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Authors: Dolejš, Petr, and Hanko, Mojmír

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# Ontogenetic development and reproduction of *Zorocrates guerrerensis* (Araneae: Zoropsidae)

### Petr Dolejš & Mojmír Hanko



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**Abstract:** Zorocrates guerrerensis Gertsch & Davis, 1940 is a Mexican cribellate spider with almost no information about its biology. As the species could potentially be of medical interest, it was decided to study basic aspects of its life history under laboratory conditions. The life cycle lasts a year, with spiders undergoing up to 12 instars to reach maturity. The courtship behaviour includes tactile communication. Copulation consists of two palpal insertions, each with a single haematodochal expansion. Both males and females can mate more than once; components of the first and second copulation do not differ. On average, the total copulation duration lasts for more than five minutes. Some details of the copulation process are discussed and compared with those of other lycosoids.

Keywords: copulation, courtship, cribellate spider, instar, life history, Mexico, polyandry, polygyny, tactile communication

**Zusammenfassung. Ontogenese und Reproduktion von Zorocrates guerrerensis (Araneae: Zoropsidae).** Die Biologie der mexikanischen cribellaten Spinne *Zorocrates guerrerensis* Gertsch & Davis, 1940 ist nahezu unbekannt. Da die Art potentiell medizinisch interessant ist, wurde ihr Lebenszyklus und ihre Lebensweise unter Laborbedingungen studiert. Ihr Zyklus dauert ein Jahr, in dem die Spinnen nach bis zu 12 Häutungen erwachsen werden. Beim Paarungsverhalten spielt taktile Kommunikation eine Rolle. Die Kopulation besteht aus zwei Insertionen der Palpen, jede mit einer einmaligen Expansion der Hämatodocha. Sowohl Männchen als auch Weibchen können sich mehr als einmal paaren; die Komponenten der ersten und zweiten Kopulation unterscheiden sich nicht. Durchschnittlich ist die Dauer der gesamten Kopulation länger als fünf Minuten. Details des Kopulationsprozesses werden diskutiert und mit dem anderer Lycosoidea verglichen.

New spider species to science continue to be discovered all over the world. Their descriptions are usually based on morphology, whereas bionomy, ecology or behaviour are rarely considered. However, behavioural data can contribute to the spider taxonomy, not only for distinguishing morphologically similar species (e.g., Kronestedt 1990, Töpfer-Hofmann et al. 2000) but also for placing them in correct genera (e.g., Rovner 1973). Surprisingly, we often lack basic information even about common, widespread species known to everybody. Recent research on long neglected animal species brought surprising results. For instance, large common (and even edible) European animals, such as the slow worm Anguis fragilis Linnaeus, 1758, the Turkish snail Helix lucorum Linnaeus, 1758 and the Roman snail Helix pomatia Linnaeus, 1758, were split up into five, two and two species respectively (Gvoždík et al. 2010, 2013, Korábek et al. 2014, 2016).

Zorocrates guerrerensis Gertsch & Davis, 1940 is also a relatively large, common, hemisynanthropic animal (Jiří Král pers. observ.), which seems to be of potential clinical importance (Sánchez-Vega et al. 2016). Despite this, *Z. guerrerensis* is a rarely studied species. Its name has appeared in only three publications: in its description (Gertsch & Davis 1940), a generic revision that provided its distribution in Mexico (Platnick & Ubick 2007) and a report on its bites (Sánchez-Vega et al. 2016). Based on morphological (Dahl 1913, Lehtinen 1967, Griswold et al. 1999, Raven & Stumkat 2005) or combined (Polotow et al. 2015) analyses, the genus *Zorocrates* has been transferred among various families (Miturgidae, Tengellidae, Zorocratide, Zoropsidae) several times. In the latest treatment (Wheeler et al. 2017), the genus was assigned to the subfamily Tengellinae of the family Zoropsidae, being

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Petr DOLEJŠ, Department of Zoology, National Museum – Natural History Museum, Cirkusová 1740, CZ – 193 00 Praha 9 – Horní Počernice, Czechia; E-mail: petr\_dolejs@nm.cz

Mojmír HANKO, Christian Grammar School, Kozinova 1000, CZ – 120 00 Praha 10, Czechia; E-mail: moj.hanko@seznam.cz

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considered a member of the superfamily Lycosoidea or the Oval Calamistrum clade. Biological and ethological observations that could support its correct taxonomic placement are, however, completely absent. Thus the aim of the present paper is to provide initial, basic information on the life history of this Mexican cribellate species.

# Material and methods

Several specimens were collected by Jiří Král in Mexico, Querétaro State, Juriquilla, in the campus UNAM (20.7036°N, 100.4474°W, 1920 m a.s.l.) on 20.vi.2009. The further breeding of Z. guerrerensis was carried out by Jaromír Hajer at the Jan Evangelista Purkyně University in Ústí nad Labem, Czech Republic. Fourteen spiderlings of the third instar from this breeding were reared from four egg sacs (constructed in 3.VII.-12.IX.2013). Exuviae of the previous two instars were available to us. Juveniles were held individually in plastic tubes (length 100 mm, diameter 15 mm; later length 115 mm, diameter 28 mm) supplied with wet cotton wool as a source of water. Spiders were reared at room temperature (20-23 °C) under natural photoperiod and fed weekly with wingless Drosophila melanogaster, Tenebrio larvae, crickets of appropriate size and seasonally available insects. Beginning with the third ecdysis, i.e. the fourth free instar, dates of each moult were recorded and the length of all shed carapaces was measured using a stereomicroscope (PZO Warszawa: MST 127) equipped with an ocular micrometer. To calculate the relative percent growth between subsequent instars for each individual, the equation of Mallis & Miller (2017) was used:  $100 \times L_N$  $L_{N-1} - 100$  (L = carapace length, N = instar). From these values, the population mean was calculated. The nomenclature and numbering of ontogenetic stages follow Downes (1987): the first instar is the stage that left the egg sac. We did not count the postembryonic moults inside the egg sacs (i.e. between the postembryo and the first nymphal instar), therefore we treated the first ecdysis as the one terminating at the first instar (after Dolejš et al. 2014).

After reaching a maturity, mating of nine females and four males (one reared juvenile died accidentally during manipulation) was observed in Petri dishes (diameter 90 mm, height 50 mm) under laboratory conditions (room temperature and a natural photoperiod). A white, moistened filter paper was placed into the Petri dish to provide a substratum suitable for spider locomotion, to improve contrast during videotaping and to allow the spiders to remain hydrated. An adult female was placed into the Petri dish 2 h before the trial to allow her to habituate to the new surroundings and to deposit silk and pheromones. After introducing a male, the spiders' behaviour was recorded for 15 min, using a digital Panasonic NV-GS400 video camera. Such a 15 min period was enough for all observed copulations to be completed. The females were paired with randomly chosen males. All but one mated female was paired in one more trial to determine whether females are monandrous or polyandrous (one female had produced an egg sac before the second trial began).

Seventeen copulations were observed and analysed. Latency (the time between introducing the male and the first physical contact), courtship and copulation duration were recorded. Numbers of insertions and of side shifts were counted. Behaviour of mating spiders was recorded. The moment when a male climbed onto a female was designated as the beginning of copulation, and the moment when the spiders physically separated as the end of copulation (Stratton et al. 1996). After copulation, each female was placed back in their plastic tube where later on they constructed their egg sacs. The production of egg sacs, the process of egg laying, hatching and postembryonic development inside the egg sacs were not investigated.

The software NCSS 2007 (Hintze 2006) was used to test the normality of continuous variables (all data were normally distributed) and to calculate descriptive statistics of the following variables: latency, courtship and copulation duration, number and duration of palpal insertions and hematodochal expansions, delay between copulation and egg sac production and number of offspring. Of the descriptive statistics, means  $(\bar{x})$  and standard errors (SE) were calculated. The first and second copulations were compared using a Paired t-test. Voucher specimens have been deposited in the National Museum, Prague (N°s P6A-6468 and P6d-14/2017).

#### Results

The life cycle of *Z. guerrerensis* lasted a year. Spiders underwent up to 12 instars; on average, the instar duration was 42.4 days (SE = 10.82) (Tab. 1). Males reached adulthood in the

10th (n = 1) or 11th (n = 3) instar, females in the 10th (n = 2), 11th (n = 4) or 12th (n = 3) instar. The between-instar growth factor was approximately constant (Tab. 1). The following instars were about 20.0 % (SE = 3.88) larger than the previous ones; the relative growths had a descending tendency (Tab. 1). Adult males were about 11.7 % smaller than females.

Pre-mating interactions between males and females started quite rapidly; the first contact occurred 28 s (SE = 34) after introducing the male into the arena with a female. Males touched the tibia or head region of the females with its first pair of legs. Females located the introduced males, raised its first or two first pairs of legs and held them either parallel to each other (Fig. 1) or at an angle of 30-60 degrees. Further contact was tactile. Males tapped (using their front legs) the patellae and tibiae of the female's front legs and also her carapace, and the femora and tibiae of the female's third and fourth legs (Fig. 2). On the average, the tactile interactions lasted for 123 s (SE = 146). During this courtship, the males waggled several times with their opisthosoma up and down and climbed onto females. When the male prosoma was above the female carapace, the females performed on average 5.5 (SE = 2.43) very vigorous jerks forwards using their third and fourth pairs of legs (the front legs were still raised) but their tarsi did not change their position, standing still on the same place. All tested females (n = 9) were receptive. Then, males mounted the females (Fig. 3) so that the male's prosoma was above that of the female, but the spiders were facing in opposite directions.

Once the males reached the copulatory position ["Position II" after Gerhardt & Kästner (1941) or "Type 3" after Foelix (2011)], they immediately inserted one palp equilaterally. The palp was inserted between the third and fourth leg of the females (Fig. 4). Just before insertion, the males briefly scraped the epigyne using rapid movements of their palps. The haematodocha was expanded (and leg spines erected) immediately after the palp was inserted (Fig. 5); during the palpal insertion, only one haematodochal expansion occurred. Males left their palps close to the epigyne (but not in direct contact with it) for some time after the haematodocha had collapsed and the spines returned to their original position (Fig. 6 and the supplementary video file). Males switched sides only once, thus each palp (right and left) was used only once. In four out of 17 copulations, the so-called 'flubs' were observed: males inserted their palps, but the haematodocha never expanded in full and males usually used the other palp. Such unsuccessful

**Tab. 1:** Summary of the ontogenetic development of *Zorocrates guerrerensis*. Carapace lengths, percentage of growth in carapace length during moults and duration of each instar given separately for males (n = 4) and females (n = 9). Means and standard errors (in parentheses) are provided.

	_		•						•			
Instar	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Carapace length (mm) \$\varphi\$	1.0 (0.06)	1.2 (0.10)	1.4 (0.07)	1.7 (0.20)	1.9 (0.27)	2.4 (0.22)	2.9 (0.22)	3.4 (0.39)	4.2 (0.52)	5.1 (0.49)	5.5 (0.34)	_
Carapace length (mm) ♂	1.0 (0.08)	1.2 (0.13)	1.5 (0.17)	1.8 (0.21)	2.2 (0.28)	2.6 (0.22)	3.3 (0.26)	3.9 (0.35)	4.5 (0.45)	5.2 (0.52)	6.0 (0.50)	6.4 (0.35)
Relative growth (%) ♀	_	21.6 (4.36)	22.2 (7.96)	20.2 (8.11)	18.8 (3.02)	21.8 (5.90)	20.3 (3.98)	20.6 (4.49)	20.3 (6.46)	22.3 (6.46)	10.3 (4.38)	_
Relative growth (%) る	_	26.5 (9.47)	20.8 (6.95)	18.4 (6.08)	20.1 (5.56)	22.3 (7.87)	19.1 (3.48)	24.3 (7.25)	18.1 (3.80)	16.8 (6.43)	19.5 (5.74)	12.7 (4.42)
Duration (days)	;	;	;	40.0 (5.488)	33.5 (4.04)	36.0 (2.71)	38.0 (4.16)	45.0 (15.78)	54.0 (11.22)	68.3 (4.51)	-	-
Duration (days) る	;	;	;	37.9 (2.09)	36.2 (4.09)	38.0 (8.12)	34.8 (3.38)	46.1 (12.47)	50.1 (7.29)	48.6 (12.04)	46.0 (15.39)	_

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Figs. 1-6: The mating of *Zorocrates guerrerensis*. 1 – Male is approaching and courting female, making contact using the first pair of legs; female orientates toward the male and raises its first pair of legs. 2 – Male and female communicate with each other using their front legs and the male is touching the female's carapace. 3 – Male is climbing onto female, reaching a copulatory position; the beginning of copulation. 4 – Insertion of the left palp, note that the palp is inserted between the third and fourth pairs of legs of the female. 5 – Haematodochal expansion, note erect leg spines. 6 – The end of palpal insertion, the haematodocha has collapsed but the palp is still close to the epigyne.

palpal insertion was not counted. The whole copulation event (i.e. including both palpal insertions but without courtship) lasted on average about 5 minutes and 18 seconds (SE = 2 min. 3 sec.). After each copulation, males spun a rectangular sperm web (Fig. 7) and charged their palps.

All but one of the females mated twice and all males mated multiply. The first copulation (of a virgin female) and the second copulation (of a mated female) did not differ in any of their components (Tab. 2). The only difference was in total copulation duration: matings of virgin females were shorter than those with already mated females, given by slower shifting of the palps from the first to the second insertion during the second copulations. However, the difference in duration of the first and second copulation was only marginally significant (Tab. 2).



Fig. 7: Sperm web of Zorocrates guerrerensis spun in a plastic tube

Fertilised females were allowed to construct up to four egg sacs under laboratory conditions. The first egg-sac was constructed in 31.9 days (SE = 12.26, n = 9) after the first mating. From each egg sac, on average 42 spiderlings (SE = 17.8, n = 17) emerged after about a two-month period ( $\bar{x}$  = 60.6 days, SE = 21.23, n = 16) of incubation.

#### Discussion

We observed the growth during a year and details of the reproductive behaviour of *Z. guerrerensis*, a Mexican cribellate spider from the family Zoropsidae. Reaching adulthood by *Z. guerrerensis* after 9-11 moults outside the egg sac is comparable with two other studied zoropsids, *Tengella perfuga* Dahl, 1901 and *T. radiata* (Kulczyński, 1909), reaching adulthood in 11-12 and 8-9 moults, respectively (Barrantes & Madrigal-Brenes 2008, Mallis & Miller 2017). The between-instar growth factor of *Z. guerrerensis* was approximately constant, contrary to *T. radiata* and *T. perfuga*, in which Barrantes & Madrigal-Brenes (2008) and Mallis & Miller (2017) observed some fluctuations in the growth factor in several instars. The observed decreasing tendency in the growth factor was caused by some individuals that needed more moults to reach maturity. Those should have also grown less during their early instars.

Males displayed the courtship of "Level I", i.e. the direct contact with the female (Platnick 1971). Communication between males and females was (beside an expected olfactory way) largely tactile. No visual or acoustic communication was recorded. Tactile communication was observed also in *T. perfuga* by Mallis & Miller (2017: video S2), but the difference was in the 'receptivity signal' (acceptance of the male and allowing it to assume a copulatory position). The 'receptivity signal' of the *Z. guerrerensis* female was in precise contacts by

**Tab. 2:** Comparison of behavioural components of mating virgin and once-mated females of *Zorocrates guerrerensis*. Means and standard errors (in parentheses) are given.

Behaviour		Copulation of mated females (n = 8)	
Courtship (s)	83.9 (121.46)	166.5 (167.47)	0.4393
First expansion of haematodocha (s)	19.6 (3.50)	16.7 (6.58)	0.1780
First palpal insertion (s)	91.9 (62.92)	98.4 (21.75)	0.9366
Second expansion of haematodocha (s)	23.0 (2.83)	22.5 (7.69)	0.4627
Second palpal insertion (s)	112.8 (46.77)	121.8 (61.03)	0.9660
Total copulation duration (s)	246.6 (53.13)	390.1 (133.75)	0.0515

legs I and II, whereas the *T. perfuga* female had their front legs stretched at the moment when the male was climbing onto it (Mallis & Miller 2017: video S5). The 'receptivity signal' of *Z. guerrerensis* was rather similar to that of the wolf spider *Arctosa* (*Tricca*) *lutetiana* (Simon, 1876) (Dolejš et al. 2010). In *T. perfuga*, Mallis & Miller (2017: video S3) observed that males spun the so-called 'bridal veil' [the silk deposited across the female's carapace and legs; also a common part of court-ship in certain *Xysticus* species (Platnick 1971)] prior to copulation. No such behaviour was observed in *Z. guerrerensis*; instead, females were jerking with their whole bodies when males were climbing onto them. A possible explanation of such behaviour occurring just prior the copulation could be that it was the female's last chance to chase away a male that for some reason would not be an ideal partner.

The copulatory position of *Z. guerrerensis* resembled that of wolf spiders and *T. perfuga*, but differed from the copulation position of *T. radiata*, in which spiders were orientated towards each other by their ventral sides (Barrantes 2008). The males of *Z. guerrerensis* inserted their palps between the third and fourth legs of the females. Such a position corresponds to what can also be seen in videos about *T. perfuga* (Mallis & Miller 2017). However, it differs from the position observed in wolf spiders in which males insert their palps behind the females' fourth legs (e.g., Montgomery 1903, Dolejš et al. 2010, 2012, Foelix 2011). Unfortunately, we are not aware of any literature dealing with this difference among various families. Thus, any conclusions about the sense, function or mechanical limitations of different ways of palpal insertions would be too preliminary now.

The pattern of copulation of *Z. guerrerensis* with a single insertion of each palp and a single expansion of haematodocha is a frequent one not only among the lycosoids but also in the unrelated cribellate genera *Amaurobius* and *Titanoeca* (Stratton et al. 1996). However, in both *Tengella* species, repeated insertions of the same palp were observed (Barrantes 2008, Mallis & Miller 2017). The second difference is in the duration of haematodochal expansion in relation to that of palpal insertion. In wolf spiders and the zoropsid *T. radiata*, almost the whole duration of palpal insertion is composed of the haematodochal expansion (Barrantes 2008, Dolejš et al. 2010, 2012). The males of *Z. guerrerensis*, however, switched

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to use the second palp sometime after the haematodocha of the first palp had collapsed. During this period, when males were apparently doing 'nothing', perhaps the copulatory courtship occurred in relation to the cryptic female choice (e.g., Huber 1998, Peretti & Aisenberg 2015).

Some males of Z. guerrerensis were observed having an obvious problem to insert their palps correctly. We call this behaviour 'flubs', despite some uncertainties existing about their definition and true meaning (e.g., Huber 1998, Barrantes 2008, Sentenská et al. 2015). In our opinion, the flubs were apparently mistakes as was defined by Watson (1991) and further observed by Dolejš et al. (2012) and Toscano-Gadea & Costa (2016). Thus, the flubs observed by us are not regularly observed palpal scraping of the epigyne just before the palpal insertion. Zorocrates guerrerensis appeared to be both polyandrous and polygynous species. Among the Lycosoidea, the same characteristics is known for the pisaurids (Nitzsche 2011 and references therein), whereas lycosid females are monandrous (Norton & Uetz 2005, Dolejš et al. 2012). Construction of the first egg sac by Z. guerrerensis and the emerging of spiderlings from it were in identical time intervals as in T. radiata (Barrantes & Madrigal-Brenes 2008).

Overall, very little is still known about the courtship and copulatory pattern of the zoropsids. The situation that some mating characteristics of *Z. guerrerensis* are more similar to *T. perfuga* and wolf spiders than to *T. radiata* is surprising because it was expected that related species would have similar behaviour. When behavioural details of more species of Zoropsidae are known, they may be of some use for improving the phylogeny of Lycosoidea.

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#### Supplementary File (\*.wmv)

Courtship and copulation of *Zorocrates guerrerensis*. The video shows all behavioural components of the mating, but several longer-lasting components of the copulation were shortened.