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

Authors: Petr Dolejš, and Mojmír Hanko

Source: Arachnologische Mitteilungen: Arachnology Letters, 55(1) : 46-51

Published By: Arachnologische Gesellschaft e.V.

URL: https://doi.org/10.30963/aramit5508
Ontogenetic development and reproduction of Zorocrates guerrerensis (Araneae: Zoropsidae)

Petr Dolejš & Mojmír Hanko

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

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 × \( L_n/L_{n-1} \) = 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 manipu-
loration) 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 substrate 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 (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 (Nos 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 s (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 haematochoda was expanded (and leg spines erected) immediately after the palp was inserted (Fig. 5); during the palpal insertion, only one haematochodal expansion occurred. Males left their palps close to the epigyne (but not in direct contact with it) for some time after the haematochoda 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 haematochoda never expanded in full and males usually used the other palp. Such unsuccessful

---

**Table 1:** Summary of the ontogenetic development of *Zorocrates guerrerensis*. Carapace lengths, percentage of growth in carapace length during molts 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   |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|
|        |      |      |      |      |      |      |      |      |      |      |      |      |
|        | 1.0  | 1.2  | 1.4  | 1.7  | 1.9  | 2.4  | 2.9  | 3.4  | 4.2  | 5.1  | 5.5  |      |
|        | (0.06)| (0.10)| (0.07)| (0.20)| (0.27)| (0.22)| (0.22)| (0.39)| (0.52)| (0.49)| (0.34)|      |
| Carapace length (mm) | 1.0  | 1.2  | 1.5  | 1.8  | 2.2  | 2.6  | 3.3  | 3.9  | 4.5  | 5.2  | 6.0  | 6.4  |
|        | (0.08)| (0.13)| (0.17)| (0.21)| (0.28)| (0.22)| (0.26)| (0.35)| (0.45)| (0.52)| (0.50)| (0.35)|
| Relative growth (%) | 21.6 | 22.2 | 20.2 | 18.8 | 21.8 | 20.3 | 20.6 | 20.3 | 22.3 | 10.3 |      |      |
|        | (4.36)| (7.96)| (8.11)| (3.02)| (5.90)| (3.98)| (4.49)| (6.46)| (6.46)| (4.38)|      |      |
| Relative growth (%) | 26.5 | 20.8 | 18.4 | 20.1 | 22.3 | 19.1 | 24.3 | 18.1 | 16.8 | 19.5 | 12.7 |      |
|        | (9.47)| (6.95)| (5.56)| (8.77)| (3.48)| (7.25)| (3.80)| (6.43)| (5.74)| (4.42)|      |      |
| Duration (days) | 40.0  | 33.5  | 36.0  | 38.0  | 45.0  | 54.0  | 68.3  |      |      |      |      |      |
|        | (5.488)| (4.04)| (2.71)| (4.16)| (15.78)| (11.22)| (4.51)|      |      |      |      |      |
| Duration (days) | 37.9  | 36.2  | 38.0  | 34.8  | 46.1  | 50.1  | 48.6  | 46.0  |      |      |      |      |
|        | (2.09)| (4.09)| (8.12)| (3.38)| (12.47)| (7.29)| (12.04)| (15.39)|      |      |      |      |
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).

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.
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 (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 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 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 S3). The 'receptivity signal' of *Z. guerrerensis* was rather similar to that of the wolf spider *Arctosa (Tricina) 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 courtship 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 *Titanova* (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...
to use the second palp sometime after the haematodocha of the first palp had collapsed. During this period, 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 mistaken 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 pisaurots (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. perjuga 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.

Acknowledgements

We are very much obliged to Jaromír Hajer for providing us with living spiderlings of Z. guerrerensis and Jiří Král for clarifying its biotope. We would like to thank Ivana Sykrová for supervising the grammar-school-thesis of the second author. We also thank Pavel Just for technical help with the video. Finally, we are obliged to two anonymous reviewers for their corrections and helpful comments on the earlier draft. This work was financially supported by the Ministry of Culture of the Czech Republic (DKRVO 2017/15, National Museum, 00023272).

References

Barrantes G 2008 Courtship behavior and copulation in Tengella radiata (Araneae, Tengellidae). – Journal of Arachnology 36: 606-608 – doi: 10.1636/St07-13.1
Barrantes G & Madrigal-Brenes R 2008 Ontogenetic changes in web architecture and growth rate of Tengella radiata (Araneae, Tengellidae). – Journal of Arachnology 34: 545-551 – doi: 10.1636/St07-66.1
Dahl F 1913 Vergleichende Physiologie und Morphologie der Spinnentiere unter besonderer Berücksichtigung der Lebensweise. I. Die Beziehungen des Körperbaues und der Farben zur Umgebung. G. Fischer, Jena. 113 pp.
Dolejš P, Buchar J, Kubcová L & Smrž J 2014 Developmental changes in the spinning apparatus over the life cycle of wolf spiders (Araneae: Lycosidae). – Invertebrate Biology 133: 281-297 – doi: 10.1111/irvb.12055
Dolejš P, Kubcová L & Buchar J 2010 Courtship, mating and cocoon maintenance of Trica lutetiana (Araneae, Lycosidae). Journal of Arachnology 38: 504-510 – doi: 10.1636/H09-29
Dolejš P, Kubcová L & Buchar J 2012 Reproduction of Arctosa alpigena lamperi (Araneae: Lycosidae) – where, when, how, and how long? – Invertebrate Reproduction & Development 56: 72-78 – doi: 10.1080/07924259.2011.617072
Downes MF 1987 A proposal for standardization of the terms used to describe the early development of spiders, based on a study of Theridion ruppius Lucas (Araneae: Theridiidae). – Bulletin of the British arachnological Society 7: 187-193
Foelix R 2011 Biology of spiders. Third Edition. Oxford University Press, New York. 419 pp.
Gerhardt U & Kästner A 1941 Araneae. In: Kükenthal W & Krumback T (eds.) Handbuch der Zoologie 3, Chelicerata. Walter de Gruyter & Co., Berlin. pp. 394-656
Gertsch WJ & Davis LJ 1940 Report on a collection of spiders from Mexico. III. – American Museum Novitates 1069: 1-44
Griswold CE, Coddington JA, Platnick NI & Forster RR 1999 Towards a phylogeny of Entelegyne spiders (Araneae, Araneomorphae, Entelegynae). – Journal of Arachnology 27: 53-63
Gvoždík V, Benkovský N, Crottini A, Bellati A, Moravec J, Romano A, Sacchi R & Jablonski D 2013 An ancient lineage of slow worms, genus Anguis (Squamata: Anguidae), survived in the Italian Peninsula. – Molecular Phylogenetics and Evolution 69: 1077-1092 – doi: 10.1016/j.ympev.2013.05.004
Gvoždík V, Jandzik D, Lymberakis P, Jablonski D & Moravec J 2010 Slow worm, Anguis fragilis (Reptilia: Anguidae) as a species complex: genetic structure reveals deep divergences. – Molecular Phylogenetics and Evolution 55: 460-472 – doi: 10.1016/j.ympev.2010.01.007
Hintze J 2006 NCSS, PASS, and GESS. NCSS, Kaysville, Utah. – Internet: http://www.ncss.com (October 15, 2017)
Huber BA 1998 Spider reproductive behaviour: a review of Gerhardt’s work from 1911–1933, with implications for sexual selection. – Bulletin of the British arachnological Society 11: 81-91
Korábek O, Juríčková L & Petruske A 2014 Resurrecting Helix stramina, a forgotten escargot with trans-Adriatic distribution: first insights into the genetic variation within the genus Helix (Gastropoda: Pulmonata). – Zoological Journal of the Linnean Society 171: 72-91 – doi: 10.1111/zol.12122
Korábek O, Juríčková L & Petruske A 2016 Splitting the Roman snail Helix pomatia Linnaeus, 1758 (Stylommatophora: Helicidae) into two: redescription of the forgotten Helix thesaliae Boettger, 1886. – Journal of Molluscan Studies 82: 11-22 – doi: 10.1093/mollus/eyv048
Kronestedt T 1990 Separation of two species standing as Alopecosa acuta by morphological, behavioural and ecological characters, with remarks on related species in the paturomenat group. – Zoologica Scripta 19: 203-225 – doi: 10.1111/j.1463-6409.1990.tb00256.x
Lehtinen PT 1967 Classification of the cribellate spiders and some allied families, with notes on the evolution of the suborder Araneomorpha. – Annales Zoologici Fennici 4: 199-468
Mallis RE & Miller KB 2017 Natural history and courtship behavior in Tengella perjuga Dahl, 1901 (Araneae: Zoropsidae). – Journal of Arachnology 45: 166-176 – doi: 10.1636/15-004.1
Montgomery TH 1903 Studies on the habits of spiders, particularly those of the mating period. – Proceedings of the Academy of Natural Sciences of Philadelphia 55: 59-149
Nitzsche ROM 2011 Courtship, mating and agonistic behaviour in Pisaura mirabillis (Clerck, 1757). – Bulletin of the British arachnological Society 15: 93-120 – doi: 10.13156/arac.2011.5.4.93
Nowosielski S & Uetz GW 2005 Mating frequency in Schizocosa ocreata (Hentz) wolf spiders: Evidence for a mating system with female monandry and male polygyny. – Journal of Arachnology 33: 16-24 – doi: 10.1636/0022-8112.405
Peretti AV & Aisenberg A 2015 Cryptic female choice in arthropods. Springer, Cham Heidelberg New York Dordrecht London. 509 pp. – doi: 10.1007/978-3-319-17894-3
Platnick N 1971 The evolution of courtship behaviour in spiders. – Bulletin of the British arachnological Society 2: 40-47
Platnick NI & Ubick D 2007 A revision of the spider genus Zorocrates Simon (Araneae, Zorocoridae). – American Museum Novitates 3579: 1-44 – doi: 10.1206/0003-0082(2007)3579[1:AROTSG]2.0.CO;2
Polotow D, Carmichael A & Griswold CE 2015 Total evidence of the phylogenetic relationship of Lycosoidea spiders (Araneae,
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.