Chelicera length	0.51	1.74	0.54 (0.40–0.69)	1.39 (0.98–2.16)	0	0.00
Fang length	0.40	1.90	0.35 (0.29–0.52)	1.26 (0.96–1.77)	0	0.00
Palp length	2.03	2.11	1.82 (1.56–2.29)	1.76 (1.39–2.24)	58	0.58
Leg Ⅰ length	4.20	4.81	3.93 (3.37–4.90)	4.05 (3.20–4.99)	45	0.74
Leg Ⅱ length	3.61	3.73	3.06 (2.43–4.02)	2.97 (2.27–4.18)	56	0.68
Leg Ⅲ length	3.65	4.04	3.24 (2.46–4.22)	3.23 (2.56–4.56)	54	0.80
Leg Ⅳ length	5.80	5.66	4.81 (3.83–5.91)	4.68 (3.85–6.22)	58	0.57

Table 2 Summary of known cases of gynandromorphy and behavior in invertebrates

Class	Species	Pattern of distribution of external morphological characteristics	Observed sex-specific behavior	References
Arachnida	*Carrhotus xanthogramma* (Latreille 1819) (Araneae: Salticidae)	Bilateral gynandromorph (The left half female, the right half male)	Male-specific behavior (Antagonistic behavior to a normal male and courtship behavior to a normal female)	Maekawa and Ikeda (1992)
	Myrmarachne formicaria (De Geer 1778) (Araneae: Salticidae)	Bilateral gynandromorph (The left half female, the right half male)	Male-specific behavior (Antagonistic behavior to a normal male and courtship behavior to a normal female)	This study
	Alopecosa pulverulenta (Clerck 1757) (Araneae: Lycosidae)	Incomplete bilateral gynandromorph (The left half male and the right half female, but the right palp intersexual)	Male-specific behavior (Courtship and mating behavior to a normal female)	Gack and Helversen (1976) cited in Yoshikura (1987)
Insecta	*Bombus ignitus* Smith 1869 (Hymenoptera: Apoidae)	Bilateral gynandromorph (The left half male, the right half female)	Never showed male-specific behavior to a queen bee	Ugajin et al. (2016)
	Bombus ignitus Smith 1869 (Hymenoptera: Apoidae)	Partial bilateral gynandromorph (The left half of the abdominal tip male, the right half female)	Abnormal male-specific behavior (Mating behavior to a queen bee)	Matsuo et al. (2018)
	Schistocerca gregaria Forsskål 1775 (Orthoptera: Acrididae)	Partial bilateral gynandromorph (The left half of the abdominal tip male, the right half female)	Male-specific behavior (Mating behavior to a normal female)	Maeno and Tanaka (2007)
	Polionemobius mikado (Shiraki 1913) (Orthoptera: Trigonidiidae)	Patchily distributed gynandromorph (Complete male forewings and a female ovipositor at the end of the abdomen)	Male-specific behavior (Antagonistic behavior to a normal male but not a normal female)	Taniyama et al. (2018)

Supplemental data

Video S1 Agonistic behavior (normal male vs. normal male)

Video S2 Courtship behavior (normal male vs. normal female)

Video S3 Normal male vs. gynandromorph

Video S4 Normal female vs. gynandromorph

Fig. 1

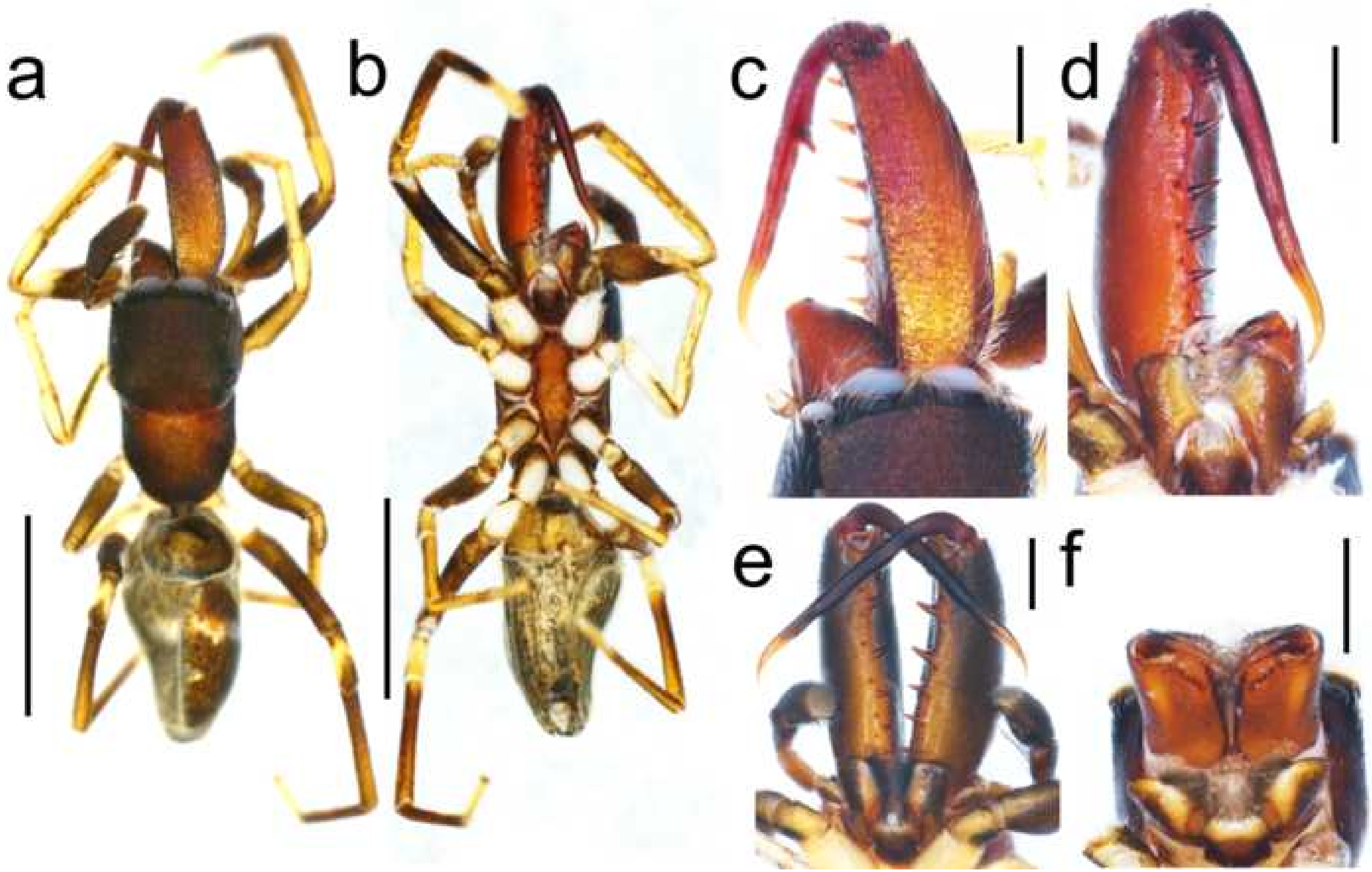

Fig. 2

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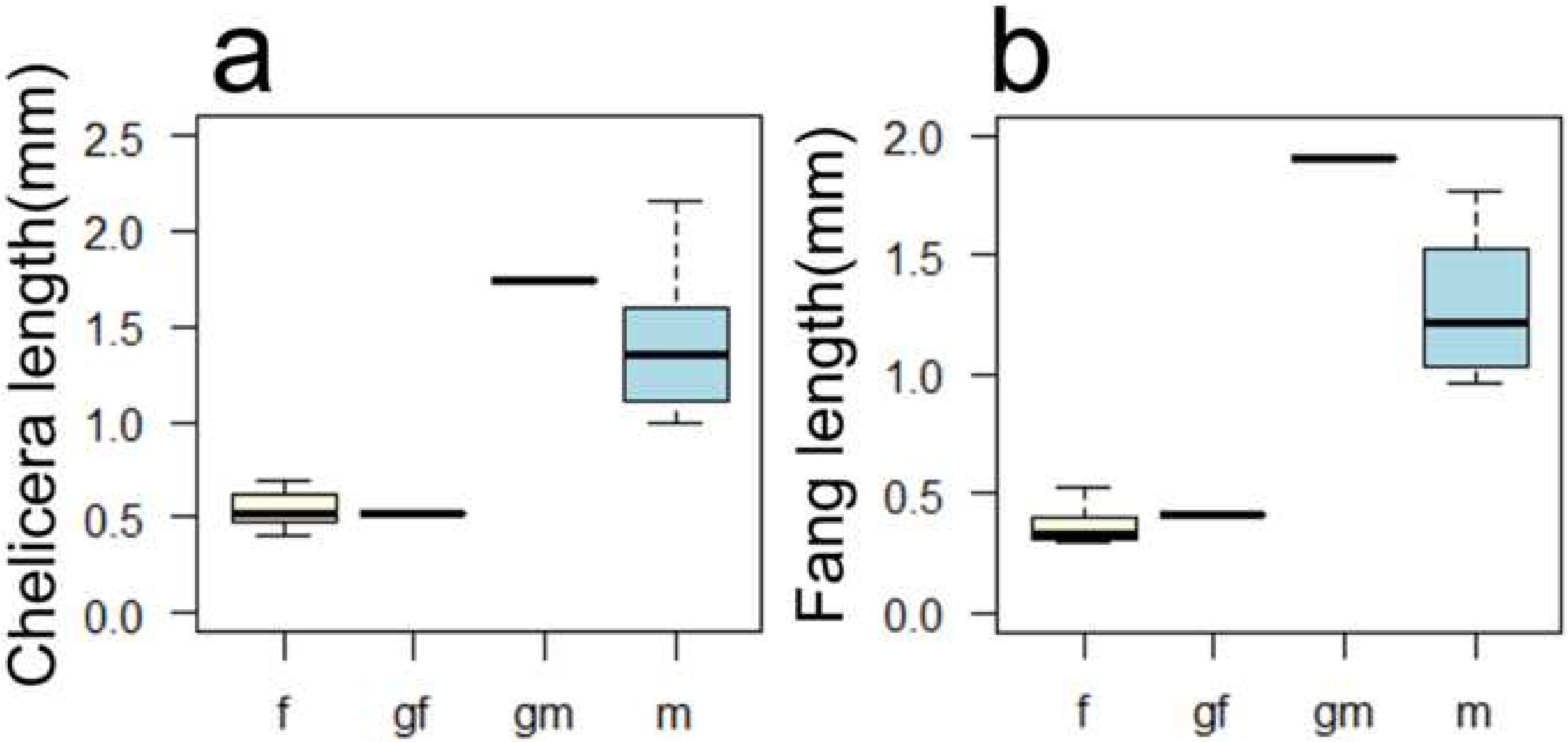


Fig. 3

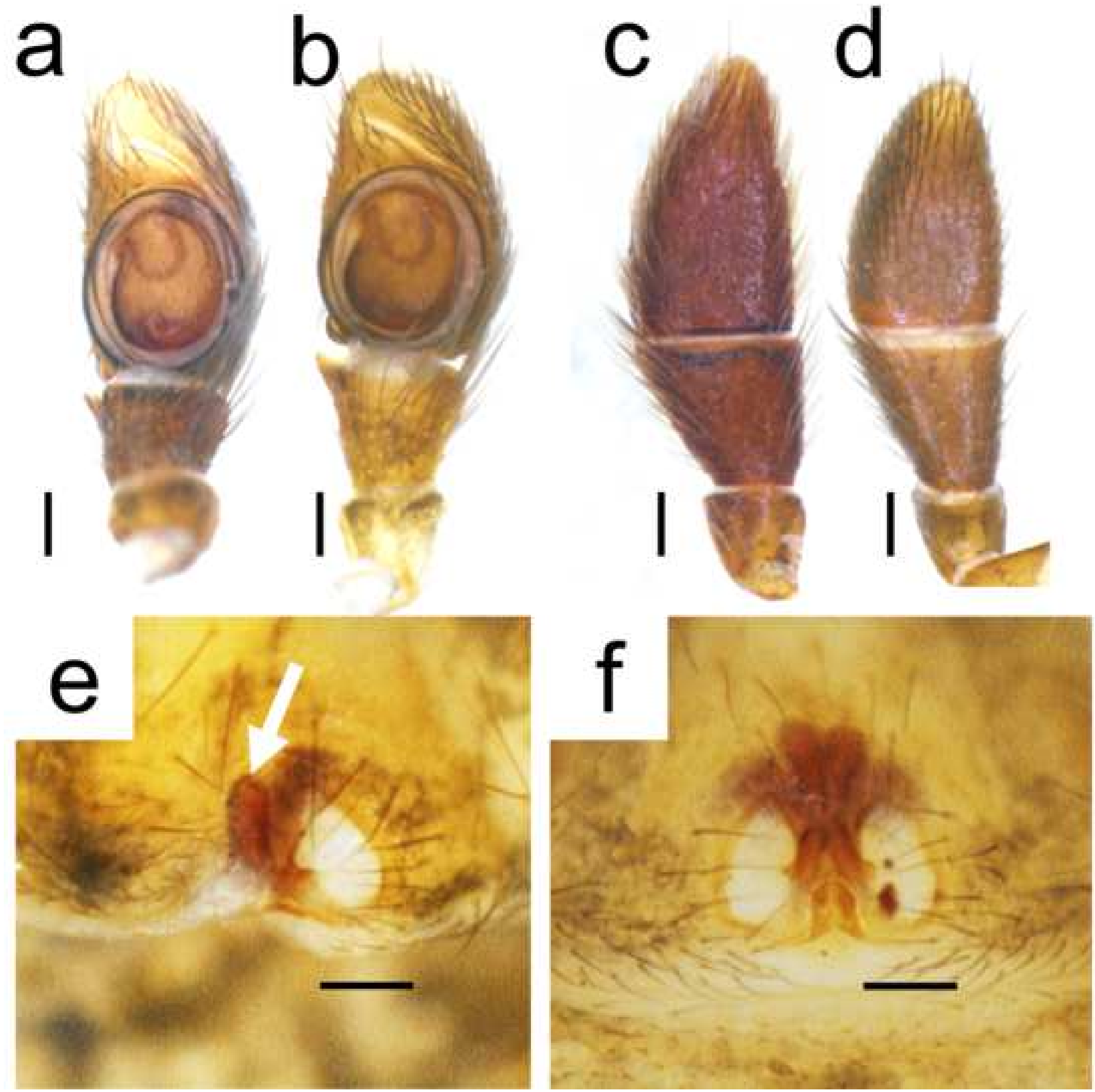
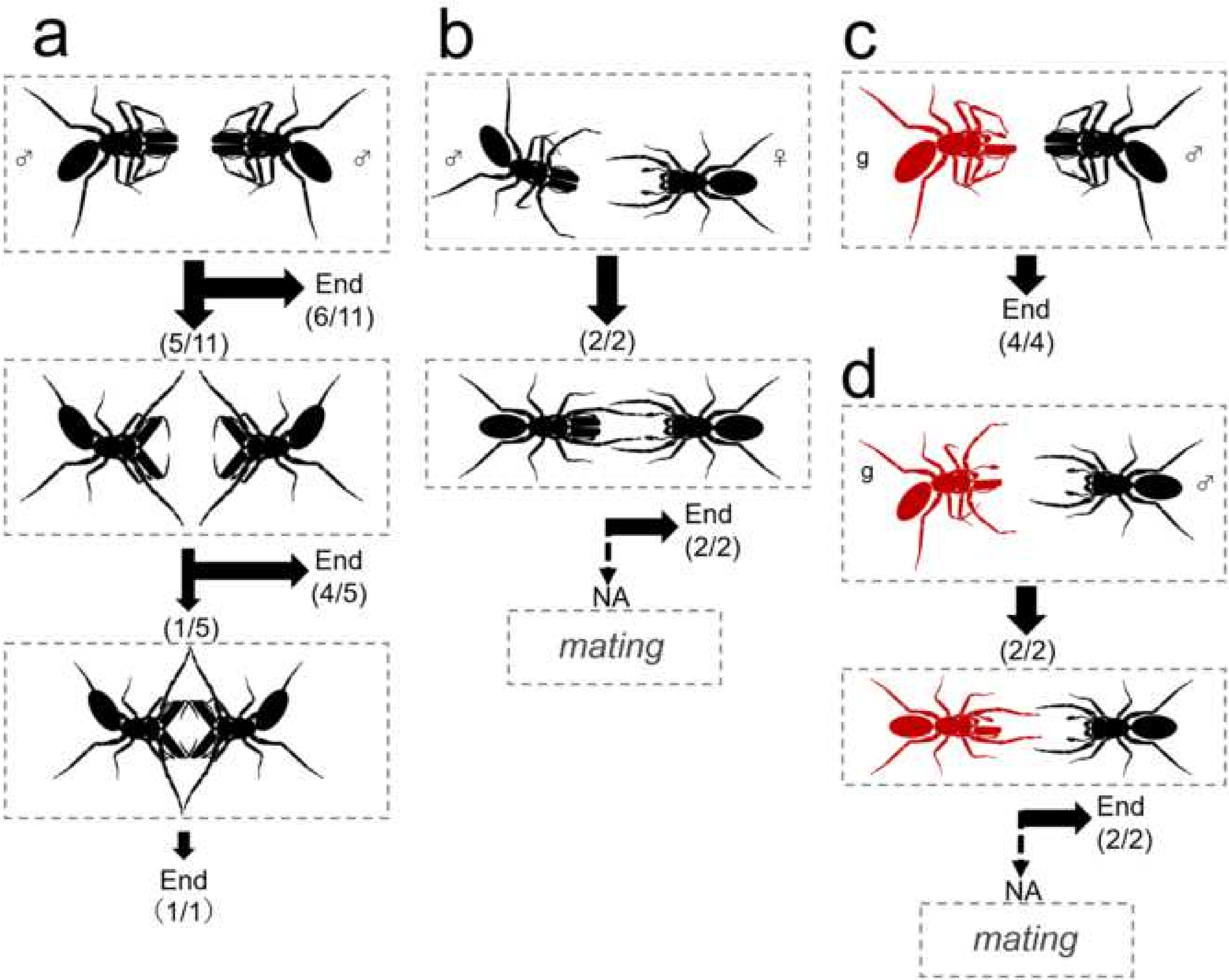


Fig. 4



a
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b
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g
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